Journal of Bone Oncology 13 (2018) 11–54

Contents lists available at ScienceDirect

Journal of Bone Oncology

journal homepage: www.elsevier.com/locate/jbo

MolPharm/CABS 2018

Invited speaker abstracts

IS2

Overview of current treatments and the potential role of combination therapies in osteoporosis

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The current therapies of osteoporosis can be divided into antiresorptive and bone forming therapies. The antiresorptive therapies comprise bisphosphonates, denosumab, SERMs, and HRT. The most efficient antiresorptive therapies reduce the risk of vertebral, non-vertebral and hip fractures by approximately 70%, 20% and 40%, respectively. The bone forming therapies are teriparatide and abaloparatide (currently only available in the US). Both stimulate the osteoblast through the PTH receptor and reduce the risk of vertebral and non-vertebral fractures by approximately 80% and 50%, respectively. Thus, efficient therapies are available for the treatment of osteoporosis, however, there are still unmet needs. Antiresorptive therapies only increase bone mineral density to a certain extent and reduce the risk of non-vertebral fractures by 20%, and the effect of bone forming therapy seems to level off over time. At least in theory, combination therapy targeting both resorption and formation could be a solution. Studies have investigated combinations of teriparatide with orally and intravenously administrated bisphosphonates and denosumab. In the PaTH trial the combination of teriparatide and alendronate did not improve BMD more than with either drug alone. In fact alendronate even appeared to impair the bone forming effect of teriparatide. A combination of teriparatide and zoledronic acid results in the best of both therapies: the increase in hip BMD seen with zoledronic acid combined with the increase in spine BMD seen with teriparatide. In contrast, the combination of denosumab and teriparatide appears to have an additive effect. None of the studies investigating combination therapy were powered to allow for conclusions regarding anti-fracture efficacy.

Conclusion: Efficient antiresorptive and bone forming therapies for osteoporosis are available and while studies investigating combination therapies have shown interesting results pertaining to BMD, the lack of evidence for anti-fracture efficacy of combination therapies makes sequential treatment the currently preferred option for the management of osteoporosis.

DISCLOSURE

Advisory boards and speakers bureau: Amgen, Eli Lilly, Merck, UCB, Teva Research Funding: Amgen, Novo Nordisk

IS3

Lessons from the SCOOP Study

Eugene McCloskey

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The SCreening for Osteoporosis in Older women for the Prevention of fracture [SCOOP] study was a community-based screening intervention, in women aged 70 to 85 years in the UK, in which osteoporosis treatment was recommended those at high risk of hip fracture using the FRAX risk assessment tool. The study delivered an average 28% reduction in the incidence of hip fracture in the screening arm, an effect that was significantly greater in those at higher risk targeted for appropriate treatment

The SCOOP results have significant impact on future healthcare policy. A potential screening approach was recommended by NICE in 2012, when it proposed that all women aged 65 years or older and men aged 75 years or older should have a fracture risk assessment using the FRAX or QFracture tools. The SCOOP study readily demonstrates the reversibility of high risk identified by FRAX. Health economic analysis of the study indicates that the cost per prevented hip fracture is less than £8 000 and that the cost per QALY gained is less than £20 000. If the SCOOP strategy was applied across the whole population of women in this age group in the UK, then almost 8000 hip fractures could be prevented each year; this could be further enhanced by mechanisms that extended the strategy to the two-thirds of eligible women who did not participate in the screening study, as well as combining osteoporosis treatment with falls prevention in eligible individuals.

Future studies should examine how this FRAX-based approach can be made available to, or accessible by, the wider community to achieve greater reductions in the number of hip fractures in the UK and elsewhere.

IS4

Zoledronate every 18 months for 6 years in osteopenic postmenopausal women: effects on fractures and non-skeletal endpoints

Ian Reid, Anne Horne, Borislav Mihov, Mark Bolland, Sonja Bastin, Gregory Gamble

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Bisphosphonates prevent fractures in patients with osteoporosis, but their efficacy in women with osteopenia is unknown. Most fractures in postmenopausal women occur in osteopenic individuals, so if phar-

https://doi.org/10.1016/j.jbo.2018.07.009

maceutical intervention is to impact significantly on total fracture numbers, therapies with efficacy in osteopenic postmenopausal women are needed.

We report a double-blind trial of 2000 osteopenic, postmenopausal women, randomly assigned to receive 4 infusions of either zoledronate 5mg, or normal saline at 18-month intervals. Each was followed for 6 years. Monthly vitamin D supplements were provided but not calcium supplementation. Women were recruited using electoral rolls. Inclusion criteria were age >65 years, hip T-score between -1.0 and -2.5. Exclusion criteria were: lumbar spine T-score <-3.0, eGFR <30 mL/minute, major systemic disease, metabolic bone disease, or regular use of bone-active drugs in the previous year. The study has 80% power to detect a decrease in osteoporotic fractures of 30%.

At baseline, age was 71 (SD 5) y, BMI 27 (5), femoral neck T-score -1.5 (0.5), and 95% were white. Non-vertebral fractures (excluding skull, face, hands and feet) occurred in 148 women in the placebo group and in 101 in the zoledronate group (ITT analysis, hazard ratio 0.66 [95%CI 0.51, 0.85], P = 0.0014, NNT = 22). Height loss, a surrogate for vertebral fracture, was 9.3 (8.7, 9.9) mm in the placebo group and 7.4 (6.9, 8.0) mm in the zoledronate group (P < 0.0001). Odds ratio for prespecified adverse events were as follows: death, 0.65 (0.40, 1.046); myocardial infarction, 0.61 (0.36, 1.02); cancer 0.67 (0.50, 0.90). Odds ratio for breast cancer was 0.58 (0.34, 0.98).

These results suggest this less intensive zoledronate regimen is effective for fracture prevention in osteopenia, and that it has beneficial effects on cancer risk and, possibly, mortality. These findings have the potential to substantially broaden the target population for pharmaceutical intervention to prevent fractures, and suggest that zoledronate should be further explored for the prevention of cancer and vascular disease.

DISCLOSURE:

IRR has received research funding and/or honoraria from Novartis, Amgen, Merck & Lilly

IS5

Update on teriparatide and the VERO clinical trial

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Clinical trials comparing the anti-fracture efficacy of osteoporosis drugs as the primary outcome are lacking. We compared the anti-fracture efficacy of teriparatide with risedronate in patients with severe osteoporosis.

In this double-blind, double-dummy trial, we enrolled postmenopausal women with at least two moderate or one severe vertebral fracture (VFx) and a bone mineral density (BMD) T-score of less than -1.50.

680 women were randomly assigned to receive 20 μg of teriparatide once daily plus oral weekly placebo, and 680 to receive 35 mg of oral risedronate once weekly plus daily injections of placebo for 24 months. The primary outcome was the incidence of new radiographic VFx. Secondary, gated outcomes included new and worsened radiographic VFx, clinical fractures, and nonvertebral fractures. A prospectively planned subgroup analyses of fracture data across subgroups predefined by the following baseline characteristics: age, number and severity of prevalent VFx, prevalent nonvertebral fractures, glucocorticoid use, prior osteoporosis drugs, recent bisphosphonate use, clinical VFx in the year before study entry, and baseline BMD was carried out.

At 24 months, new VFx occurred in 5.4% of patients in the teriparatide group, as compared with 12.0% in the risedronate group (risk ratio: 0.44; 95% confidence interval [CI]: 0.29 to 0.68; p < 0.001). Clinical fractures occurred in 4.8% of patients in the teriparatide group, compared with 9.8% in the risedronate group (hazard ratio: 0.48; 95% CI: 0.32 to 0.74; p < 0.001). Nonvertebral fragility fractures occurred in 4.0% of patients in the teriparatide group and 6.1% in the risedronate group (p = 0.10). The rate ratio of all nonvertebral fragility fractures estimated with a Poisson regression model was significant in favour of teriparatide (rate ratio 0.56; 95% CI 0.35 to 0.90; p = 0.017). More patients treated with teriparatide had at least one high value of serum calcium or uric acid. 25-OH-vitamin D serum levels were lower in the teriparatide group.

Amongst postmenopausal women with severe osteoporosis, the risk of new VFx and clinical fractures was significantly reduced in patients receiving teriparatide compared with those receiving risedronate by 56% and 52%, respectively. The anti-fracture efficacy of teriparatide compared with risedronate was consistent within the various predefined subgroups.

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DISCLOSURE:

Employee and shareholder Eli Lilly and Company

IS6

Non-invasive imaging of therapeutic responses in bone

Ken Poole

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Human imaging techniques now permit the evaluation of bone treatment responses in great detail. Advances in 3D histology might have a role in elucidating the response of bone to treatment, especially in the rarer causes of bone pathology such as osteomalacia. With potentially curative treatments such as anti-FGF23 on the horizon for XLH and TIO, there are good reasons to consider what 3D histological imaging techniques can provide by way of unmineralised and mineralised tissue quantification. Considering emerging osteoporosis therapies, novel non-invasive imaging techniques have shown that key skeletal regions such as the hips and spine become denser, thicker and importantly, stronger within as little as 12 months. Monthly anti-sclerostin antibody therapy and daily teriparatide injections have both been demonstrated to have rapid effects both on bone turnover, with particularly beneficial effects in osteoporotic vertebrae. In this session the advances in imaging that permit non-invasive estimation of vertebral strength, thickness and compartment-specific density will be discussed.

IS7

Understanding fibrodysplasia ossificans progressiva (FOP): genetics and biological consequences

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During embryonic development, the induction of bone formation is spatially and temporally regulated to specify the skeletal elements. After birth, new bone formation is normally limited to regeneration during fracture repair, a process that is also precisely regulated.

Fibrodysplasia ossificans progressiva (FOP) is a rare human genetic disease in which extensive and progressive heterotopic ossification – the formation of bone outside of the normal skeleton – occurs in soft connective tissues such as skeletal muscle. At birth, there is usually little indication of the disease, except for a characteristic malformation of the great toes. However during early childhood, episodes of bone formation that are frequently associated with swelling and inflammation begin within soft tissues. In most patients examined to date, FOP is caused by a single nucleotide change in the BMP type I receptor ACVR1 (Activin A receptor, type 1; alias, ALK2) that causes enhanced pathway activation. All patients with a classic clinical presentation of the disease possess a recurrent heterozygous c.617G > A (R206H) mutation.

Heterotopic ossification in FOP frequently forms in response to tissue injury, although also develops in the absence of overt tissue trauma. Within the tissue, heterotopic bone formation in FOP patients progresses through well-described changes in affected tissue: initial catabolic events are followed by an anabolic stage and formation of endochondral bone. In vivo mouse models have been instrumental in providing a more detailed understanding of the tissue, cellular, and molecular impact of the ACVR1 R206H mutation and the structural changes in the soft connective tissues that are transitioning to cartilage and bone. In response to injury, initial steps of wound healing in mutant tissue appear to be normal, including an early immune response that leads to tissue degradation and removal of damaged tissue. However, this tissue repair trajectory rapidly diverges and, instead of repairing and regenerating the injured muscle tissue, ectopic cartilage and bone are formed. Understanding these events are providing more detailed insight into the tissue, cellular, and molecular impact of the ACVR1 R206H mutation and the mechanisms that regulate tissue maintenance, repair, regeneration, and heterotopic bone formation.

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IS8

Activin A and ACVR1 variants as tools to explore Activin A's ability to inhibit BMP-mediated signaling via ACVR1

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The genetic disorder Fibrodysplasia Ossificans Progressiva (FOP) is caused by mutations in the intracellular domain of the type I Bone Morphogenetic Protein (BMP) receptor ACVR1. ~97% of FOP patients carry the variant $ACVR1^{R206H}$. A significant breakthrough in understanding FOP was the discovery that Activin A, a BMP/TGFbeta family member, is a required factor for the development of HO in FOP by acting as an agonistic ligand on $ACVR1^{R206H}$ (Hatsell, Idone et al, 2015). In contrast, Activin A normally antagonizes BMP signaling mediated via wild type ACVR1. Outside of FOP, Activin A has been studied as an agonist of type I receptors ALK4 and ALK7; hence, its antagonistic properties remain largely unexplored. We are now addressing this knowledge gap by generating Activin A 'muteins' that cannot antagonize ACVR1, as well as ACVR1 muteins that cannot recognize Activin A. The learnings obtained thus far with these muteins will be presented in the context of FOP, to inform the question of whether BMPs can bypass the apparent requirement of Activin A as an HO-causing ligand in FOP, and beyond FOP, to address the question of whether the ability of Activin A to act as an inhibitor of BMP signaling mediated by Acvr1 has physiological significance.

DISCLOSURE

Except for Alexandra Dimitriou (who is a student), all the other authors are employees for Regeneron Pharmaceuticals, and own common stock in the company.

IS9

FOP and HME: Rare pediatric disorders with distinct causes, but amenable to a similar treatment?

Maurizio Pacifici

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Abstract

Fibrodysplasia Ossificans Progressiva (FOP) is a rare pediatric disorder caused by gain-of-function mutations in *ACVR1* that encodes the bone morphogenetic protein (BMP) receptor ALK2. FOP is characterized by formation and accumulation of extraskeletal endochondral bone at the expense of muscles and other connective tissues, resulting in progressive impairment of body function and even premature death. Hereditary Multiple Exostoses (HME) is a rare pediatric disorder linked to loss-of-function mutations in *EXT1* or *EXT2* that encode Golgi enzymes responsible for heparan sulfate (HS) synthesis, resulting in HS deficiency. HME involves the formation of benign cartilaginous-bony tumors developing next to the growth plate in long bones, ribs and vertebrae that cause multiple skeletal abnormalities and problems and can transition to malignancy in some cases. Despite their obvious genetic and clinical differences, FOP and HME share certain basic pathogenic mechanisms. Chief among them is the fact that both require the reprogramming and developmental redirection of progenitor cells to form ectopic cartilage and bone at extraskeletal sites and within neighboring tissues. Important also is the fact that both disorders rely on abnormal and exuberant action by protein signaling pathways, including the BMP pathway. These and other significant findings have resulted in a far better understanding of the cellular and molecular pathogenesis of FOP and HME, with contributions by many research groups. I will summarize salient data from such studies as well as new data from ongoing studies, pointing to druggable therapeutic targets for these diseases one of which is currently been investigated for clinical relevance and effectiveness.

DISCLOSURE

Consultant for Clementia Pharmaceuticals

IS10

Clinical trials in rare bone diseases: challenges and revelations

Richard Keen

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Fibrodysplasia ossificans progressive (FOP) is rare disorder, affecting approximately 1 in 2 million people (1). Since the discovery of the ACVR1 gene, advances in our understanding about disease pathology have led to the identification of possible drug targets which are currently be studies in clinical trials. There are, however, considerable challenges in undertaking trials in such a rare disease (2). Disease registries are important and how they can help identify subjects who may be suitable participants in a trial, but also give useful information about the natural history of the disease (3). It can be useful to have the backing of patient support groups and charities to help publicise these registries and aid recruitment. When designing a trial, the end points of both clinical and regulatory significance need to determined (4,5), and there will need to be discussion with the appropriate authorities. Designation of the drug in an orphan disease indication will help these discussions and fast-track the process. With small samples sizes, the classical randomised, placebo-controlled trial may not be possible, and there are challenges in study design and in whether a historical control group or a natural history study can be used as the comparator. With limited patient numbers, participants often have to be recruited across a number of countries and sites, and there is a large administrative burden in managing these studies. Despite these challenges, progress is being made and some of the preliminary data from these studies will be shared.

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DISCLOSURE:
I am an investigator for clinical trials in FOP which are sponsored by Clementia Pharmaceuticals and Regeneron Pharmaceuticals

IS11

Novel approaches to the treatment of inherited disorders of phosphate metabolism: FGF23 and other targets

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Hypophosphatemic rickets due to excess urinary excretion of phosphate occurs in several related disorders. X-linked hypophosphatemia (XLH), caused by loss-of-function mutations in *PHEX*, is the most common of these, and is the prototype disorder of excess FGF23 activity. Impaired renal tubular phosphate reabsorption and altered vitamin D metabolism are the cardinal physiologic sequelae of excess FGF23 activity in XLH, thus conventional therapy has consisted of frequent administration of oral phosphate together with 1,25 dihydroxyvitamin D (1,25D) as a replacement strategy. This approach has been the mainstay of therapy for four decades, but the therapy is cumbersome, fraught with complications, and often leaves patients with incomplete correction of deformity.

A humanized monoclonal antibody (burosumab) designed to inhibit FGF23 activity has been developed as a novel approach to treat XLH. We present here a summary of our extended clinical trial experience with burosumab in children and adults: In the first pediatric trial, 5-12 year old children with XLH received burosumab every 2 or every 4 weeks; with both groups showing improvements in biochemical, radiographic, and patient-reported outcomes despite prior conventional therapy. Next, a trial in 1-5 yr old children with XLH given burosumab every 2 weeks improved biochemical and radiographic outcomes by week 24 of therapy. A study of burosumab in adults with XLH has shown improved biochemical and patient-related outcomes compared to a placebo-treated group, with increased healing of fractures and pseudofractures identified at baseline.

Children and adults affected with XLH would benefit from a more effective and better-tolerated therapy than currently available approaches. Administration of burosumab every 2-4 weeks improved clinically important outcomes in growing children, and burosumab treatment of affected adults improved biochemical, radiographic, and patient-reported outcomes. Inhibition of FGF23 activity using an anti-FGF23 inhibitory antibody appears to be a safe and effective strategy.

DISCLOSURE:
I have served as consultant for and received research support from Ultragenyx.

IS12

Treating skeletal dysplasia and achondroplasia

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Abstract

Skeletal dysplasia refers to abnormal development of the skeleton, usually owing to pathogenic variants in genes encoding proteins essential to growth plate function, effective ossification, bone modelling and turnover, and skeletal patterning. There are known to be over 450 distinct types of skeletal dysplasia condition, with a range of clinical expression and severity. The *FGFR3*-associated condition achondroplasia is the most common type of skeletal dysplasia that causes disproportionate short stature (dwarfism) As our knowledge of the causative molecular mechanisms continues to improve and better appreciation of the underlying disruption of key pathways in skeletal development is determined, opportunities to manipulate these networks and develop new therapeutic strategies emerge. Success of these potential new treatments though is contingent upon our full understanding of the natural history of skeletal dysplasia conditions. Indeed this knowledge constitutes the mainstay of current medical management in the multidisciplinary setting.

In this talk, current practices in clinical care in skeletal dysplasia, with focus upon achondroplasia as an exemplar, will be outlined. A overview of current clinical trials will be provided and a look towards the future will provide glimpse of what lies on the horizon in treating skeletal dysplasia.

DISCLOSURE
Melita Irving holds a consultancy agreement with BioMarin

IS13

New approaches to treating disorders of the calcium-sensing receptor

Fadil Hannan
University of Liverpool, Liverpool, United Kingdom. University of Oxford, Oxford, United Kingdom

The calcium (Ca^{2+})-sensing receptor (CaSR) is a family C G-protein coupled receptor (GPCR) that senses extracellular concentrations of $Ca^{2+}$and amino acids. The CaSR is most highly expressed in the parathyroid glands and kidneys, where it regulates parathyroid hormone (PTH) secretion and urinary Ca^{2+} excretion, respectively. Germline loss- and gain-of-function CaSR mutations cause familial hypocalciuric

hypercalcaemia (FHH) and autosomal dominant hypocalcaemia (ADH), respectively. CaSR signal transduction is regulated by G-protein subunit α_{11} ($G\alpha_{11}$) and adaptor-related protein complex-2 sigma subunit (AP2σ), and recent studies have identified germline mutations of these proteins as a cause of FHH and/or ADH. Calcimimetics and calcilytics are positive and negative allosteric modulators of the CaSR that have potential efficacy for symptomatic forms of FHH and ADH. Cellular studies have demonstrated that these compounds correct signalling defects caused by mutant CaSR, $G\alpha_{11}$ or AP2σ proteins. Moreover, mouse model studies indicate that calcilytics can rectify the hypocalcaemia and hypercalciuria associated with ADH, and patient-based studies reveal calcimimetics to ameliorate symptomatic hypercalcaemia caused by FHH. Thus, calcimimetics and calcilytics represent targeted therapies for inherited disorders of the CaSR and its partner proteins.

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DISCLOSURE

Dr Hannan has received grant funding from NPS/Shire Pharmaceuticals and GlaxoSmithKline for studies involving the use of calcilytic drugs.

IS14

Which therapeutic interventions are, or will be, beneficial to patients with Osteogenesis Imperfecta, and how do we determine this?

Matthew Warman

Boston Children's Hospital and Harvard Medical School, Boston, USA

Osteogenesis Imperfecta (OI) comprises a heterogeneous group of Mendelian genetic disorders that cause skeletal fragility. Most patients with OI have dominant forms of the disease caused by type I collagen mutations. Mutations in collagen chaperones or in signaling pathway proteins cause recessive forms of OI seen more commonly in populations with higher coefficients of inbreeding. A major focus of therapy for all forms of OI is to prevent fracture and skeletal deformity. However, these clinical problems are not the only ones that affect an OI patient's quality of life. In order to improve bone strength in patients with OI, drugs approved for osteoporosis are being used off-label; the OI community is also anxious to participate in clinical trials for newer bone anabolic agents. However, companies have been slow to embrace OI as a target for clinical trials even though OI is among the more common of the rare genetic diseases affecting bone. This presentation will address the challenge of performing clinical trials in patients with OI, the missed opportunities that may result from conflating patients with OI and those with osteoporosis, and the ethical conflict of waiting for a drug's safety and efficacy to first be confirmed in a common condition before clinical trials are initiated in a rare condition for which the therapeutic effect might be larger.

KEY REFERENCE

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IS15

Hunting for a therapeutic for sclerosteosis: a personal story

Timothy Dreyer[1, 2], Mittal Shah[2], Carl Doyle[2], David McMillan[2], Alistair Henry[2], David Ke[2], Gill Holdsworth[2], Vinny Naidoo[1]

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Sclerosteosis is a severe autosomal recessive sclerosing skeletal dysplasia that is characterised by excessive bone overgrowth. It is caused by mutations in the *SOST* gene which lead to loss of expression of the protein sclerostin (Scl). The condition has provided great insights into the importance of sclerostin as a negative regulator of bone formation through its actions as an inhibitor of the canonical Wnt signalling pathway. Antibodies to inhibit sclerostin are currently in development for the treatment of low bone mass conditions such as osteoporosis. In contrast, the clinical management of sclerosteosis is limited to surgical intervention. I am a sclerosteosis patient, and the aim of this study is to explore exogenous recombinant Scl as a potential therapeutic approach in sclerosteosis.

Recombinant wild type Scl and various fusions thereof were produced by mammalian expression and were purified using standard chromatography approaches. High affinity (nM) binding of recombinant Scl proteins to the Wnt co-receptor, LRP6, was demonstrated in vitro, and addition of purified recombinant Scl proteins inhibited mineralisation of MC-3T3 cells cultured in osteogenic conditions.

The pharmacokinetic properties of these proteins will now be explored in vivo and the skeletal consequence of administration of these proteins in a mouse model of sclerosteosis (*SOST* knock out mice) will be examined. This approach will provide insights into the potential of exogenous recombinant Scl as a treatment strategy for sclerosteosis.

This work is part-funded by UCB Pharma and the National Research Foundation of South Africa

IS16

The genetics and pathophysiology of Paget's disease

Luigi Gennari

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Paget's disease of bone (PDB) is a chronic bone disorder which typically results in enlarged and deformed bones in one or more regions of the skeleton. The characteristic feature of the disease is an increased resorption followed by an increase in bone formation. At affected sites, excessive bone breakdown and formation produces a disorganized and structurally abnormal bone. As a result, bone pain, arthritis, noticeable deformities and fractures can occur. In a small proportion of cases (< 1%) neoplastic degeneration in osteosarcoma, or, less frequently, giant cell tumor has been described. Over the last two decades, thanks to the development in technology, remarkable advances have made on the genetic causes of PDB. Together with the discovery of the genes associated with the pathogenesis of PDB-related syndromes (e.g. *TNFRSF11A, TNFRSF11B*, and *VCP*) mutations in *SQSTM1* gene were described in up to 50% of familial and 15% of sporadic cases by a wide variety of investigators in several populations. This gene encodes for p62 protein, that acts as a scaffold in a range of signaling and ubiquitin binding pathways. Relevant to bone biology, p62, through its interaction with TRAF6, acts as a scaffold in signaling pathways involved in

the activation of NFkB transcription factor, including the pathway which mediates RANKL activated osteoclastogenesis. More recently, other candidate genetic variants were associated with PDB by a large genome-wide approach, explaining about 13% of the familial risk of PDB in *SQSTM1* negative patients, and mutations in at least two additional genes (*ZNF687*, *FKBP5*) were identified in a limited number of cases. In particular, a recurrent mutation of *ZNF687* has been described in severe, early onset, cases with neoplastic degeneration in giant cell tumor. As a counterpart to the genetic hypothesis, the focal nature of skeletal lesions together with the decline in prevalence rates and the incomplete penetrance of the disease among family members all suggest that one or more environmental triggers may play a role in the pathophysiology of PDB. However, the exact nature of these triggers and how they might interact with the genetic factors in the pathogenesis of PDB is less understood.

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IS17

Secular changes in Paget's disease: lessons from Medieval times

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Although both the incidence and severity of newly diagnosed cases have declined over the past few decades, and reasons for these profound secular changes are unclear, Paget's disease of bone (PDB) remains a relatively common condition. PDB-like changes have previously been noted in archaeological remains dating to Roman times, however accurate diagnoses of ancient forms of the disorder are lacking. Here we report highly unusual features within a collection of 130 articulated skeletons from the North-West of England dating to the late Medieval period, with pathological changes resembling PDB. In contrast to contemporary forms of PDB, this ancient bone disease was very extensive throughout individual skeletons, with a high prevalence, often with low age-at-death estimations, but with very few skeletal complications. Despite these clear phenotypic differences, direct sequencing of ancient proteins extracted from the remains indicates that the subjects were likely affected with a precursor of contemporary PDB, with detection of a modified form of a critical PDB-associated protein. Thus, even greater secular changes may have occurred in PDB since the late Medieval period. Our work provides new insights into the natural history of PDB and supports the notion that ancient forms of the disorder may have been potentiated by as yet unidentified genetic factors or environmental triggers. Further knowledge of these triggers may be relevant in understanding disease aetiology and the more recent secular changes observed in contemporary PDB.

IS18

Sarcopenia, ageing and frailty: defining the scope and opportunities

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Musculoskeletal disease constitutes a major health burden worldwide. The principal chronic musculoskeletal disorders are osteoporosis, sarcopenia and osteoarthritis; these conditions increase in frequency with advancing age, and understanding their epidemiology throughout the life course is critical to the development of effective preventive strategies. Osteoporosis contributes to disability and death through its association with age related fractures. These fractures typically occur at the hip, spine and distal forearm. It has been estimated from incidence rates derived in North America that the lifetime risk of a hip fracture in Caucasian women is 17.5% with a comparable risk in men of 6%. Sarcopenia refers to an age related loss of skeletal muscle mass and function. Between the ages of 20 and 80 years, a decline in muscle fibre size and number causes a loss of muscle mass (30%), with a greater accompanying loss of muscle strength (60%). The origins of sarcopenia are multifactorial and include biological senescence, muscle disuse, endocrine dysfunction, comorbidity, inflammation and nutritional deficiency. While the clinical relevance of sarcopenia is widely recognised, there remains no universally accepted definition of the term. Recent approaches to definition incorporate combinations of decline in fat free mass by DXA; strength assessments using isometric dynamometry; and poor physical performance using observational tests (gait speed, sit to stand time and standing balance). The establishment of these recent methods for the assessment of sarcopenia has led to a characterisation of the prevalence of this disorder with advancing age in men and women. Modifications of the definition will inform outcome studies and future randomised controlled trials. Finally, shared aetiological mechanisms underpinning the senescence of bone, muscle and joint, will open an arena in which novel therapeutic strategies for musculoskeletal disease will become available.

IS20

The Role of Senescence in Bone Biology

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With the aging of the population and projected increase in osteoporotic fractures, coupled with the declining use of osteoporosis medications, there is a compelling need for new approaches to treat osteoporosis. Given that age-related osteoporosis generally co-exists with multiple other co-morbidities (e.g., atherosclerosis, diabetes, frailty), all sharing aging itself as the leading risk factor, there is growing interest in the "Geroscience Hypothesis", which posits that manipulation of fundamental aging mechanisms will delay the appearance or severity of multiple chronic diseases because these diseases share the same underlying risk factor – age. In this context, one fundamental aging mechanism that has received considerable attention recently as contributing to multiple age-related morbidities is cellular senescence. There is now convincing evidence that senescent cells accumulate with age and drive age-related tissue dysfunction. Consistent with this, senescent cells have been shown to increase with aging in the bone microenvironment in mice and in humans. These cells produce a pro-inflammatory secretome that leads to increased bone resorption and decreased bone formation, and approaches that either eliminate senescent cells or impair the production of their pro-inflammatory secretome have been shown to prevent age-related bone loss in mice. Moreover, targeting senescent cells leads to a reduction in bone resorption and either a maintenance (trabecular bone) or increase (cortical bone) in bone formation, thus making this approach

fundamentally different from conventional anti-resorptive therapy, which leads to a reduction in bone resorption and a coupled decrease in bone formation. Thus, targeting cellular senescence represents a novel therapeutic strategy to prevent not only bone loss but potentially multiple age-related diseases simultaneously.

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IS21

Addressing fracture risk in the elderly with novel therapeutics

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Therapeutic approaches for osteoporosis (OP) have followed a discovery and development paradigm that highlighted the imbalance of exuberant bone resorption at the onset of this disease. This led to the successful launch and treatment of numerous anti-resorptives, spearheaded by bisphosphonates as first line therapies. However, as we approach the new millennium, OP patients have exhausted their potential interventions to rescue their bone strength to prevent future incident fracture. These are driven by limitations on use of anabolic therapies such as teriparatide ($\leq$ 2 yrs.), and the emerging rare but relevant risks associated with anti-resorptives because of suppression of bone turnover. In addition, the elderly patients who suffer from lower limb skeletal muscle strength tend to be pre-disposed to fall-related fragility fractures. We will highlight the development of therapies that target this most exigent patient population who are at a high risk of fall related injuries including fractures. The therapy will discuss the concomitant improvement in skeletal muscle power and bone strength that could offer a unique protection from fall-related fractures for a sub-set elderly patients.

IS22

Effects of bone active drugs on health and life span

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Osteoporosis is an interesting stage in its management worldwide. We now have available a variety of effective the well-tolerated treatments that have good safety profiles albeit with some potential adverse effects of low frequency. Hence it is surprising that a small proportion of women, perhaps 20 to 30%, and an even smaller proportion of men, perhaps 5 to 10%, receive effective anti-osteoporosis therapy even after well-recognised fragility fractures.

Many possibilities have been proposed for this low level of treatment. They include lack of understanding of the significance of the osteoporotic fractures with respect to future fragility fractures and early mortality and concerns about side effects despite their very low frequency. In this context, evidence of the excess mortality after all types of fragility fracture and the potential reduction of that excess mortality with effective anti-osteoporosis therapy has major clinical significance. In one large randomised controlled trial of intravenous zoledronic acid after hip fracture in both men and women, there was a 28% reduction in mortality in the treated individuals. A subsequent meta-analysis of all of the pivotal studies of all the anti-osteoporosis drug indicated a 10% lower mortality in the treated individuals. There are now a number of observational studies that show a similar outcome, often with even larger reductions in mortality.

Despite these findings, this is not considered part of the therapeutic paradigms post fragility fracture. The challenge is the lack of a validated pharmacological mechanism for these apparent benefits. Possible mechanisms include prevention of release of bone sequestered toxic factors, including lead, and or inflammatory factors as well as possible actions in other tissues outside the skeleton.

This presentation will review both the randomised controlled trial and cohort data and discuss potential biological mechanisms for this effect.

DISCLOSURE

Consulting and research support from Actavis, Amgen, Aspen, Eli Lilly, Merck Sharp and Dohme, Novartis, Sanofi-Aventis, Servier and Theramex.

IS23

Clinical trials for patients with co-morbid conditions

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Conventional randomized trials have proved the efficacy of several drugs for the “treatment of osteoporosis.” These drugs are underused particularly by patients with comorbid conditions who have the highest risk of fracture. This is partly a consequence of the design of those conventional trials. They enrolled people “with osteoporosis” by screening general populations and practices of osteoporosis specialists with BMD, risk assessment, and spine imaging, intended to maximize the efficacy and minimize the sample size to demonstrate reduction in new vertebral deformities from radiographs. Consequently, patients deserving treatment are also sought (or not) by generalists or osteoporosis specialists by screening with BMD, risk assessment, and occasionally spine imaging. The yield of this strategy has been shrinking and at least half of those discovered to “need treatment” stop taking it within a year.

In the U.S. there are more patients who have a substantially increased risk of hip fracture because they have a disease, such as Parkinson’s disease, than there are patients with a T-score $<$ -2.5. Evidence that drugs are efficacious for these older sicker patients is typically missing because they have been excluded from conventional trials. These patients would warrant treatment at lower thresholds of fracture risk because the preventable consequences of fractures are more severe. However, very few receive and even fewer continue oral drug treatments.

Older patients with Parkinson’s disease (PD) have a very high risk of fracture but rarely receive treatments for osteoporosis. We have designed a US-wide trial to be conducted without clinical sites to test the approach of simply treating older Parkinson’s patients with zoledronate at home by a visiting nurse without BMD testing or individual risk assessment. If successful, it could lead to nearly universal long-lasting treatment of older patients with Parkinson’s disease. The trial could provide a model for reaching many more patients who could have the greatest benefits of drug treatment that reduces fracture risk.

IS24

Ageing and musculoskeletal function: an update on the work of the Centre for Musculoskeletal Ageing Research

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The MRC-ARUK Centre for Musculoskeletal Ageing Research was awarded in 2012 and renewed in 2017 for a further 5 years. It is a partnership between the Universities of Birmingham and Nottingham and aims to understand the mechanisms underlying age-related musculoskeletal decline with a focus on sarcopaenia. Furthermore in its second phase the centre will investigate the links between age and musculoskeletal diseases, notably rheumatoid arthritis. The ultimate goal is to develop interventions to prevent or reverse musculoskeletal decline with age and the centre includes a theme on interventions which includes lifestyle, nutrition and pharmacological approaches. To date the Centre has revealed some of the mechanisms that drive anabolic resistance in muscle, shown that obesity may not in fact accelerate sarcopaenia but instead modify the metabolic quality of muscle, developed a technology platform to use isotope labelling methods to asses tissue turnover in community dwelling adults, co-developed novel MRI methods to assess brain oxygen uptake in adults during exercise, investigated the role of the age-related decline in immune function with the increased systemic inflammation found in ageing, carried out trials with nutriceuticals to improve muscle metabolism in older adults, determined just how much of age-related musculoskeletal decline is driven by reduced physical activity with age.

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IS25

Big Data: integrating omics and cohorts

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Many if not all human diseases and phenotypes have a genetic component. Twin studies show that in particular the so-called complex diseases and traits are heritable and that a certain fraction of the variance among individuals is genetically determined varying typically between 20-80%. The Human Genome Project and its sequela have shown that the sequence of the human genome is in fact characterized by numerous genetic variations (ranging from small Single Nucleotide Polymorphisms to very large Copy Number Variations).Wwe currently estimate that ~5% of all 3.3 billion nucleotides in the human genome varies between subjects. In parallel, DNA analysis technology has undergone several revolutions whereby it is now possible to sequence a complete human genome in <24hours, and to analyze millions of SNPs in millions of DNA samples. Together with the existence and creation of large and well-characterized longitudinal cohorts studies and biobanks, this DNA analysis technology has resulted in the identification of thousands of genetic factors by Genome Wide Association Studies (GWAS). Such genetic factors are now beginning to find their way into clinical, forensic, and societal practice (such as with Direct To Consumer (DTC) Companies as 23andme). GWAS and studies using other genomic technologies based on analysis of DNA methylation, RNA expression, and/or microbiomes, have led to global collaborative consortia. In these consortia the best possible way to execute scientific experiments is taking place including replication of discoveries in one and the same collaborative study (and resulting manuscript). This new culture of doing scientific research is not common in all scientific disciplines and so several learning curves are being followed currently. I will describe some aspects of these developments, highlight a few illustrative examples and provide an outlook on future developments and opportunities.

IS26

Bone biomarkers

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Bone turnover markers reflect the work of the osteoblast and the osteoclast. They can be measured in blood or urine and allow for an inexpensive and non-invasive way to study bone metabolism. They have been evaluated for their use in predicting risk of fracture, accelerated bone loss or the presence of secondary osteoporosis, but for all these uses they are not yet established. They are useful in monitoring the response to treatment of osteoporosis, especially with drugs such as oral bisphosphonates. One study focused on the clinical utility of modern spectrum of bone turnover markers for monitoring oral bisphosphonate therapy (alendronate, ibandronate, risedronate) in women with postmenopausal osteoporosis. The study concluded that two approaches could be used to identify response, namely a change beyond the least significant change or a change to below the mean value of bone turnover in healthy young women (1). This approach identified about 90% of women from the study as responding by 12 weeks on treatment. The International Osteoporosis Foundation and European Calcified Tissue Society proposed that a bone marker measurement made after 12 weeks was a good way to identify patients who are not adhering to therapy, as non-adherence is the commonest reason for non-response (2). Bone turnover markers have proven useful in drug development. They provide information about the mechanism of action of a drug, for example anabolic or anti-resorptive, and for the optimal dose and schedule of treatment.

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DISCLOSURE

I consult and receive research funding from Roche Diagnostics, Immunodiagnostics, Nittobo.

IS27

Clinical utility of circulating microRNAs for diagnosis of bone diseases

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MicroRNAs are small RNA molecules that control changes in cell function by selectively regulating the synthesis of proteins. Circulating microRNAs, which are secreted into the blood by many cell types, are associated with physiologic and pathologic processes at the cellular level, and thus may allow conclusions on the development and progression of diseases before clinical symptoms are evident. MicroRNAs fulfil key requirements of diagnostic tools such as i) non or minimally invasive accessibility, ii) robust, standardized and non-expensive quantitative analysis, iii) rapid turnaround of the test result and iv) most importantly, because they provide a comprehensive snapshot of current physiologic process in cells and tissues that package and release microRNAs into cell-free space. These characteristics have established circulating microRNAs as extremely promising biomarker candidates.

We have investigated the role of microRNAs in the context of bone function and dysfunction and have found that serum concentrations of certain microRNAs differ between fractured and non-fractured patients of osteoporosis [1,2], are responsive to anti-resorptive as well as anabolic treatment, and are associated with parameters of bone quality as detected by imaging techniques [3].

Recently, using OVX rats we were able to investigate time-dependent effects of anti-resorptive treatment as well as anabolic treatment on microRNA levels in serum and femoral bone. Furthermore we could show that patients with monogenetic (WNT1) diseases show an aberrant serum microRNA pattern that overlaps partially with that of postmenopausal osteoporosis [4].

Based on our research findings, we have developed a prototype fracture risk assessment tool (the osteomiR™ test) based on a panel of microRNAs measured in blood. In order to generate evidence of the test's usefulness in terms of benefit to the patient and resource consumption, we have conducted a health economic study. Our simulations provide evidence for the utility of the osteomiR™ test for fracture risk assessment, and, furthermore, highlight the potential of circulating microRNAs to be exploited as minimal-invasive diagnostic tools [5].

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IS29

Regenerative medicine and bone tissue engineering: clinical translation

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Cell based therapies, classified in Europe as Advanced Therapy Medicinal Products, have been explored for more than 2 decades now as potential therapeutics in a large variety of clinical indications, in particular also skeletal tissue repair. Their clinical impact has so far been limited and this is due to many factors such as our lack of understanding of the mechanisms of action, the poor correlation between in vitro cell characteristics and in vivo outcome in patients, and the lack of robust manufacturing (1).

Recent scientific insights indicate that expanded mesenchymal stromal cells (MSC) are affecting tissue repair by paracrine mechanisms thereby boosting the endogenous repair potential. However in large (bone) tissue defects and in compromised conditions, the data indicate that the generation of cell based tissue engineered implants is required, where the stem/progenitor cells serve as "raw materials" to make the construct. Indeed, the cells are contributing to the in vitro production of living tissue intermediates or provisional tissues that upon implantation direct a repair process in vivo with restoration of tissue function.

We have been focusing on the in vitro manufacturing of cartilage tissue intermediates, following our developmental engineering paradigm (2,3), to try to repair large long bone defects in vivo. We have chosen the human periost as tissue source, and using periosteal derived cell populations (hPDC), we have generated distinct cartilage tissue intermediates in vitro, leading to successful ectopic bone formation and orthotopic bone bridging in vivo in nude mice. Examples will be discussed including combination products using hPDCs, low dose Bone Morphogenetic Proteins (BMP) on optimized scaffolds; implants produced using pre-conditioning media and BMP priming of periosteal cells on a scaffold (4); and spheroid based micro-tissues assembled into cartilaginous macro-tissues. Similar approaches have been explored successfully using induced pluripotent stem cells (iPS). The successful implants in the small animal models have been transferred into a preclinical track into large animal models, being a critical size long bone defect model and a non union long bone defect model in sheep (5), with the intention to initiate a clinical explorative trial in patients.

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IS30

BMPs and bone repair

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A novel BMP6 based autologous bone graft has been developed with superior healing results and reduced side effects in preclinical studies. It was tested in Phase I/II clinical studies in patients with a distal radius fracture (DRF) and high tibial osteotomy (HTO). It comprises of an autologous carrier made from the peripheral blood (ABC – autologous blood coagulum) and of rhBMP6. Such formulation circumvents the use of animal-derived materials, significantly limits inflammatory processes and renders the carrier flexible and injectable ensuring the ease of use. The primary objectives of the DRF first-in-human study were safety, tolerability and systemic pharmacokinetics. There were three groups of patients enrolled: standard of care, ABC alone and ABC + rhBMP6. In Phase I 36 patients have been enrolled, while additional 39 patients will be enrolled in Phase II. The Osteogrow device was formulated from 1 mL of autologous blood and 250 μg of rhBMP6, while placebo was formed from 1 mL of autologous blood. No measurable plasma amounts of rhBMP6 were detected after 5 min to 7 days, or BMP6 antibody after 13 weeks. In addition, no serious adverse effects (SAE) have been reported, including swelling, redness of the skin, edema, distant soft tissue ossification, pain or systemic side effects. The use of a small dose of rhBMP6 minimized the risk of side effects and antibody production as opposed to available therapy of DRF patients with a large dose of BMP2 or BMP7 in a similar application.

In patients with HTO the weight bearing part of the knee was shifted from overloaded knee compartment to realign the knee pressure. A randomized, double blind, placebo controlled trial was conducted in two stages in 20 patients treated with 10 ml ABC containing placebo or 1 mg rhBMP6. Phase I included 6 patients and in Phase II the remaining 16 patients were enrolled. No SAEs have been reported, and at 24 weeks of radiological follow up there were different patterns of bone healing within the osteotomy wedges observed. The results are currently evaluated and will be presented at the meeting.

IS31

Clinical trials in degenerative disc disease

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We will conduct a clinical program for the evaluation of a novel bone-regeneration product OSTEOproSPINE as a treatment for intractable chronic back pain. OSTEOproSPINE is a novel bone regeneration therapy composed of OSTEOGROW (recombinant human bone morphogenetic protein 6 [rhBMP6] delivered in autologous peripheral blood coagulum) reinforced with allograft (a compression resistant matrix). Preclinical results showed promising results of postero-lateral spine fusion procedure in rabbits and sheep. OSTEOproSPINE in humans is designed to guide the formation of new bone at extraskeletal site and replace autograft harvested from patient's iliac crest for the fusion of lumbar vertebrae. By generating new bone, OSTEOproSPINE will restore the spine's weight bearing function, reduce the severity of back pain and improve the success rate of posterolateral spinal fusion surgery. The program consortium of 13 partners from 6 EU member states has been assembled to conduct a Phase II, randomized, patient- and evaluator-blinded clinical trial of OSTEOproSPINE. Three clinical centers will enroll 192 patients suffering from degenerative disc disease to assess OSTEOproSPINE efficacy and safety in comparison with Standard of care (autograft). A positive outcome of proposed trial will confirm OSTEOproSPINE potential to form a functioning new bone in human and by this restore the spine's function and improve the quality of life in patients with degenerative disc disorders using the ground principle of regenerative medicine: "provide the correct molecular signals to a population of presumptive cells in a permissive micro-environment".

IS32

Update on the pathogenesis of pain in OA

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How pain arises in OA has divided the community for the past 30 years. Epidemiology demonstrates independent correlations between pain and synovitis, bone marrow oedema and cartilage loss. Causative evidence for any of these tissues has been missing. We have studied spontaneous painful behaviour in surgical models of murine OA. We have observed that pain behaviour occurs late in disease, long after cartilage damage and bone remodelling has started, and in the absence of significant synovitis. Careful molecular analysis reveals that key pain sensitising molecules, including nerve growth factor (NGF) are regulated exclusively in articular cartilage at the time of pain. Pain behaviour in murine OA is abrogated by NGF neutralisation. These data will be reviewed and new data on novel methods for suppressing NGF will be presented.

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IS33

Marrow lesions, pain and osteoarthritis

Nidhi Sofat

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Osteoarthritis (OA) is a condition affecting the whole joint and is the most prevalent arthritis worldwide. A number of pathological changes are recognised in OA, including deterioration of cartilage integrity, subchondral bone changes and synovitis. Although advances have been made in our understanding of OA pathophysiology, there are no current treatments that halt the progression of the disease. Treatments are largely based upon physical therapies to improve function, anti-inflammatory agents for pain and joint replacement surgery for late stage disease in larger weight bearing joints. There is an urgent need to better

understand OA pathophysiology, which could help in the development of new treatments. Historically, evidence of OA structural damage was established using plain radiography of affected joints. In recent years, more advanced imaging techniques including magnetic resonance imaging (MRI) have led to an improved understanding of changes at the bone-cartilage interface in OA, with recognition that loss of integrity at the cartilage-bone junction and development of bone marrow lesions (BMLs) in the subchondral bone is associated with OA pain from large epidemiological studies. One of the next big challenges in OA BML research has been to identify the structural characteristics, gene and protein expression in OA BMLs. Gene analyses of BMLs have demonstrated that they are highly metabolically-active structures, demonstrating evidence of angiogenesis, new bone/cartilage formation and expression of neurotrophic factors. Findings from gene and protein studies of BMLs will be discussed in this talk. The gene signature of BMLs may assist in identification of new molecular targets in OA pathophysiology and treatment.

IS34

Zoledronic acid in arthritis

Erik Fink Eriksen[1], Maziar Shabestari[2]

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Bisphosphonates have been shown to reduce erosions in rheumatoid arthritis (RA) in combination with methotrexate, but are not generally used for this indications because biologics and denosumab have been shown to more efficaceous. The focus of this overview will therefore be osteoarthritis, where no current disease modfying treatment option exists. In several different animal OA models, bisphosphonates have demonstrated efficacy.

Active RA and OA are both characterized by the presence of bone marrow lesions in subchondral bone (BMLs), and these lesions signal progression and increased pain. Histologically BMLs are characterized by pronounced increases in bone turnover and vascularity (1). Bisphosphonates, probalby via their anti-angiogenic effects, reduce the size and pain associated with BMLs, which may the principal effect in OA. Effects on synovitis and cartilage protection have also been invoked, however.

The first human trial using oral risedronate was, however, negative both in terms of symptomatic relief and joint space narrowing (2). Another study using four iv. doses of Neridronate in OA patients with demonstrable BMLs, however, showed reduction of bone marrow lesions, 80% reduction of pain and improved function (3). Another more recent study using one dose of iv. zoledronic acid demonstrated symptomatic relief at 6 months, but not at 12 months (4). Thus, iv. bisphosphonate represnet promising candidates as disease modifying drugs for the treatment of OA, but further studies are needed to establish optimal dosing fand duration of effects.

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IS35

Epigenetics and osteoarthritis -the interplay between DNA methylation, DNA polymorphism and gene activity

John Loughlin

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Molecular genetic investigations of osteoarthritis (OA) have highlighted cellular processes and pathways involved in disease development. These studies include high-throughput genetic and genomic screens that offer genome-wide coverage and in recent years have involved epigenetic analyses of regulatory RNAs, histones and DNA methylation. My group has a particular interest in the interaction between epigenetics and OA genetic susceptibility, including the interplay between DNA methylation, DNA polymorphism and gene activity.

In this presentation, I will present our current work on *PLEC* as an example of how epigenetic studies enable us to prioritise genes from genetic scans for subsequent functional investigation. These involve an analysis of cell lines and primary cells from patients undergoing orthopaedic procedures, the latter used, amongst other things, to map methylation and expression quantitative trait loci (mQTLs and eQTLs). *PLEC* codes for plectin, a multifunctional cytoskeletal linker that acts as a scaffold for signalling proteins and as an organiser of cytoskeletal filaments. Plectin is particularly abundant in tissues that are subjected to mechanical stress and load, including cartilage.

PLEC resides within a gene rich region of high linkage disequilibrium, with DNA polymorphisms flanking the gene being associated with hip OA at genome-wide significance. An array analysis identified a compelling mQTL operating on the locus, with the methylation of 15 intergenic CpGs correlating with genotype at the association signal. Twelve of the CpGs are within an interval of 27kb located in the body of *PLEC* and map to regions that have transcriptional regulatory functions. A cartilage eQTL that correlates with the association signal was also found to operate on the gene, with the OA risk-conferring allele resulting in reduced *PLEC* expression. An analysis of OA versus non-OA cartilage then revealed an increased expression of the gene in diseased tissue. We posit that in a joint predisposed to OA, *PLEC* expression increases to combat aberrant bio-mechanics and that this is epigenetically regulated. However, carriage of the low expressing allele hinders this response leading to an increased risk of disease development.

Our studies demonstrate the utility of epigenetic investigation and its power to prioritise genes and their encoded proteins.

IS36

Cancer treatment induced bone loss (CTIBL): antiresorptive treatment effects and more….

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Osteoporosis is one of the most frequent diseases in postmenopausal women leading to an increased fracture risk due to the physiologic loss of the bone protective effects of estrogen. Hereby, several risk factors for fracture such as prevalent fracture, low BMD, age, low BMI, family history, tendency to falls, smoking, use of SSRIs, glucocorticoid use etc. have been identified. Additionally, the further reduction of endogenous estrogens with chemotherapy (CHT), GnRH-analoga or aromatase inhibitors (AI) continuously increases fracture risk. Breast cancer (BC) on the other hand is the most frequent cancer type in women. Recent reports indicated a continuous increased incidence while mortality, due to early diagnosis and treatment improvements is decreasing.

Dependent on specific tumor characteristics, radiation, chemotherapy (CHT), antibody treatment as well as endocrine treatment has been introduced into the adjuvant clinical treatment setting.
Some but not all of this cancer specific treatments interfere with bone turnover leading to an accelerate bone loss referred to as cancer treatment induced bone loss (CTIBL). Whereas CHT leads to an unspecific increased of bone resorption, Aromatase inhibitor (AI) reduces residual serum endogenous estrogen level and is associated with a decrease of bone mineral density (BMD) and increased fracture risk. Independent of the type of AI administered, bone loss is 2-3 fold increased compared to healthy, age matched postmenopausal controls. Therefore several guidelines have emerged to help managing CTIBL in women with BC including strategies to identify and treat those at highest risk for fractures.
Recently, several studies and a meta-analysis have investigated the additional effect of Bisphosphonates on breast cancer outcomes leading to a 34% decreased rick of bone recurrence and a 17% decreased breast cancer mortality risk. The workshop will summarizes the current knowledge on CTIBL and fracturing risk and indicates current treatment guidelines and intervention options as well as the additional effects of adj. antiresorptive treatments.

IS38

Myeloma bone disease

Andrew Chantry
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Patients with Multiple Myeloma develop a devastating bone disease driven by the uncoupling of bone remodelling, excess osteoclastic bone resorption and diminished osteoblastic bone formation. The bone phenotype is typified by focal osteolytic lesions leading to pathological fractures, hypercalcaemia and other catastrophic bone events such as spinal cord compression. This causes bone pain, impaired functional status, decreased quality of life and increased mortality. Early in the disease, malignant plasma cells occupy a niche environment that encompasses their interaction with other key cellular components of the bone marrow microenvironment. Through these interactions, osteoclast activating factors and osteoblast inhibitory factors are produced, which together uncouple the dynamic process of bone remodelling, leading to net bone loss and focal osteolytic lesions.
Current management includes anti-resorptive therapies i.e. bisphosphonates, palliative support and orthopaedic interventions. Bisphosphonates are the mainstay of treatment for myeloma bone disease, but are only partially effective and do have some significant disadvantages, for example, they do not lead to the repair of existing bone destruction. Thus, newer agents to prevent bone destruction and also promote bone formation and repair existing lesions are warranted. This presentation summarises conventional treatments and novel ways that myeloma bone disease is being therapeutically targeted.

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IS39

Targeting integrin-mediated chemoresistance in breast cancer bone metastases

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Abstract
Bone metastases occur in approximately 70% of metastatic breast cancer (BC) patients, often leading to skeletal injuries. Current treatments are mainly palliative and underscore the unmet clinical need for improved therapies. It is known that integrin β3 (β3) is expressed on bone metastases and plays a role in metastatic tumor cell colonization. Analysis of patient matched bone and primary breast tumors from 42 patients, demonstrated markedly increased β3 expression in bone metastases compared to matched primary biopsies across all BC subtypes: luminal A, luminal B, HER2-enriched, and triple-negative. Likewise, we found increased expression of β3 on BC bone metastases as compared to primary tumor or visceral metastases in 3 preclinical BC models: 4T1, MDA-MB-231, and PyMT-BO1(luminal B ER +). Importantly, we found that avb3 was expressed on microscopic bone marrow disseminated tumor cells (DTC) from mice after primary tumor resection without bone metastases. Mechanistic investigations revealed that TGFβ signaling through SMAD2/SMAD3 was necessary for induction of β3 within the bone. Using a micelle-based nanoparticle therapy that recognizes activated integrin αvβ3 (αvβ3-MPs of ~12.5 nm), we demonstrated specific localization to BC bone metastases. Using this system for targeted delivery of the chemotherapeutic docetaxel, we showed that bone tumor burden was significantly reduced with less bone destruction and less hepatotoxicity compared with equimolar doses of free docetaxel.
We found that bone metastases were more resistant to docetaxel compared to lung metastases. Several groups have demonstrated that cancer stem cells express αvβ3 and are resistant to chemotherapy. We examined the role of αvβ3 in chemoresistance in bone metastases. We found that bone metastases from BO1 cells with genetic deletion of β3 by CRISPR (BO1-$\beta3^{-/-}$) had increased sensitivity to DTX treatment *in vivo*, while re-expression of β3 in β3-/- BO1 cells restored the relative DTX resistance, but re-expression of signaling defective β3 did not. RNA sequencing analyses point to anti-apoptosis mechanisms. Taken together, our results offer preclinical proof of concept for a method to enhance delivery of chemotherapeutics to breast cancer cells within the bone by exploiting their selective expression of integrin αvβ3 at that metastatic site.

IS40

Lung cancer and bone metastases

Sarah Danson
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Bone metastases are common in lung cancer with an incidence of 40%. Current national and international guidelines recommend the use of denosumab or bisphosphonates in lung cancer patients with bone metastases. However, these treatments are not always utilised as patients with lung cancer have typically presented late with advanced disease, poor performance status and limited life expectancies. This landscape is changing with more lines of standard anticancer therapies now being available.
The presence of bone metastases and the occurrence of skeletal related events (SREs) are associated with reduced survival in non-small cell lung cancer (NSCLC) [1]. Zoledronic acid reduces the risk of SREs in solid tumours, including NSCLC [2]. In a large trial of patients with solid tumours other than breast cancer and prostate cancer, a post-hoc subset analysis of the NSCLC patients with bone metastases treated with zoledronic acid or denosumab showed significantly better overall survival in the group of patients treated with denosumab [3]. A subsequent phase III study of denosumab in combination with first line chemotherapy compared with first line chemotherapy alone in NSCLC,

with a primary objective to assess the effect of denosumab on overall survival, has just closed to accrual and results are awaited. Other possible bone-targeting strategies for patients with lung cancer, such as radium-223, will be discussed.

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DISCLOSURE

Professor Danson is EORTC co-ordinator for the SPLENDOUR trial of denosumab in advanced lung cancer. She has received travel support/honoraria from Amgen, BMS, MSD, Boehringer Ingelheim, Lilly, Abbvie and GSK. She has carried out consultancy work for GSK and Incanthera.

IS41

Advances in detection and treatment of prostate cancer

Freddie Hamdy

University of Oxford, UK

The management of prostate cancer has seen unprecedented change and evolution in recent years. Over three decades of prostate-specific antigen (PSA) testing and opportunistic screening led to over-detection and over-treatment of low-risk disease using radical treatments with unnecessary side-effects. At the same time lethal disease has been under-treated, with androgen deprivation therapy prevailing in the management of advanced, metastatic and cancer induced bone disease. Findings in new, large randomized controlled trials - from testing the effectiveness of screening, to the treatment of PSA-detected, high-risk and advanced prostate cancer as well as the development of cutting-edge imaging and minimally invasive techniques for partial tissue ablation - provide new transformational evidence which is changing practice.[1-5] In addition, recent investigations using high-throughput platforms bring new insight into the complex biology and genomic diversity of prostate cancer, our Achilles heel in the management of this common and ubiquitous malignancy. An overview of these changes and transformation in the management of prostate cancer, and key new trial findings will be presented and discussed.

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IS42

Recent advances in bone sarcomas: new therapeutic targets and biomarkers

Dominique Heymann

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Bone sarcomas and more specifically osteosarcomas are highly heterogeneous. This heterogeneity between the tumours (inter-tumour heterogeneity) and within the tumours (intra-tumour heterogeneity) can be related to genetic and non-genetic factors and introduces significant challenges for classifying patients that might benefit from targeted therapies. A new model for osteosarcoma development has been recently proposed in which a *TP53* and/or *RB1* mutant cell initiated a monoclonal disease. This cell population exhibited higher chromosomic instability, leading to both the emergence of new cell clones and polyclonal disease associated with these secondary genetic events and the development or lung metastases and drug resistance. The combination of multiple genetic events and a favourable microenvironment facilitate tumour growth. All of these events are regulated by epigenetic events that may define new biomarkers and may be used as new therapeutic targets. It has been also suggested that Circulating Tumour Cells (CTCs) may reflect the biological evolution (e.g. new mutation events) of primary tumours and associated metastases. Unfortunately, in contrast to carcinomas, in which CTCs have been isolated from epithelial makers (e.g. Epcam), there are no specific makers expressed by sarcoma cells. However, sarcoma cells like other cancer cells show frequently a differential size compared to normal cells and a lower deformability. Size and deformability criteria have been used in pre-clinical models for isolating CTCs and could serve as proof-of-concept for pilot clinical trials in bone sarcoma.

IS43

Inflammatory macrophages, efferocytosis, and skeletal metastasis

Hernan Roca, Laurie McCauley

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Tumor associated macrophages are immune cells found in high numbers in a wide variety of tumors where their infiltration has been correlated with poor prognoses. They are involved in cancer-related inflammation yet the precise mechanisms remain unclear and knowledge of their impact in skeletal metastasis is lacking. When tumor cells disseminate to the skeleton, they are bathed in a rich milieu of bone marrow myeloid cells. Differentiation into bone marrow macrophages suggests they are poised to engulf apoptotic tumor cells in a manner similar to when they engulf normal apoptotic cells during development and homeostasis, a process termed efferocytosis. A unique transcriptional profile and cytokine production occurs in macrophages that engulf apoptotic prostate cancer (PCa) cells versus non-cancer cells. Upon efferocytosis of apoptotic cancer cells macrophages activate NF-κB and Stat 3 leading to pro-inflammatory cytokine production, especially CXCL5, versus anti-inflammatory cytokines normally attributed to efferocytosis. A novel inducible gene-targeted apoptosis construct in PCa cells resulted in pro-inflammatory cytokine production in efferocytosing macrophages *in vitro*, and triggering apoptosis *in vivo* led to further cancer cell growth. Diminished tumor growth in CXCL5 deficient mice further implicated the tumor associated macrophage efferocytic pathway in supporting tumorigenesis. Peripheral blood mononuclear cells from human patients with PCa skeletal metastases showed increased numbers of non-classical CD14 + CD16 + monocytes with high efferocytotic potential, and PCa patients had higher circulating levels of CXCL5. Such a tumor associated macrophage destructive cascade via apoptotic cell clearance provides clues for potential therapeutic interventions that will maintain the critical clearance of apoptotic tumor cells while diminishing the pro-inflammatory landscape.

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IS44

Mechanisms that drive skeletal pain

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Skeletal pain is common and frequently difficult to fully control. Recent data suggests that the skeleton is innervated by a restricted set of nociceptors and that many skeletal pains have both a nociceptive and a neuropathic component. Significant progress has been made in defining the mechanisms that drive skeletal pain. These mechanisms include; peripheral sensitization, translational changes in sensory neurons, ectopic sprouting of sensory and sympathetic nerve fibers and central sensitization. These insights have the led to human clinical trials of bisphosphonates, anti-RANKL and anti-NGF antibodies for the relief of acute and chronic skeletal pain. Understanding which therapies are most efficacious in relieving different types of skeletal pain may allow more effective control of pain while minimizing unwanted side effects.

DISCLOSURE
Rinat, Pfizer, Lilly, Amgen

IS45

Pharmacology of bisphosphonates in pain

Thomas Tzschentke
Grünenthal GmbH, Aachen, Germany

The treatment of chronic pain remains a clinical challenge particularly for pain associated with rare conditions e.g. vulvodynia or CRPS. Recently, the search for new analgesics has turned towards bisphosphonates. There is a small literature on antinociceptive and anti-hypersensitive effects of bisphosphonates in animal pain models. There is also evidence for clinical efficacy of bisphosphonates in chronic pain states. However, the pharmacological/mechanistic rationale for bisphosphonate activity against pain is still elusive.

Several studies have shown positive effects for bisphosphonates in animal-models of inflammatory pain. Where measured, the effect appeared to coincide with a reduction of inflammatory markers. Very limited data exist on the effects of bisphosphonates in animal-models of neuropathic pain, where bisphosphonates had no or only very moderate effects. On the other hand, zoledronate and alendronate had anti-allodynic effects in a rat model of CRPS, and neridronate showed clinical efficacy in CRPS patients. Furthermore, zoledronate, alendronate and ibandronate were shown to reduce allodynia and hyperalgesia in bone cancer pain models, which may at least partly be related to a direct anti-tumor effect. Bisphosphonates have also been characterized in animal-models of osteoarthritis pain, and were usually found to reduce pain-related behavior. Overall, this effect appeared to coincide with reduced cartilage degradation. A clinical study of zoledronate in osteoarthritis pain is ongoing.

Regarding the analgesic mechanism of action, a few studies have suggested pain-relevant ´off-target´ effects, e.g. via P2X2/3 receptors for minodronate, or inhibition of vesicular ATP release for clodronate. Although such activity may contribute to the effects of individual bisphosphonates, they cannot explain the analgesic effects in their entirety. It rather appears that pain reduction is often secondary to the bisphosphonates´ primary effects on osteoclasts and/or chondrocytes (osteoarthritis pain), effects on inflammatory mediators (inflammatory pain) and effects on tumor-growth (bone cancer pain). Taken together, current evidence suggests that bisphosphonates can exert analgesic effects, and that these effects may largely be secondary to their anti-osteolytic/anti-chondrolytic, anti-inflammatory and anti-tumor activity. The degree to which a particular bisphosphonate affects pain may depend on the potency and efficacy with which the bisphosphonate influences the relevant underlying primary mechanisms.

DISCLOSURE:
The author is an employee of Grünenthal GmbH. Grünenthal develops neridronate for the treatment of CRPS.

IS46

Monoclonal antibody antagonists of GFRα3 significantly attenuate nociceptive responses in mouse models of joint pain

Michael LaCroix-Fralish, Luz Cortes-Burgos, Emily Thayer, Min Gao, LiQin Xie, Ashique Rafique, Jeanette Fairhurst, Ashok Badithe, Robert Babb, Frank Delfino, Charleen Hunt, William Poueymirou, Andrew Murphy, Lynn Macdonald, Susan Croll
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Artemin is a member of the glial-derived neurotrophic factor (GDNF) family of growth factor ligands (GFLs). It binds with high affinity to the GFL family receptor GFRα3. Upon binding to artemin, GFRα3 forms a complex with the RET tyrosine kinase receptor, leading to activation of intracellular signaling. GFRα3 is preferentially expressed in sensory and sympathetic neurons of the peripheral nervous system, and has been implicated in the initiation, sensitization, and maintenance of nociceptive responses. We generated high affinity mouse and human monoclonal antibodies against GFRα3 that prevent artemin binding, and have used these antibodies in two mouse models of joint pain. In the DMM (Destabilization of the Medial Meniscus) model of osteoarthritic-like pain, knees of adult male C57Bl/6 mice were surgically destabilized. Sixteen weeks after surgery, mice exhibited significant tactile allodynia, measured using Von Frey Hairs, and marked development of knee joint osteophytes, evaluated by μCT scan. Both mouse and human monoclonal antibodies against GFRα3 prevented and reversed tactile allodynia in this model. Administration of anti-GFRα3 did not appear to be disease-modifying, because withdrawal of antibody treatment restored the allodynic response. In addition, knee joint osteophyte burden was similar for animals treated with anti-GFRα3, PBS, or an isotype control antibody. A second model of joint pain, the intra-articular CFA (Complete Freunds' Adjuvant) model, was also used to evaluate the efficacy of anti-GFRα3. In this model, CFA was injected into the knee joint of adult male C57Bl/6 mice and nociceptive responses were evaluated for 5 weeks. Anti GFRα3 significantly prevented both tactile allodynia and thermal hyperalgesia (evaluated with the Hargreaves' Test) in this model. Knee joints were collected at the end of the experiment and inflammation was scored by a blinded evaluator using a subjective rating scale. No differences were observed among the treatment groups in inflammation scores, suggesting that the antibody reduced nociceptive responses without reducing CFA-induced inflammation. Our data suggest that inhibition of artemin binding to GFRα3 may be efficacious against chronic joint pain.

DISCLOSURE
Authors are employees and hold equity in Regeneron Pharmaceuticals

IS47

Skeletal pain, Radium-223 and bone metastases

Øyvind Bruland
University of Oslo and Norwegian Radium Hospital, Oslo, Norway

The presentation will briefly address key pathophysiological aspects related to skeletal pain in patients with bone-metastatic prostate cancer and also some clinical experiences with beta-emitting bone-seeking

radiopharmaceuticals. Main focus will be on results from various clinical trials during the development of radium-223 dicloride (Xofigo).
Already in the phase 1 trial with single-dosage administration of increasing amounts of Ra-223, pain relief was indicated in more than half of the 25 patients. In a subsequent phase 2 dose-response study, pain palliation was seen in up to 71% of 100 pts with castration-resistant prostate cancer (CRPC) and painful bone metastases. The primary endpoint was pain index (visual analogue scale and analgesic use) used to classify patients as responders or non-responders. The pivotal randomized phase 3 study, ALSYMPCA study, included 921 CRPC pts with bone mets (Ra-223, n = 614; placebo, n = 307). Here six doses of Ra-223 given monthly significantly improved overall survival vs placebo, delayed subsequent symptomatic skeletal related event and was well tolerated. QoL was assessed with the Functional Assessment of Cancer Therapy—Prostate (FACT-P) instrument. The pain analysis was based on 4 pain-related questions from the FACT-P prostate cancer subscale (PCS) using ANCOVA. Time to initial opioid use was analysed using the log-rank test.
Baseline pain characteristics were similar between Ra-223 and placebo groups. The FACT-P PCS pain scores revealed that Ra-223 pts had a significantly greater increase in pain-related QoL than did placebo pts at both pre-scheduled treatment visits (week 16, $P = 0.010$; week 24, $P = 0.005$). Fewer pts in the Ra-223 group (36%) than in the placebo group (50%) required opioid use for pain relief. Furthermore, the difference in time to initial opioid use was statistically significant between the treatment groups ($P = 0.0023$).
Results from ALSYMPCA support the positive effect of Ra-223 on pain relief in CRPC pts with bone mets, with less opioid use and improved pain-related QoL compared with placebo.

DISCLOSURE
Øyvind Bruland has served as a consultant (paid to Institution) for Bayer AS, was one of the co-founders of Algeta ASA, and has had patents related to radium-223

IS48

Systemic effects of the tumor-bone microenvironment: mechanisms and therapeutic approaches

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Breast and other cancers commonly metastasize to bone to cause bone destruction, pain, fractures hypercalcemia and muscle weakness. Recently, we described a specific molecular mechanism by which bone-derived transforming growth factor (TGF)-beta, released as a consequence of tumor-induced bone destruction causes muscle dysfunction, before the loss of muscle mass. Circulating TGF-beta induces oxidation of the ryanodine receptor (RYR1) on the sarcoplasmic reticulum of skeletal muscle to induce calcium leak and muscle weakness. Blocking TGF-beta, or its release from bone (with bisphosphonates), preventing oxidation of or stabilizing RyR1 all prevented muscle weakness in mouse models of breast cancer bone metastases. In addition to these effects on skeletal muscle, circulating TGF-beta may act on beta cells of the pancreas to impair insulin secretion and result in glucose intolerance. These and other potential systemic effects of TGF-beta released from the tumor-bone microenvironment or from cancer treatment-induced bone destruction implicate bone as a major source of systemic effects of cancer and cancer treatment. Therapy to block the systemic effects of the bone microenvironment will improve morbidity associated with bone metastases and cancer treatment.
Funding sources: NIH/NCI: U01 CA143057, R01CA206025; Susan G. Komen Foundation; DoD: BC150678

IS49

Cancer: the evil companion corrupting good behaviour

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The tumour microenvironment or niche is the vital non-cancerous compartment of the tumour structure. Tumour cells and their microenvironment establish a synergistic cooperation that characterizes all aspects of tumour growth, from onset to metastasis. Importantly, this ever-changing interaction is a key source of cancer cells' plasticity and plays an important role in every aspect of tumour progression. Indeed, cancer cells with higher ability to establish favourable crosstalk with their surrounding are more tumorigenic (1). Thus, targeting non-tumour-derived cellular components represents a promising avenue to better therapeutic interventions. For instance, we identify neutrophils as the main component and driver of metastatic establishment within the pre-metastatic lung microenvironment in mouse breast cancer models. Importantly, we find that neutrophil-derived leukotrienes aid the colonization of distant tissue by selectively expanding the sub-pool of cancer cells that retain high tumorigenic potential. Pharmacologic inhibition of the leukotriene-generating enzyme arachidonate 5-lipoxygenase (Alox5) reduces metastasis, revealing the efficacy of using targeted therapy against a specific tumour microenvironment component (2).

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IS50

Influence of sympathetic nerves on the bone metastatic niches.

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Bone metastasis is a complication of many cancers such as lung, prostate and breast. Once the tumor grows in bone, treatment options are limited and clinical management can only be palliative. Identifying the mechanisms driving the engraftment of metastatic cancer cells into the skeleton is thus crucial to design efficacious therapeutic options. Progression and recurrence of breast cancer, as well as reduced survival of patients with breast cancer, are associated with chronic stress, a condition known to stimulate sympathetic nerve outflow. In this context, our previous work has shown that β-adrenergic stimulation in bone-forming cells leads to a VEGF-dependent increase in bone vascular density and to a RANKL-mediated migration of metastatic breast cancer cells toward the osteoblastic niche. The impact of sympathetic nerves on the bone marrow environment in the context of the early stages of bone metastasis will be summarized, including new data related to aging and role of pro-inflammatory cytokines into the equation.

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IS51

Tumour-intrinsic interferons as key dictators of bone metastasis

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Prostate and breast cancer have 10-year patient survival rates as high as 90%. Despite this, they remain the 2nd leading cause of cancer-related deaths in men and women due to metastatic spread to distant tissues, particularly bone. Bone-metastatic disease can develop decades after primary tumour diagnosis and treatment. This is largely due to the early escape of cancer cells beyond the primary tumour site to the bone marrow, where they can exist in a dormant state fora prolonged period until they become activated in some patients to form deadly metastases. Predicting and targeting this reactivation phase is critical for impacting patient mortality rates. Using metastatic prostate cancer models, we recently discovered that the switch from dormancy to tumour cell activation in bone is accompanied by the loss of the type I Interferon (IFN) pathway, a pathway well known for its role in activating an immune response to protect against diseases, including cancer[1]. This supports our previous investigations in breast cancer demonstrating that tumour-inherent type I IFN signalling activates immune surveillance mechanisms to block metastatic spread in breast cancer,and that loss of thesesignals promotes bone metastasis[2,3,4]. Given very little is known about what mechanisms control the dormancy switch, current studies are focused on the role of IFN signalling in the earliest stages of metastatic outgrowth in bone and the identification of IFN signatures as biomarkers to predict bone metastatic spread.

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IS52

New approaches in in vivo imaging. New whole body optical imaging and multi-modality imaging tools and their use in cancer research.

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In my presentation new bioluminescent and fluorescent based gene reporters will be discussed that can be used for imaging stem cell differentiation, tumor progression and metastasis in mice. Also, the use of a triple multi-modality gene reporter to image bone metastasis in combination with CT and MRI. We have also generated a new mutated codon optimized Click Beetle Red2 luciferase and substrate that allows more sensitive and deeper imaging with higher resolution in living mice. We also developed transgenic dual-color T-cell reporter mice that can be used to study T-cell migration and activation. I will also discuss the use of new luciferin based caged-substrates which now, together with the new dual-color mutated luciferases, enables a wide variety of multiplexing. Together with my colleagues we have developed software for automated registration of whole-body follow up micro-CT data in mice with bone metastases.

I will also briefly discuss new clinical developments on Near Infra-Red Fluorescent (NIRF) probes that can be used for image guided surgery of tumor tissue and to detect sentinel lymph nodes.

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IS53

Single cell analysis of MPN stem cells

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Single-cell RNA-sequencing has emerged as a powerful tool to resolve transcriptional heterogeneity. However, its application to study cancerous tissues is currently hampered by the lack of coverage across key mutation hotspots in the vast majority of cells, which prevents correlation of genetic and transcriptional readouts from the same single cell. Therefore, it remains unclear to what degree transcriptional heterogeneity in myeloproliferative neoplasm stem/progenitor cells is driven by somatic mutations versus other factors. To overcome this, we developed TARGET-seq, a method for the high-sensitivity detection of multiple mutations within single-cells from both genomic and coding DNA, in parallel with unbiased, high-depth whole transcriptome analysis. Using this technique, we achieve a 98% sensitivity in the detection of multiple mutations within single-cells, which allows accurate reconstruction of tumour phylogenetic trees at single cell resolution. We demonstrate how TARGET-seq uniquely resolves transcriptional and genetic heterogeneity in myeloproliferative neoplasm stem/progenitor cells, providing insights into deregulated pathways of both mutant and non-mutant cells. Specific examples of how this approach might be applied clinically for risk stratification, minimal residual disease monitoring and tracking of clonal evolution will be presented. In summary, TARGET-seq provides a powerful tool to resolve molecular signatures of genetically distinct subclones of tumour cells.

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megakaryocyte-erythroid progenitors identifies distinct megakaryocyte and erythroid differentiation pathways. Genome Biol. 2016;17:83.. +,* Equal contribution.

IS55

Epigenetic suppression in myeloma bone disease

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Multiple myeloma bone disease (MMBD) results from markedly increased bone resorption and severely suppressed bone formation, causing lytic lesions that rarely heal. These lesions can persist even when patients are in complete remission. We showed, using *in vivo* models of MM and patient samples that *Runx2* and *Osterix* expression remained suppressed in bone marrow stromal cells (BMSCs) from MM-bearing bones, and that MM-exposed BMSCs failed to differentiate into osteoblasts (OBs) weeks after removal of MM cells. We hypothesized that MM cells induce repressive epigenetic changes in the *Runx2* and *Osterix* promoters that inhibit OB differentiation of BMSCs which persists in the absence of MM cells. Therefore, we characterized these promoters in murine pre-osteoblast MC4 cells and patient BMSCs by chromatin immunoprecipitation (ChIP). The *Runx2 and Osterix* transcriptional start sites (TSSs) in untreated MC4 cells were co-occupied by transcriptionally active histone-3 lysine-4 tri-methylation (H3K4me3) and transcriptionally repressive histone-3 lysine-27 tri-methylation (H3K27me3) modifications, termed "bivalent domains". Co-culture of MC4 cells with MM cells or treatment with TNF-α increased H3K27me3 levels in these bivalent domains making them transcriptionally silent. This occurred because MM cells induced the Runx2-transcriptional repressor Gfi-1 in BMSCs via p62/sequestosome 1 to suppress OB differentiation of BMSCs. Gfi-1 directly bound the *Runx2* promoter and recruited histone co-repressors (HDAC1, LSD1, and EZH2) to the promoter, thereby decreasing H93K9ac and H3K4me3 and increasing H3K27me3 modifications, to block *Runx2* expression. These repressive chromatin changes in *Runx2* persisted after MM cell removal. Further, Gfi-1 knockdown, selective pharmacological inhibition of HDAC1 or EZH2, or treatment of BMSCs with a p62-ZZ domain small molecule inhibitor (XRK3F2) both prevented and reversed MM-induced *Runx2* repression and rescued OB differentiation. These results suggest that HDAC1 or EZH2 inhibitor treatment or targeting the p62-ZZ domain in BMSCs of MM patients with XRK3F2 may restore OB function in MMBD.

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1 J. Adamik[1], R. Silbermann[2], D. Galson[1] and G.D. Roodman[3,4]. Departments of Medicine, [1]University of Pittsburgh School of Medicine, Pittsburgh, PA, [2]Oregon Health and Science University, Portland, OR, 3Indiana University School of Medicine and 4Roudebush VA, Indianapolis, IN USA

IS56

Bisphosphonates for Delivering Drugs to Bone

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An ideal therapeutic drug or diagnostic for use in the management of bone diseases should be bone tissue specific with no pharmacological activity at other anatomical sites. A drug or diagnostic that targets the most diseased sites of the skeleton is even more desirable, and is an attribute that bisphosphonates (BPs) can provide with their propensity to favor sites of high bone turnover. For imaging, we have been capitalizing on this property to develop new fluorescently labeled BP analogs as well as novel PET scanning probes, with non-releasable BP linked conjugates, particularly for use in multiple myeloma. For drug development we have been utilizing a "target and release" linker strategy designed to have serum-stable BP linkers that metabolize and release drugs at the bone surface. We have also been designing our conjugates with antiresorptive inactive or less active bisphosphonates to avoid confounding our study conclusions with BP pharmacology. Our teams has recently described a proof of concept study to develop new therapies for osteomyelitis, where inadequate efficacy has been ascribed to the limited access of current systemically administered antibiotics to sites where causative bacteria (eg. S. aureus) can reside in biofilms on bone and even within the osteocytic canalicular network. We recently reported impressive in vivo efficacy with a novel bisphosphonate-ciprofloxacin conjugate, BV600022. (Sedghizadeh et al, J Med Chem, 2017). An in vitro comparison of our new bone targeted antibiotic conjugates further demonstrates their efficacy advantages vs commonly used antibiotics such as minocycline and vancomycin. In addition, the rate of release of these conjugates can be adjusted to maximize efficacy and duration of action. Our work also continues toward the utilization of these approaches in the management of other bone diseases such as multiple myeloma, bone metastases, and arthritis. In particular, new mechanistic understanding and an interesting drug development approach for a potentially improved treatment for multiple myeloma has evolved from this work by targeting proteasome inhibitors that should provide greater efficacy as well as greater safety at non skeletal sites.

SUPPORT:
NIH/NIDCR: R42-DE025789-02; NIH/NIAID: R43AI125060-01; NIH/NIDCR: R44DE025524-02, 1R21AR070984-01, NIH/NIAMS

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DISCLOSURE:
Ebetino, Sun, McKenna are stockholders in BioVinc
CABS oral communications and posters

OC1

10-year patterns of recurrence from early breast cancer: Analyses of the AZURE (BIG 01/04) study of adjuvant zoledronic acid.

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INTRODUCTION: The AZURE clinical trial recruited 3360 patients with early breast cancer at moderate/high risk of recurrence, randomised 1:1 to receive standard adjuvant therapy +/-zoledronic acid (ZOL). The study offers an excellent opportunity to record patterns of relapse in the modern treatment era and the impact of adjuvant bisphosphonate treatment, not only in bone but also in local and other distant sites.
METHODS: Patients were regularly reviewed during the 5-year treatment phase and then annually to 10 years (total median follow-up: 117 months) or until recurrence in bone or death. Local and distant recurrences were recorded and classified according to recurrence sites (with further breakdown of anatomical site for recurrence in bone), expression of estrogen receptor (ER) in the primary tumour and patient's menopausal status at diagnosis. Data were adjudicated with trial site where necessary to remove errors or ambiguities.
RESULTS: At 10 years, 1191 patients had developed disease recurrence (ZOL arm: 577 relapsed pts out of 1681; Control arm: 614 relapsed patients out of 1678). A summary of the data according to ER and menopausal status is listed below:

- In control group, patterns of relapse were similar in postmenopausal (PM: at least 5 years since menopause) and non-postmenopausal (NPM) patients, with distant recurrence prevailing over loco-regional one;
- In the same arm, bone recurrence (± extra-skeletal relapse) was the most frequent in ER positive ladies;
- As expected, visceral relapse was more common in the ER negative group;
- In ZOL group, reduction in bone metastases (especially "bone only", as compared with bone and other sites at first recurrence) was greater in NPM patients (ER positive: 8.1% vs 11.1% of controls; ER negative: 3.6% vs 6.5% of controls);
- ZOL increased extra-skeletal disease free survival (DFS) events in PM patients;
- Little effect of ZOL was found on bone metastases occurring after a non-skeletal first relapse.

Conclusions: These analyses yield valuable new data on the patterns of local and distant recurrence occurring over 10 years of post-diagnosis follow-up in patients with early breast cancer.

DISCLOSURE
Janet Brown: Advisory Boards and Speaker Bureaux for Amgen, Novartis and Bayer. Walter Gregory: consultancy fees from Cologne and Janssen; patent with Inbiomotion for a related biomarker. Robert Coleman: Institutional research grants from Amgen and Bayer; fees from Novartis for expert testimony; lecture fees from Amgen.

OC2

ERRα in primary breast tumours promotes tumour cell dissemination to bone by regulating RANK

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Bone is the most common metastatic site for breast cancer. Because Estrogen-Related-Receptor alpha (ERRα) has been implicated in cancer cell invasiveness and bone metastasis progression, we determined whether ERRα expression in primary breast tumours could modulate the molecular mechanisms that drive breast cancer cell dissemination to bone. In breast cancer patients, we showed that high ERRα expression level in primary tumours (n = 295) was associated with relapse to bone but not to lungs. ERRα expression was also detected in the circulating tumour cells from metastatic breast cancer patients. Using a murine 4T1 breast cancer cell line that spontaneously metastasizes to lung and bone when inoculated orthotopically to animals, we found that ERRα overexpression in 4T1 cells (4T1-ERRα) promoted spontaneous bone micro-metastasis formation whereas lung metastasis formation remained unchanged. RANK was identified as an ERRα regulated gene in 4T1-ERRα primary tumours both as transcriptomic and protein levels. Concomitantly, we showed that RANKL stimulated 4T1-ERRα cells migration as well as the phosphorylation of mTOR *in vitro*. In line with these data, RANK was up-regulated in human MCF7 breast cancer cells overexpressing ERRα. A positive correlation was also observed between high ERRα/RANK expression levels in primary tumours from patients with breast cancer (n = 446) and occurrence of bone metastases (n = 248). Furthermore, in order to determine whether inhibition of ERRα abrogates primary tumour outgrowth and tumour cell dissemination to bone, the pharmacological effect of the ERRα inverse agonist C29, which is known to block ERRα transcriptional activity, was studied. We first showed that C29 inhibited RANK mRNA expression in 4T1 and MCF7 breast cancer cells *in vitro*. Then, 4T1-ERRα cells were inoculated orthotopically and mice were treated with the C29. Reinforcing our data, we found that the pharmacological inhibition of ERRα reduced primary tumour growth, bone micro-metastasis formation *in vivo*, and decreased RANK expression *in situ*. In conclusion, our results reveal a novel ERRα/RANK axis through which ERRα in primary breast cancer promotes dissemination of tumour cells to bone. We propose that ERRα may be a useful biomarker to identify breast cancer patients at high risk of relapse in bone.

OC3

HDAC inhibitors stimulate LIFR and other pro-dormancy genes in dormant and aggressive breast cancer cells

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Breast cancer cells often metastasize to the bone, where they remain

dormant for years. Our laboratory previously found that loss of leukemia inhibitory factor receptor (LIFR) in breast cancer cells results in poor patient survival and promotes tumor cell exit from dormancy in the bone marrow. Treatment with the pan-histone deacetylase inhibitor (HDACi), valproic acid, induces LIFR expression and other pro-dormancy genes through an undefined mechanism. We hypothesize that additional HDACi (romidepsin, panobinostat, vorinostat, entinostat) can be used to directly stimulate LIFR expression through histone acetylation on the *LIFR* promoter and maintain tumor cells in a chronic dormant state. HDACi dramatically induced *LIFR* mRNA levels (up to 9.5-fold, $p < 0.05$-0.0001) and protein levels by 24 hours in breast cancer cells with low (MCF7, SUM159, D2.0R) and high metastatic potential (MDA-MB-231b, D2A1, 4T1BM2). MCF7 cells treated with HDACi showed a significant enrichment of acetylated-H3K9 along the *LIFR* promoter (up to 10.9-fold, $p < 0.05$-0.001), indicating that HDACi directly stimulate *LIFR* transcription. HDACi also induced other pro-dormancy genes including *AMOT*, *TGFB2*, and *IGFBP5* (up to 5.5-fold, $p < 0.05$-0.0001), but did not enrich for acetylation of the promoters, suggesting HDACi indirectly regulates these dormancy genes. Furthermore, using MCF7shLIFR cells, we determined that induction of *AMOT*, *TGFB2*, and *IGFBP5* is not dependent on *LIFR* expression. Together, these data indicate that HDACi enhance promoter acetylation and expression of *LIFR* while also upregulating other pro-dormancy genes in a *LIFR*-independent manner. To determine whether HDACi treatment promotes tumor dormancy *in vivo*, mice were treated with VPA (~2mg/kg; 3x/wk) following intracardiac inoculation of MDA-MB-231b cells, but there was no significant difference in tumor-induced osteolysis or tumor burden with HDACi treatment. However, VPA treatment in naïve mice revealed a significant reduction in bone volume, suggesting that VPA negatively impacts bone remodeling and should be combined with a bone protective agent in future *in vivo* experiments. Importantly, these studies suggest that HDACi can stimulate pro-dormancy gene expression and therefore may have clinical implications for the use of HDACi as a means to maintain breast cancer cells in a chronic dormant state.

OC4

TGFβ inhibition in combination with chemotherapy repairs existing lytic bone lesions in a novelplateau phase model of multiple myeloma

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Multiple myeloma (MM) causes a destructive bone disease in >85% of patients and current therapies do little to repair existing bone damage. We previously identified that combined bone anabolic and anti-resorptive therapy repairs osteolytic lesions in mice with high tumour load. In patients, if bone repair agents were given, they would be administered in combination with chemotherapy. Thus, this study aimed to determine if bone recovers after chemotherapy and if this is enhanced by bone anabolic therapy.

Human U266-GFP-luc MM cells were i.v. injected into NSG mice (n = 5-7/group). After tumour and lytic bone lesion development, mice were administered first-line chemotherapeutics (bortezomib ± lenalidomide) ± a bone anabolic (SD208; transforming growth factor β receptor 1 inhibitor) or vehicles for 2 weeks. Tumour and bone lesions were monitored *in vivo* by bioluminescence imaging (BLI), serum paraprotein ELISAs and μCT. Flow cytometry, histomorphometry, μCT, TRAP and P1NP ELISAs and QPCR were performed for endpoint analyses.

Chemotherapy significantly reduced total body tumour burden and paraprotein, and increased survival. Combined chemotherapy was more effective than either given alone, reducing tumour to levels undetectable by BLI and paraprotein. However, flow cytometry revealed low tumour levels of 100MM cells/10^6 bone marrow cells. Lytic bone lesions developed ~8 weeks after tumour inoculation. Vehicle treated mice exhibited progressive bone lesion development and virtually no trabecular bone at endpoint. Lesions in mice administered bortezomib ± lenalidomide were unchanged after 1 week but began to repair after 2 weeks, with significantly reduced TRAP+ osteoclasts and increased osteoblasts, indicating recovery of bone. Mice treated with chemotherapy + anabolic SD-208 exhibited enhanced repair of bone lesions, with partial repair of perforating cortical lesions on all tibial surfaces within 1 week and complete repair of lesions within 2 weeks. SD-208 also significantly increased trabecular bone volume after 2 weeks.

This study identified SD-208 enhances MM bone lesion repair when combined with first-line chemotherapeutics. Future studies combining SD-208 and chemotherapy with anti-resorptive therapy will identify optimum treatment regimens for translation of bone anabolic therapy into MM clinical trials.

OC5

Bone microenvironment induction of tumoral integrin β3 expression promotes resistance to chemotherapy in breast cancer bone metastases.

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Breast cancer (BC) cells that have disseminated to the bone microenvironment exhibit relative resistance to treatment with chemotherapy compared to primary breast tumors. Given that over two-thirds of patients with metastatic BC will develop bone lesions at some point in the clinical course, understanding of the mechanisms responsible for bone-induced therapeutic resistance in BC cells represents an urgent and unmet clinical need. We have previously shown that expression of the β3 integrin subunit is increased in bone-residing tumors compared to matched primary breast cancers in both preclinical models and patient samples. This upregulation of integrin β3 in BC bone metastases was dependent on TGF-β signaling through SMAD2/3. Finally, integrin αvβ3-targeted nanoparticles (~12.5nm) harboring the microtubule-stabilizing chemotherapy docetaxel were significantly more effective than free docetaxel in treating bone metastases. In the present study, we next investigated whether signaling through integrin β3 (β3) itself has a functional role in mediating chemotherapy resistance in bone metastases. *In vitro* docetaxel treatment of either bone-avid MMTV-PyMT-derived BC cells (BO1-FL-GFP) or 4T1 BC cells enriched for populations with higher β3 expression. Chemotherapy reduced BrdU incorporation in β3lo BC cells but not in β3hi cells, suggesting a relative resistance to chemotherapy in the β3hi population. To evaluate the necessity of β3 expression for chemoresistance *in vivo*, β3 was genetically deleted in BO1 cells using CRISPR/Cas9 (β3-/- BO1). Bone metastases established with β3-/- BO1 cells were more sensitive to docetaxel treatment compared to the relative docetaxel resistance observed in wild type bone metastases. Retroviral rescue of β3-/- BO1 cells with human integrin β3 (hβ3) restored docetaxel resistance in bone metastases, while β3-/- BO1 cells expressing either a signaling-deficient mutant integrin β3 (Δβ3) or empty vector (pMx) remained sensitive, indicating that integrin β3 signaling promotes a chemotherapy resistance phenotype. *In vitro*, hβ3-expressing cells demonstrated increased viability, higher BrdU incorporation, and reduced caspase-3/7 activity compared to either Δβ3-expressing cells or pMx cells in the presence of docetaxel or the microtubule-destabilizing agent eribulin. RNAseq and qPCR analysis of hβ3-expressing cells demonstrate upregulation of multiple genes associated with chemoresistance, including anti-apoptotic factors. Collectively, this study demonstrates

that integrin β3 expression on BC bone metastases promotes resistance to potent anti-breast cancer, microtubule-targeted chemotherapies.

OC6

Development of ROS-responsive and Bone-Targeted Nanocarriers of Gli-Inhibitors for Treatment of Tumor-Induced Bone Disease

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In multiple cancer types including breast and lung, increased expression of Gli2, a Hedgehog family transcription factor, has been shown to contribute to tumor-induced bone disease (TIBD). Small molecule drug candidates have been identified to antagonize the aberrant activation of the Hedgehog pathway; however, these molecules are hydrophobic and exhibit poor pharmacokinetic properties. Here, we have developed reactive oxygen species (ROS, prevalent in tumor and inflamed sites) responsive and bone-targeted polymeric nanocarriers to encapsulate the small molecule Gli2-inhibitor, GANT58, to create GANT58 nanoparticles (GANT58-NPs). A block-copolymer formulation of poly(propylene sulfide)-poly(oligoethylene glycol acrylate) (PPS-b-POEGA) was synthesized using reversible addition fragmentation chain transfer (RAFT) polymerization, and NPs encapsulating GANT58 were fabricated by an oil-in-water solvent evaporation method. The GANT58-NPs were tested in a mouse model of breast and lung cancer invasion into the tibia. MDA-MB-231 human breast cancer cells and RWGT2 human lung cancer cells were injected into the tibiae of athymic nude mice, and GANT58-NPs were delivered by tail vein injections daily. GANT58-NP treatment significantly reduced bone destruction in both the MDA-MB-231 and RWGT2 models, and the biodistribution of the GANT58-NPs demonstrated preferential uptake in the tumor site. To further improve the site specificity of the GANT58-NPs toward the bone microenvironment, a new polymer formulation incorporating the bone-binding bisphosphonate alendronate has been developed. *In vitro* hydroxyapatite (HA) binding assays show the bone-targeted GANT58-NPs (GANT58-BTNPs) have a tunable bone-binding affinity dependent on the alendronate content in the polymer formulation. *In vivo* biodistribution confirmed the *in vitro* findings, with the GANT58-BTNPs showing over a two-fold increase in bone localization compared to the non-targeted formulation. It is hypothesized that these GANT58-BTNPs will not only improve the effective dosage of GANT58 to the bone-tumor site, but could also be used as a preventive treatment, as GANT58 has shown antitumor effects *in vitro* and thus could prevent tumor establishment in bone. In summary, the polymeric nanocarriers developed here have successfully encapsulated the Gli2-inhibitor GANT58 and reduced bone destruction in mouse models of TIBD, and the bone-targeting of these nanocarriers is hypothesized to provide a platform for prevention of tumor establishment in bone.

OC7

A novel osteolineage-derived cancer associated fibroblast population in primary tumors expresses Dkk1 and enhances tumor growth

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We recently described that mice bearing extra-skeletal tumors have increased levels of the Wnt/β-catenin inhibitor Dkk1 in circulation and bone marrow fluid. Although Dkk1 is known to modulate bone remodeling, during tumor progression Dkk1 induces the expansion of myeloid derived suppressor populations to inhibit anti-tumor T cell responses. The goal of this study was to determine the source of Dkk1 and whether Dkk1 acts locally or systemically to exert immune suppressive functions. Dkk1 was highly expressed by osteoblasts (OBs) and osteocytes in mice with extra-skeletal tumors. Although we did not detect expression of Dkk1 in our tumor lines, we found expression in the tumor mass by the cancer-associated fibroblasts (CAFs). Unexpectedly CAFs also expressed two OB-specific markers Osterix (Osx) and Osteocalcin (Ocn), leading to the hypothesis that Dkk1 at tumor site is produced by a novel osteolineage-derived fibroblast population. To test this possibility, we turned to a mouse model carrying the R26R-tdTomato reporter protein (TdTomato) under the control of a TET-off doxycycline Osx-Cre (TdT^{Osx}) driven promoter. Doxycycline was administered to moms and pups until weaning to prevent Osx-Cre activation in perinatal mesenchymal stem cells and, at 8 weeks of age, mice were orthotopically inoculated with B16 or PyMT cell lines. We found that 3-9% cells in the tumor mass were TdT^{Osx+}. This population expressed several fibroblast markers and Dkk1. Importantly, TdT^{Osx} cells isolated from B16 tumors and co-injected together with B16 cells into WT recipient mice significantly increased tumor growth compared to the tumor line alone. Next, to determine whether Osx+ cells support tumor progression via production of Dkk1, we generated Osx-Cre;Dkk1fl/fl mice and observed a significant reduction in tumor size compared to control mice. Since Osx-Cre targets also the OBs, to specifically evaluate the contribution of CAF-derived Dkk1, we generated Fsp1-Cre;Dkk1fl/fl mice. Strikingly, tumor growth was also significantly reduced in the Fsp-Cre;Dkk1fl/fl mice indicating that fibroblast-derived Dkk1 plays an important role during tumor progression. In summary, we have identified a novel population of CAFs that expresses the osteolineage marker Osx and contribute to Dkk1 production and strongly support tumor growth.

OC8

ROUNDABOUT 4 MEDIATES THE HOMING OF HUMAN BREAST CANCER CELLS IN THE BONE MARROW

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Bone metastases contribute substantially to morbidity and mortality in women with advanced breast cancer and current treatments are only palliative. In order to prevent bone metastasis, it is vital to increase our understanding of the molecular mechanisms that control cellular events preceding the development of overt skeletal lesions. A comparative transcriptional analysis was performed between human MDA-MB-231 breast cancer cells that metastasise to several organs in animals, and a sub-population of the MDA-MB-231 cell line (B02) that metastasises to bone only. Compared to MDA-MB-231, B02 cells overexpressed Roundabout 4 (Robo4), a cell surface receptor belonging to the Roundabout family of axonal guidance receptors. Robo4 is involved in angiogenesis, blood vessel integrity and homing of hematopoietic stem cells in the osteoblastic niche. To determine whether Robo4 could also mediate the homing of tumour cells in the bone marrow, we invalidated *ROBO4* in B02 cells using a CRISPR/Cas9 strategy. *In vitro*, the interaction of *ROBO4*-depleted B02 cells (B02-ROBO4$^-$) with pre-osteoblasts (MC3T3-E1) was decreased, compared to that observed with parental B02 cells (B02-C). After culture of B02-C or B02-ROBO4$^-$ cells in suspension, tumour cells formed mammospheres. Their size was however substantially reduced upon *ROBO4* depletion. Similar findings were observed when co-culturing B02-C or B02-ROBO4- cells with murine MC3T3-E1 cells or human mesenchymal cells. *Ex vivo*, the number and size of tumour cell colonies in the bone marrow from animals inoculated with B02-ROBO4$^-$ cells were significantly decreased compared to that observed with B02-C cells. *In situ* visualization by dual-photon microscopy of DiD-labelled B02-ROBO4$^-$ and B02-C cells in the hind limbs from tumour-bearing animals showed that *ROBO4* depletion impeded the homing of tumour cells in the bone marrow. Furthermore, compared to B02-C cells, the homing of B02-ROBO4$^-$ cells in a humanized osteoblastic niche implanted subcutaneously in NOD/SCID mice was also markedly reduced. Overall, these results suggest that ROBO4 mediates the early seeding of human breast cancer cells in bone.

OC9

Potent therapeutic impact of TAK1 inhibition on myeloma progression and bone destruction

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Multiple myeloma (MM) has a unique propensity to develop and expand in the bone marrow with devastating bone destruction. We have reported that MM cells constitutively overexpress PIM2, and that bone marrow stromal cells (BMSCs) as well as osteoclasts (OCs) further upregulate PIM2 as an anti-apoptotic mediator in MM cells. Besides, the interaction with MM cells induce PIM2 in BMSCs and OCs to progress bone destruction, indicating the critical role of PIM2 in MM tumor growth and bone destruction. We recently identified TGF-β-activated kinase-1 (TAK1) as an upstream mediator responsible for PIM2 upregulation in these cells. Therefore, we aimed to clarify the role of TAK1 in MM growth and bone destruction, and the therapeutic impact of TAK1 inhibition on MM. TAK1 was constitutively overexpressed and phosphorylated in MM cell lines tested. Immunoreactivity of phosphorylated TAK1 was apparently observed in MM cells in 36 out of 50 MM patients, but not in normal peripheral blood mononuclear cells. The TAK1 inhibitor LLZ1640-2 reduced the PIM2 expression and induced apoptosis in MM cells. Although TNF-α and IL-6 upregulated PIM2 expression in MM cells, LLZ1640-2 abolished TNF-α-induced NF-κB activation and IL-6-induced STAT3 activation in MM cells to reduce PIM2. The TAK1 inhibition suppressed VEGF production and the expression of BCMA and TACI, receptors for BAFF and APRIL. TAK1 phosphorylation was induced in BMSCs when the cells were co-cultured with MM cells; however, LLZ1640-2 suppressed PIM2 up-regulation and induced apoptosis in MM cells in cocultures with BMSCs. TAK1 inhibition reduced VCAM-1 and RANKL expression and IL-6 production by BMSCs, which impaired adhesive interactions between MM cells and BMSCs, and blunted protective activity of BMSCs for MM cells against anti-MM agents. LLZ1640-2 abolished the PIM2 induction in MC3T3-E1 cells by MM cells to restore osteoblastogenesis. Furthermore, LLZ1640-2 suppressed osteoclastogenesis by RANKL as well as MM cells. Treatment with LLZ1640-2 suppressed MM growth and prevented bone destruction in mouse MM models. These results demonstrate that TAK1 plays a pivotal role in tumor progression and bone destruction in MM. TAK1 inhibition may become a unique anti-MM therapeutic option with bone-modifying activity.

OC10

Dual BRD4/autophagy inhibitor as novel strategy for the treatment of multiple myeloma

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The bromodomain and extra-terminal (BET) family of proteins are important epigenetic regulators of oncogenes, including c-Myc. JQ1 is a potent BRD4 inhibitor and the focus of current clinical trials for the treatment of hematologic malignancies. Unfortunately, resistance to BET inhibitors is acquired, indicating that single agent therapies may not provide durable therapeutic responses.

To overcome this limitation, we have developed a new class of dual inhibitors, that simultaneously target BRD4 and a panel of tyrosine kinases highly expressed in cancer including JAK2, FLT3, RET, FGFR1, ULK1 and ULK3.

Preliminary *in vitro* screening identified SG3 as the lead compound with BRD4 and c-Myc IC_{50}'s similar to JQ1 (nM). SG3 however, was also inhibiting JAK2, FLT3, ULK1 and ULK3 in the nM range. We initially explored the efficacy of SG3 on MM. *In vitro* assays on cell lines revealed higher SG3 sensitivity (5TGM1, 0.85mM; U266, 0.99mM) compared to JQ1 (5TGM1, 4.7mM; U266, 16mM). *In vivo* studies with 5TGM1-Luc cells demonstrated that SG3 treatment significantly contributes to overall survival compared to control (median survival CTRL = 40.5; SG3 = 50.5; JQ1 = 46 days).

Post-study analyses demonstrated a significant reduction in myeloma-induced bone disease in the SG3 treated mice, quantified by X-ray/mCT/ histomorphometry. No toxicities or adverse effects were noted.

As expected, SG3 inhibited c-Myc expression. To identify potentially additional mechanisms of action we explored the prevalence of SG3 target kinases in publicly available datasets. We identified ULK3 as a candidate kinase of SG3 targeting activity and correlated its expression with MM disease stages. High basal levels of autophagy are present in MM and ULK3 is a key regulator of the process. We therefore explored whether the additional efficacy of SG3 was being mediated by autophagy inhibition. We noted, *in vitro*, that within 6 hours, SG3 completely shut down autophagy with decrease levels of ULK1, beclin-1, ATG16L-1, ATG12, ATG3, p62 and increased LC3I/II ratios (additionally confirmed by microscopy and flow cytometry). These effects were not noted with JQ1.

In summary, we believe SG3 is an exciting new dual inhibitor that will be effective for the treatment of treatment-naïve and potentially refractory multiple myeloma.

OC11

Two-way Signalling between Multiple Myeloma Cells and Bone Marrow Adipocytes

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Multiple myeloma is a plasma cell cancer within the bone marrow (BM). Myeloma cells have a dependency on the BM microenvironment for survival and proliferation, and BM adipocytes (BMAs) demonstrate a unique, endocrine signaling capacity and lipid composition that is likely to cause unique cross-talk between myeloma cells and BM adipose tissue (BMAT).

We generated BMAT from human BM-derived stromal cells (BMAT) using adipogenic media. We performed proliferation, adhesion, gene expression, lipid accumulation, adipokine array, and drug resistance screens to study the relationship between BMAT and myeloma cells. Myeloma cells were cultured on, or with conditioned media (CM) from, BMAT + /-chemotherapies. We also developed a tissue-engineered 3D BMAT-myeloma model using silk scaffolds.

Direct culture with BMAT rescued OPM2 myeloma cells from dexamethasone- and bortezomib-induced apoptosis and cell death. We explored changes in cell-cell adhesion genes using qRT-PCR and found that VCAM-1 was increased in OPM2 cells after co-culture with BMAT, and this was further increased after addition of bortezomib, while other genes (e.g., N-cadherin) were not changed. MM1S cells also showed significant dexamethasone resistance and decreased apoptosis when co-cultured with BMAT CM. IL-6, resistin, and leptin showed promise as molecules that contribute to this. Our 3D BMAT models also induced OPM2 dexamethasone resistance.

We also observed the phenomenon "adipomimicry" in tumor cells. OPM2 cells expressed FABP4, PPARγ and ADIPOQ when co-cultured with BMAT, and these were further increased upon treatment with bortezomib. Myeloma cells were also observed to contain lipid droplets only when co-cultured with BMAT. Whether these changes are governed by direct lipid transfer, exosome (miRNA/mRNA) transfer, or cell-cell soluble or adhesion-based activation of downstream signalling pathways is under investigation in our lab. In both 2D and 3D co-cultures, we also found a reduction in BMAT lipid content using Oil-Red-O staining. Lastly, an adipokine array revealed significant increases in tumor supportive secreted factors (AgRP, IGF2, IL6, Lipocalin-2, RAGE, RANTES, and RBP4) from 3T3L1 adipocytes after transwell co-culture with myeloma cells.

Overall, we propose a positive feedback loop between myeloma cells and BMAs, which has great potential for targeting with novel therapeutics in multiple myeloma.

OC12

The small molecule adiponectin receptor agonist AdipoRON exhibits anti-myeloma activity *in vitro* and *in vivo*.

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Multiple Myeloma (MM) is a plasma cell malignancy which interacts with the bone microenvironment, inducing bone lesions and pain. Adiponectin is an adipocyte-secreted cytokine which is thought to have beneficial effects against obesity, diabetes and cardiovascular health. Serum levels of adiponectin are negatively correlated with MM progression in patients, while bone microenvironment-produced adiponectin is protective against myeloma in mouse models. AdipoRON, a recently developed orally-available adiponectin receptor agonist compound, binds both adiponectin receptors AdipoR1 and AdipoR2 and activates downstream signalling. We examined the effect of AdipoRON on MM growth *in vitro*, using both primary MM samples and MM cell lines, and used the C57Bl/KaLwRij strain of mouse with 5TGM1-GFP + MM cells to examine the effect of AdipoRON as a therapeutic *in vivo*, in comparison to the widely used therapeutic bortezomib.

Treatment with AdipoRON reduced viability and induced cleaved PARP and apoptosis in a panel of MM cell lines *in vitro*, and death of patient-derived MM cells as revealed by flow cytometry. *In vivo*, IgG2b paraprotein (a marker of tumour burden) was significantly reduced by either oral AdipoRON treatment or bortezomib three times per week ($p < 0.01$) relative to vehicle control. Bone marrow and spleen GFP + tumour cell percentages by flow cytometry confirmed MM invasion to both sites. Spleen weights were significantly increased in MM-bearing mice after control or bortezomib treatments ($p < 0.01$ and $p < 0.001$ respectively), but not AdipoRON treatment. Preliminary micro-CT analysis indicated a beneficial effect of both AdipoRON and bortezomib on trabecular number and BV/TV in the tibia of MM-bearing mice. Neither treatment had a significant effect on cortical bone strength *in vivo* at this time point as measured by 3-point bend analysis, however *in vitro* AdipoRON increased 2T3 mouse osteoblast mineralisation, suggesting a potential secondary effect of long-term AdipoRON treatment. This work further underscores the potential of adiponectin-based therapeutics in MM treatment.

OC13

MSCs promote the evolution of apoptosis resistant bone metastatic prostate cancer via interleukin-28

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Bone metastatic prostate cancer is incurable and often has osteoblastic phenotype. Osteoblast precursors, mesenchymal stem cells (MSCs), reside in the bone marrow and are thought to promote cancer growth. We therefore hypothesized that prostate cancer interactions with MSCs in the bone microenvironment would lead to an osteoblastic phenotype and accelerated tumor growth. Rag2 immunocompromised mice (n = 8/group) were intratibially inoculated with primary MSCs ($2x10^4$), PaIIILucs ($2x10^4$), or a 1:1 ratio of MSCs:PaIII ($4x10^4$ total). Surprisingly, we observed an initial suppression of prostate cancer growth by MSCs until day11 ($p < 0.05$) after which we noted rapid expansion of the prostate cancer cells. *Ex vivo* analyses demonstrated that co-injection of MSCs significantly reduced tumor induced osteolysis (X-ray) and promoted trabecular bone formation (μCT). *In vitro*, co-culture of prostate cancer cells (PaIII, DU145, MycCAP) with MSCs or MSC conditioned media (CM) induced prostate cancer cell apoptosis as measured by cleaved caspase-3. However, not all cells succumbed and two further rounds of selection in MSC CM yielded sub-populations (F2) of prostate cancer cells resistant to MSC CM, etoposide and docetaxel. Repeat of our *in vivo* study now demonstrated that MSC educated prostate cancer cells lines grew significantly faster than in the presence of MSCs compared to the parental (F0) cells lines. Cytokine array analyses of MSC CM revealed interleukin 28 (IL-28) as a candidate factor promoting prostate cancer cell apoptosis. We found that depletion of IL-28 from MSC CM prevented F0 prostate cancer cell apoptosis with no effect on F2 populations. This was further validated using shRNA approaches. Analysis of downstream effectors of the IL-28 receptor revealed rapid phosphorylation of STAT3 in the F2 cell lines. Using a STAT3 inhibitor (S3I-201) we observed that F2 prostate cancer cell lines were more sensitive than their parental counterparts *in vitro*. *In vivo*, we observed MSC educated F2 PAIII cells were significantly inhibited by S3I-201 compared to vehicle control. Taken together, our results indicate that bone MSCs promote the evolution of apoptosis resistant prostate cancer cells via chronic exposure to IL-28 but the evolved cells are sensitive to STAT3 inhibition.

OC14

The Physical Bone Microenvironment Modulates Osteolytic Gene Expression in Metastatic Breast Cancer

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Tumor-induced bone disease is a common comorbidity in patients with metastatic breast cancer. Patients suffer from debilitating skeletal-related events primarily because the bone microenvironment, including resident bone cells and soluble factors, provides a fertile niche for breast cancer cells to promote osteoclast-mediated bone resorption. Specifically, our group has established that bone-metastatic breast cancer cells overexpress the Hedgehog (Hh) transcription factor Gli2 in response to transforming growth factor beta (TGF-β), which induces parathyroid-related protein (PTHrP) secretion and bone destruction. However, Gli2 expression is not solely regulated by soluble factors like Hh ligands or TGF-β. Our previous investigations show that matrix rigidity can also regulate tumor cell expression of Gli2 and PTHrP. Breast cancer cells cultured on 2D polyurethane films with elastic moduli ranging from the basement membrane to cortical bone had significantly increasing mRNA levels of Gli2 and PTHrP as well as the mechanosensitive gene integrin beta 3 (ITGB3), which has been implicated in breast cancer metastasis to bone. Other parameters of the physical and structural bone microenvironment and their effects on tumor cell gene expression and behavior have yet to be explored. We hypothesized that the trabecular architecture of bone can modulate the expression of osteolytic genes, including Gli2, PTHrP, and ITGB3, to promote the bone-destructive phenotype of breast cancer cells. To test this, we created novel 3D tissue-engineered bone constructs (TEBCs) that mimic the trabecular structure of human bone at different anatomical sites such as the femur, tibia, and vertebrae. There were significant differences in the expression of Gli2, PTHrP, and ITGB3 by bone-metastatic breast cancer cells (MDA-MB-231) cultured on TEBCs, even when compared to that of cells seeded on a scaffold with uniform pore size. Interestingly, breast cancer cells with relatively low metastatic potential (MCF-7 and SUM159) also differentially express Gli2, PTHrP, and ITGB3 on these bone constructs. These data indicate that bone microarchitecture regulates osteolytic gene expression in breast cancer cells and underscores the potential of the 3D bone microenvironment to promote breast cancer-induced osteolysis. A better understanding of tumor cell response to the bone microenvironment can help investigators develop new therapies to treat tumor-induced bone disease.

OC15

Epigenetic targeting of the myeloma-bone microenvironment in 3D

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EZH2, the methyltransferase subunit of Polycomb Repressive Complex 2 (PRC2), catalyzes H3K27me3 histone modification and epigenetically regulates genes involved in cellular pluripotency and differentiation. EZH2 regulates MM cell survival, and its elevated expression correlates with poor prognosis in MM patients. EZH2 inhibitors including GSK126 in combination with conventional therapies showed promising anti-myeloma effects.With a focus on reversal of MM osteolytic bone disease, we showed that GSK126 rescued MM-induced suppression of BM-MSC osteogenic differentiation. We provide new evidence that GSK126 blocks MM-induced hyperactivation of osteoclast precursors (OCLp). RNA-seq profiling revealed that inhibition of EZH2 re-activated 115 genes associated with bivalent and/or H3K27me3 promoter signatures including OCL inhibitory factors *MafB*, *Irf8, Bcl6b* and *Arg1*. In contrast, we found that OCLp expansion in MM1.S-conditioned media (MMCM) induced significant gene expression changes, which correlated with TNF and IKK signaling, inflammatory responses and CXC-chemokine receptor pathways. We evaluated the effectiveness of GSK126 in the context of the bone microenvironment in a novel 3-dimensional (3D) model of MM co-cultures (3D-MM). We combined basement membrane extract (BME) hydrogels with devitalized bone slices to mimic the 3D setting of MM with OCL-resorbing endosteal surface. This enabled us to test the effetcs of GSK126 alone or in combination with bortezomib simultaneously on MM survival and OCLp differentiation and bone resorption. While GSK126 exhibited potent anti-MM effects and blocked OCL differentiation, mature OCL added to 3D-MM co-cultures increased the IC_{50} MM inhibition dose of both bortezomib and GSK126 by 40% and 50% respectively. The synergistic effect of these two drugs on MM inhibition was further reduced by 70%. 3D-MM co-cultures with total bone marrows of differentially (1-12 months old) aged mice showed selective protection from GSK126, but not bortezomib, on MM viability. Furthermore, the resistance to GSK126 was age-dependent, with the 12-month-old bone marrows causing the greatest protection of the MM cells. Using confocal fluorescence microscopy, we found that the resistant MM cells were in close proximity to osteoprogenitor cells, suggesting that this interaction reduces the effectiveness of epigenetic drug targeting against MM. This novel 3D-MM system enables us to rapidly screen drug combinations, and simultaneously evaluate the influence of bone-microenvironmental interactions on MM drug resistance and bone marrow cell responses to the drugs.

OC16

Blocking Cysteinyl leukotriene receptor type 1 (CysLT1) inhibits blood platelets' prometastatic activity.

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Metastases are the main causes of death in cancer patients because current primary tumor-targeted therapies are missing of specificity against the metastasis process. Therefore, development of targeted anti-metastasis therapeutics is urgently required. Blood platelets contribute to the poor survival of patients with advance cancers. Unfortunately platelets are mandatory for human life due to their paramount function in hemostasis. The main objective of our project was to define new anti-metastasis therapies exhibiting a high specificity for the pathological tumor cell–blood platelet interaction without abrogating normal platelet function. The project was based on an *in vitro* high-throughput screening of a bank of molecules comprising 1280 compounds showing that pharmacological blockade of cysteinyl leukotriene receptor 1 (CysLT1) with two already FDA-approved drugs in a asthma (Zafirlukast, Montelukast), inhibited specifically the pro-tumoral activity of human blood platelets on MDA-B02 human breast cancer cells with an IC50 around 0.5μM. CysLT2 inhibitor HAMI3379 had no effect on this assay confirming the specific involvement of CysLT1. CysLT1/2 receptors are restricted to the myeloid cell lineage. We confirmed that MDA-B02 cells did not express these receptors and were insensitive to stimulation with CysLT1 ligand, LTD4. MDA-B02 cell conditioned media (MC) added alone did not induce platelet aggregation. Remarkably, MDA-B02 cell MC potentiated the mild platelet agonist activity of a low dose of ADP (1μM) from a reversible to a full platelet aggregation that was totally blunted with Zafirlukast. This result suggested that cancer cells produced CystLT1 ligands (LTC4, LTD4 or LTE4) that were active on platelet aggregation in presence of ADP. We then addressed in the impact of Zafirlukast on platelet function in vivo. Based on a bleeding test we confirmed that blocking CysLT1 in mouse had no impact on hemostasis. However, we found that the treatment with Zafirlukast (0.4mg/kg/day per os) for 7 days decreased by 60% the incidence of metastasis in mice after intra-arterial injection of MDA-B02 cells and inhibited by 90% the number of tumor cells that colonized the bone. Our study revealed for the first time that CysLT1 receptor is a potential new target for blocking early events of the bone metastasis cascade by targeting a new partner involved in blood platelet and tumor cell connections.

OC17

Inhibition of multiple myeloma exosomes prevents bone loss and reduces tumor growth

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Multiple myeloma, one of the most common hematological malignancies, will often manifest osteolytic lesions during disease development, throughout the whole body.

MM is still considered an incurable malignancy, with a supportive micro-environment in the bone marrow. Extracellular vesicles such as exosomes are known to play an important tumor-promoting role in this micro-environment, by inducing angiogenesis and immune suppression. The aim of our study was to examine the effects of MM exosomes on osteolysis in vitro and in vivo, and to determine whether inhibition of exosome secretion can lead to a delay in tumor growth and prevent bone loss.

We used the 5TGM1 model which is a syngeneic murine MM model, presenting typical MM characteristics such as osteolysis, angiogenesis and a serum M-spike. We examined the effects of conditioned medium from these MM cells and MM-derived exosomes on cell viability and proliferation of the MC3T3 pre-osteoblast cell line and on their ability to produce alkaline phosphatase (ALP). We saw a decrease in viability and ALP activity when adding exosomes or conditioned medium of the 5TGM1 myeloma cells to the osteoblasts. Moreover, RT-PCR analysis showed a decrease of various osteogenic differentiation genes when osteoblasts were exposed to 5TGM1 exosomes, demonstrating a

diminished capacity of osteoblastic differentiation.
We next added the exosome secretion inhibitor GW4869 to the 5TGM1 cells, thereby reducing the number of exosomes in the conditioned medium. This was confirmed through western blot of the typical exosome markers. The negative effects of the 5TGM1 conditioned medium on osteoblasts could be overturned by reducing the exosome content therein.
In vivo, we injected 5TGM1 exosomes intravenously in healthy mice for three weeks and examined the effects on osteolysis by microCT and TRAP staining. We saw an increase in osteolysis, comparable to the typical osteolytic lesions in tumor-bearing mice. Furthermore, when inhibiting the exosome secretion in vivo in 5TGM1 mice, we observed a significantly reduction in tumor load.
Through this study we can conclude that exosomes from multiple myeloma cells can induce osteolytic lesions by inhibiting osteoblast proliferation and differentiation. These effects can be reversed by inhibiting the secretion of exosomes.

P1
ABSTRACT WITHDRAWN

P2
REFILLING OF BONY MYELOMA LYTIC LESIONS MEDIATED BY TOTAL THERAPY 4 TREATMENT

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Multiple myeloma (MM) is a disease characterized by proliferation of clonal plasma cells. At diagnosis, 70% of MM patients with extensive osteolytic bone lesions. Aim of this study was to investigate the effect of chemotherapy and autologous stem-cell transplantation on bone formation at lytic bone sites with more than one centimeter in largest diameter.
We included 233 patients (144 males and 89 females, age range: 38-84 years) enrolled in the Total Therapy (TT4) trial. TT4 encompassed induction chemotherapy, tandem autologous stem-cell transplantation and maintenance with proteasome inhibitors and immunomodulatory drugs. All patients shared low risk according to the gene expression profiling (GEP70 risk-classifier). Lytic lesion were distributed in flat and long bones in 96% and 4% of cases, respectively. Remineralization/refilling was defined as a sclerotic CT changes within lytic lesion. Healing at the lytic site was calculated as a percentage, using the initial lesion size reference.
With a median follow-up of 3 years we observed a decreased lesion size in 147 patients (63%), with 13 individuals (5%) demonstrating complete remineralization. An improvement between 25-50% was documented in 26 subjects (11%), 50-75% in 19 patients (8%), and 75-100% in 18 patients (8%). The healing status had no significant impact on overall or event free survival. We compared the parameters as gender, age, race, LDH, B2M, albumin, and international staging system (ISS) between patients with vs. without healing. The proportion of patients with ISS stage III was significantly lower in the group of healing patients (24 vs 38%). Comparison of gene expression profiles indicated higher expression in the gene for Vitamin D receptor in non-healing subjects.
This retrospective analysis of 233 myeloma patients demonstrated a significant healing of lytic lesions in two third of patients treated with intensive chemotherapy. The underlying mechanism remains elusive, however our results suggest that the grade of disease and the expression level of the Vitamin D receptor impact this phenomenon.

P3
Bone seeking MMP-2 inhibitors can prevent bone metastatic breast cancer

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Bone metastasis is a common event during breast cancer progression. The resultant lesions are painful and currently incurable. The advancement of bone metastatic breast cancer is critically dependent on interactions with the surrounding microenvironment. Therefore, identifying the underpinning molecular mechanisms is vital for the development of new therapies.
Gene expression analysis and validation in human and murine specimens of bone metastases revealed that MMP-2 is highly expressed in the bone metastatic microenvironment. These data support the rationale for the development of a highly specific MMP-2 inhibitor for the eradication of active bone metastatic breast cancer.
Given that previous broad-spectrum MMP inhibitor trials were unsuccessful due to dose limiting systemic side effects, we utilized a novel chemical approach to synthesize bone seeking MMP inhibitors (BMMPIs) on a bisphosphonic backbone, with specificity for MMP-2 (IC_{50} = 140 nM).
Based on our previous data about BMMPI's ability to significantly reduce breast cancer growth in the bone microenvironment, we decided to investigate the role of BMMPI such as ML115 in preventing dissemination of cancer cells into the skeleton.
Balb-c female mice (n = 10/group), pretreated for 7 days with vehicle, standard of care bisphosphonate (Zoledronate = 1 mg/kg) and BMMPIs (ML115 = 1 mg/kg), were randomized and intra-cardiac injected with luciferase expressing 4T1 ($2x10^5$) breast cancer cell line. Tumor growth was assessed via luminescence quantitation overtime. At day 14, mice were sacrificed and tibias were collected for ex vivo analysis. Our preliminary total body bioluminescent data suggest that pretreatment with ML115 can significantly reduce the amount of breast cancer cells homing into the bone, compared to vehicle and standard bisphosphonate. Cancer induced bone disease was measured ex vivo by μCT, Xray and histomorphometry. *Ex vivo* analysis confirmed the significant beneficial effects of the BMMPIs in reducing the size of osteolytic lesions. We are currently measuring MMP activity in vivo and ex vivo via specific activatable MMP probes, in order to confirm BMMPI mechanism of action.
Given the well tolerated nature of bisphosphonates in the clinical setting, we predict that BMMPIs could be used as preventive treatment to impair breast cancer metastasis to bone.

P4
PRECLINICAL TESTING OF TRAIL THERAPEUTICS FOR SARCOMA

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Despite intensive, multimodal therapy, patients with bone sarcomas have poor 5-year survival, close to 50%. TNF-related apoptosis-inducing ligand (TRAIL) can induce apoptosis in cancer cells, via the extrinsic pathway, after binding to its death receptors: DR4 and DR5, while sparing nontransformed cells. TRAIL provides an approach that can overcome current therapeutic limitations, such as drug resistance and toxicity associated with high doses, when administered alone or combined with conventional therapies.
To characterise sarcoma cells for Death Receptor (DR) expression and factors that render them susceptible to TRAIL therapy, and to assess the effectiveness of different forms of TRAIL.
We characterized osteosarcoma cell lines (U2OS, SAOS-2 and SJSA-1), Ewing's sarcoma cell line (TC-71), bone fibrosarcoma cell line (HT1080), chondrosarcoma cell line (SW1353), for Death Receptor 4

(DR4) and Death Receptor 5 (DR5) expression at the RNA and protein levels. Sarcoma and normal human cell lines were exposed to different forms of TRAIL.

DR5 was found to be upregulated in sarcoma cell lines. Crosslinked forms of TRAIL were more cytotoxic when compared to non-crosslinked forms of TRAIL. Fibroblasts and stem cells were resistant to TRAIL therapy; however, human hepatocytes cells were sensitive. SuperKillerTRAIL (SKT) and a newly designed scFv:TRAIL variant targetting NG2, also upregulated in U2OS and SW1353 cell lines, demonstrated significantly enhanced cell killing when combined with doxorubicin.

Our pilot in vitro data indicate that osteosarcoma cell lines such as U2OS can be killed by pre-oligomerised versions of TRAIL and more resistant cell lines such as SJSA-1 and SW1353 can be sensitised to TRAIL by current chemotherapeutics. Furthermore, resistant cell lines such as SW1353 can also be sensitised by Birinapant, which is an improvement on previous smac mimetics. We plan to test the therapeutics in vivo in an orthotopic mouse model of bone sarcoma.

P5

Humanized Mouse Models of Triple-Negative and Triple-Positive Breast Cancer Bone Metastasis for Preclinical Validation of Novel Immuno-Oncology Therapies

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Immunotherapy provides promising results in the treatment of breast cancer, but there is a high variability in response between different subtypes. Triple-negative breast tumors have a high number of tumor infiltrating lymphocytes (TILs) and respond well to immunotherapy. Conversely, hormone receptor positive breast tumors attract fewer immune cells and are less sensitive to immunotherapy. Based on the phenotype these tumors are referred as “hot” and “cold”. In both breast cancer subtypes, the patients typically develop bone metastases that are currently incurable. Novel immunotherapies hold the potential for more effective treatment of patients with bone metastatic disease. The aim of this study was to establish two different preclinical breast cancer models in humanized mice to be used in preclinical evaluation of the efficacy of new immunotherapies in bone metastatic setting.

Female CIEA NOG mice engrafted with human CD34+ hematopoietic stem cells (huNOG, Taconic Biosciences) were inoculated with human BT-474 (ER+, PR+, HER2+) or MDA-MB-231(SA) (ER-, PR-, HER2-) cells into the bone marrow of tibia. Tumor-induced changes in bone were monitored by X-ray imaging. Tumor-induced bone alterations were analyzed by micro-computed tomography (μCT) imaging. Histological analyses were performed for quantitation of tumor area in bone and immunohistochemical analyses for quantitation and localization of TILs.

BT-474 cells induced osteoblastic bone growth in the mice. Tumor-induced changes in bone were visible in X-ray imaging at 4 weeks after inoculation. At 8 weeks, the μCT analysis showed an increased bone volume compared to intact tibia. MDA-MB-231(SA) cells induced osteolytic bone loss. In a 3 weeks study, the osteolytic lesions were moderate in size and the mice exhibited cachectic features including decreased body weight, curved spine, and muscle loss. μCT quantitation of bone volume showed a moderate decrease compared to intact tibia. The BT-474 model resembled a “cold” tumor, and a low number of TILs, CD4+ and CD8+ cells were observed in this model. In contrast, the MDA-MB-231(SA) model resembled a “hot” tumor, and a large quantity of CD4+ TILs were seen in the tumor with CD8+ cells.

P6

Preventing*and*repairing myeloma bone disease by combining conventional anti-resorptive treatment with a novel bone anabolic treatment

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Multiple myeloma is a plasma cell malignancy, which develops in the bone marrow and frequently leads to severe bone destruction. Current anti-resorptive therapies to treat the bone disease do little to repair damaged bone; therefore, new treatment strategies incorporating bone anabolic therapies are urgently required. We hypothesised that combination therapy using the standard of care anti-resorptive zoledronic acid (Zol) with a bone anabolic (anti-TGFβ/1D11) would be more effective at treating myeloma-induced bone disease than Zol therapy alone. JJN3-bearing mice (n = 8/group) were treated with vehicle, Zol or 1D11 alone or Zol and 1D11 combined. Bone loss was analysed at the end stage of disease by *ex vivo* micro-CT and histomorphometry. U266-bearing mice (n = 8/group) with established lytic bone lesions were treated with vehicle, Zol or Zol and 1D11 combined. Bone changes were monitored overtime by *in vivo* micro-CT, serum bone markers and dynamic histomorphometry. JJN3 myeloma-bearing mice treated with combined Zol and 1D11 resulted in a 48% increase ($P \leq 0.001$) in trabecular bone volume compared to Zol alone and a 65% ($P \leq 0.0001$) increase compared to 1D11 alone. The most significant finding was the substantial repair of U266-induced lytic bone lesions with combination therapy, which resulted in a significant reduction in lesion area compared to vehicle ($P \leq 0.01$) or Zol alone ($P \leq 0.01$). These results reveal a novel finding and demonstrate that combined anti-resorptive and bone anabolic therapy are significantly more effective at treating established myeloma-induced bone disease than Zol alone. This is a highly translational strategy which could significantly improve bone outcomes and quality of life in myeloma patients.

P7

Inhibiting IL-1B signalling increases therapeutic efficacy of doxorubicin and zoledronic acid in immunocompetent models of mammary cancer bone metastases.

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We have identified IL1B as a biomarker for breast cancer patients who develop metastasis to bone. Our previous studies have shown that inhibiting IL-1 signalling using the IL-1R antagonist, Anakinra, inhibits bone metastases. In the clinic patients with this condition are commonly treated with a combination of chemotherapy (such as doxorubicin) and zoledronic acid (ZA). We are therefore investigating whether addition of Anakinra to this combination can further increase efficacy.

Investigate the effects of Anakinra, doxorubicin and ZA alone and in combination on breast cancer bone metastases.

Mouse syngeneic E0771 GFP-Luc or 4T1 Luc2 mammary cancer cells were administered to 6-8 week old C57BL/6 (n = 14/group) or BALB/c (n-16/group) mice via intra-cardiac injection. Treatment with Anakinra (1mg/kg/day), doxorubicin (2mg/kg/ week), ZA (100ug/kg/ week – 24h post dox) or placebo, alone or in combination, commenced 3 days after tumour cell injection. Tumour size and location were monitored using IVIS bioluminescent detection. Effects on bone were measured by uCT, P1NP, TRAPc.

Treatment with a combination of all three drugs reduced the number mice with bone metastases from 50% to 0% in mice injected with E0771 cells and from 25% to 14.2% in mice injected with 4T1 cells. Adding Anakinra to doxorubicin and ZA also reduced non-bone metastases from 50% to 16.67% in mice injected with E0771 cells and from 87.5% to 57.1% in mice injected with 4T1 cells. Anakinra alone reduced bone metastases to 16.67% (P = 0.0294) and 12.5% (P = 0.0.0201) in mice injected with E0771 and 4T1 cells respectively. Neither Anakinra alone or doxorubicin followed by ZA reduced non-bone metastases. Administration of Anakinra or ZA alone or in combination had significant anabolic effects on bone inhibiting tumour associated bone loss.

Administration of Anakinra inhibits breast cancer growth and bone metastases in vivo. When added to a combination of doxorubicin and ZA this further increased the efficacy reducing bone and non-bone metastases. Our data indicate that inhibiting IL-1B signalling in patients with breast cancer metastasis may increase the therapeutic potential of their current standard of care.

P8

Tumour derived IL-1B Induces differential tumour promoting mechanisms in breast cancer bone metastasis

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Breast cancer bone metastases are incurable and associate with poor prognosis. After homing and colonising bone, breast cancer cells may remain dormant, until signals from the microenvironment stimulate their proliferation to form overt metastases. We have recently identified interleukin-1B (IL-1B) as a potential marker for predicting breast cancer patients at increased risk for developing metastasis and established a role for IL-1 signalling in tumour cell dormancy in bone. Our novel data support that tumour-derived, microenvironment-dependent IL-1B play major roles in breast cancer metastasis and growth in bone.

In tissue samples from >1300 patients with stage II/III breast cancer, active IL-1B in tumour cells correlated with relapse in bone (hazard ratio 1.73; 95% CI 0.99-3.05; P = 0.0387) and other sites (hazard ratio 1.72; 95% CI 1.04-2.84; P = 0.0016). In a model of spontaneous human breast cancer metastasis to human bone, administration of the clinically available anti-IL-1B monoclonal antibody, Ilaris, or the receptor antagonist, Anakinra, significantly reduced metastasis to bone (8/10 control, 1/10 Ilaris and 2/10 Anakinra). Both agents reduced the number of tumour cells shed from the primary site into the circulation. Genetic manipulation of breast cancer cells to overexpress IL-1B demonstrated that exogenous production of IL-1B by breast cancer cells promoted EMT (decreased E-Cadherin, N-Cadherin and G-Catenin), invasion, migration and organ-specific homing in ER-ve (MDA-MB-231) and ER + ve (T47D and MCF7) cells *in vitro* and *in vivo*.

In the bone microenvironment, contact between tumour cells and osteoblasts or bone marrow cells increased IL-1B secretion from all three cell types. Exposure of tumour cells to IL-1B in the absence of bone cells did not stimulate tumour cell proliferation. Instead, high concentrations of IL-1B caused expansion of the bone metastatic niche (increased osteoblasts and blood vessels) that in turn stimulated tumour proliferation.

Our novel data demonstrate that IL-1B/IL-1R1 signalling plays an important role in breast cancer metastasis to bone. Pharmacological inhibition of IL-1B has potential as a novel treatment.

P9

Interleukin-34 as a Potential Therapeutic Target for the Treatment of Osteosarcoma

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Interleukin-34 is a recent discovered cytokine sharing functional similarities with the macrophage-colony stimulating factor (M-CSF) in regulating the proliferation/differentiation/survival of myeloid cells. IL-34 expression is associated with an increase of osteosarcoma growth in bone site as well as the formation of lung metastases. It has already been established that recruitment of M2 macrophages, new vessel formation and extravasation of immune cells are the main mechanisms by which IL-34 plays a key role in the pathogenesis of osteosarcoma. Owing to this, IL-34 is a potential therapeutic target in osteosarcoma.

The present study aimed to determine the therapeutic effect of IL-34 targeting in osteosarcoma by using murine pre-clinical models and anti-IL34 blocking antibodies.

Human GFP-MNNG/HOS and mouse MOS-J osteosarcoma cells where used to establish human and mouse xenograft models in immunodeficient and immunocompetent environment respectively. Cells were inoculated in close proximity to the tibia, and anti-IL-34 monoclonal blocking antibodies were administered (i.p.) three times weekly at 100 microg/injection. Tumour volumes were measured twice a week with calipers, and tumour samples were processed for immunohistochemistry.

As expected the inoculation of osteosarcoma cells led to the development of palpable tumour mass after 10 days. The administration of mouse anti-human IL-34 antibodies did not modulate the tumour growth in contrast to rat anti-mouse IL-34 which markedly blocked the tumour progression.

In osteosarcoma development, IL-34 appears mainly produced by the murine tumour microenvironment. The inhibition of IL-34 specific blocking antibodies demonstrates that the therapeutic benefit to abrogate IL-34 in osteosarcoma. IL-34 may be a novel therapeutic target in bone associated-diseases.

P10

Combination of capecitabine and radium-223 for patients with breast cancer and bone metastases: Results of the CARBON trial phase IB initial safety stage

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INTRODUCTION: Median survival time in breast cancer after the development of bone metastases remains around 2-3 years. Many patients with bone involvement also have metastasis in other sites. Thus, combining a bone-targeted treatment such as radium-223 with systemic chemotherapy, may provide a more effective treatment with minimal additional side effects. In the CARBON study, we are assessing the safety and preliminary efficacy of the combination of radium-223 and capecitabine. We present data from the safety phase of the CARBON trial.

METHODS: CARBON is a randomised, multi-centre, open-label phase IB/IIA study, for female breast cancer patients with bone metastases,

with or without metastasis elsewhere. The initial safety phase employed a modified 3 + 3 design aiming to determine the feasibility and safety of administering radium-223 (55kBq/kg 6-weekly starting cycle two) with capecitabine (standard of care, 1000mg/m^2 on days 4-17 of each cycle, for up to 12 cycles). The primary endpoint was the number of patients experiencing dose limiting toxicities (DLTs) within the first two cycles. Phase IIA then aims to establish preliminary information on efficacy (bone turnover markers and progression in bone and non-skeletal sites) and further characterise the safety profile.

RESULTS: Six patients (mean age 54.2 years) were recruited to the initial safety phase. There were no DLTs and three non-trial treatment related serious adverse events in two patients. Five CTCAE grade 3 adverse events (malignant neoplasm breast, neutropenia, headache, leptomeningeal disease and hand-foot syndrome) were observed in four patients with one related to capecitabine and one related to both treatments. Four patients required capecitabine dose reductions. Patients received an average of 8.7 treatment cycles with two patients completing all 12 cycles and the remaining stopping early due to extraskeletal disease progression (n = 3) and disease progression plus toxicities (n = 1).

Discussion: As no DLTs were observed and the treatment was generally well tolerated, the Safety Review Committee approved the opening of the randomised extension IIA phase of the study. To date, of the 36 patients required for the extension phase, 12 patients have been recruited and randomised on a 2:1 basis (combination vs capecitabine alone).

DISCLOSURE

Janet Brown: Advisory Boards and Speaker Bureaux for Amgen, Novartis and Bayer

P11

Enrichment and detection of tumor cells in novel models of breast cancer bone colonization

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Breast cancer cells frequently home to the bone, but the mechanisms controlling tumor colonization of the bone marrow remain unclear. This deficit is partially due to the lack of *in vivo* models that recapitulate prolonged tumor latency and our limited ability to detect low levels of tumor burden in the bone. We therefore investigated the ability of three breast carcinoma cell lines (ER+ human MCF7, ER+ murine D2.0R, and ER- human SUM159) to disseminate to the bone following intracardiac inoculation with or without estrogen (E2) supplementation. Using flow cytometric and quantitative PCR approaches, tumor cells were detected in >80% of MCF7 tumor-inoculated mice, regardless of E2 (n = 8/10 mice -E2, n = 7/8 mice +E2), and were significantly enriched in +E2 mice (up to 46-fold, p = 0.0042-0.03). Tumor cells were detected at the histological level in +E2, but not -E2, mice. These data indicate that exogenous E2 is not required for tumor cell dissemination to the bone marrow, but is necessary for tumor cells to grow in and colonize the bone. While MCF7 cells (with E2 supplementation) have been used by multiple groups in bone colonization studies, this study is the first to describe the ability of D2.0R and SUM159 cells to home to bone following intracardiac inoculation. Tumor cells were detected in the bone marrow of up to 100% of D2.0R (n = 9 mice -E2, n = 6 mice +E2) and SUM159 (n = 8 mice)-inoculated mice, depending on the detection method. We propose these cell lines as additional models in which to study prolonged latency periods by bone-disseminated tumor cells, given that D2.0R cells exhibit a time-course similar to the MCF7 model (~7 weeks) and SUM159 cells exhibit a prolonged latency period (13 weeks). Importantly, a distinct advantage of the D2.0R model is that tumor cells are inoculated into immunocompetent mice allowing for the impact of the immune system on tumor dormancy to be investigated. These findings establish novel models of bone colonization in which to study mechanisms underlying tumor cell seeding to the marrow and prolonged latency, and provide highly sensitive methods to detect these rare events.

P12

Panobinostat significantly prevents osteosarcoma progression and metastasis

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Patients that succumb to osteosarcoma (OS), the most common primary skeletal malignancy, typically do so from lung metastatic disease. Disappointingly, overall survival rates for patients with advanced OS have remained static over the past two decades despite the development of several novel therapies for other solid malignancies. We therefore examined a battery of FDA therapies for their ability to impact the growth of OS cell lines and identified Panobinostat, a histone deacetylase (HDAC) inhibitor, has having a broad efficacy across the panel. Using the immunocompetent K7M2 OS model we addressed three questions. 1. Can panobinostat treat primary OS growth and prevent spontaneous metastases? Mice were intratibially inoculated with K7M2-Luc cells, randomized into treatment (n = 12) and control (n = 11) groups. Panobinostat (10mg/kg) or vehicle were administered intraperitoneally for 5 consecutive days followed by 2 days of rest (all *in vivo* studies). Tumor growth was measured by bioluminescence (RLU). Primary OS growth was significantly reduced by panobinostat treatment ($p < 0.05$) with the median time to our clinical endpoint (RLU = 10^6) being 21 days (control) and 53 days (panobinostat) ($p < 0.05$). The time to detection of spontaneous lung metastasis was significantly delayed from 10 days in the control group to 21 days in the panobinostat group ($p < 0.05$). 2. Can panobinostat pretreatment prevent OS lung seeding? Mice were pretreated with vehicle (n = 9) or panobinostat (n = 5) for 5 days prior to tail vein inoculation of $1x10^6$ K7M2-Luc cells. Panobinostat significantly ($p < 0.05$) delayed tumor growth compared to control animals. This translated to a 58% increase in median survival rate, 54 days (control) vs. 93 days (panobinostat) ($p < 0.05$). 3. Can panobinostat treat established lung metastases? Mice were tail-vein inoculated with $1x10^6$ K7M2-Luc cells and randomized after successful engraftment (n = 12/group). We observed panobinostat significantly reduced growth of and number of established OS lung metastases and improved overall survival times ($p < 0.05$). These data suggest FDA approved panobinostat would be useful for the treatment of OS patients. Our current efforts are focused on identifying the specific HDACs responsible for panobinostat to test more specific inhibitors, and understand the genetic program being regulated by HDACs that drive osteosarcoma progression.

P13

BMP4 gene therapy inhibits myeloma tumor growth, but has a negative impact on bone

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Multiple myeloma (MM) is a hematological cancer caused by accumulation of malignant plasma cells in the bone marrow. One of the hallmarks of MM is the loss of bone, caused by increased bone degradation

and reduced bone formation. The latter is due to reduced number of functional, mature osteoblasts. BMP-signaling is important for both pre- and postnatal bone formation. Additionally, several BMPs induce growth arrest and apoptosis in MM cells. Thus, increasing BMP-signaling in MM patients has the potential to both reduce tumor growth and to restore bone formation. We therefore investigated if BMP4 gene therapy influenced tumor growth and bone formation in a human-mouse model of MM.

$RAG2^{-/-}$ $GC^{-/-}$mice were implanted with calcium phosphate scaffolds seeded with human mesenchymal stromal cells and allowed to differentiate *in vivo* for 8 weeks. Then, adeno-associated virus serotype 8 expressing BMP4 under control of a liver specific promotor (AAV8-BMP4) or control vector (AAV8-CTRL) were administered by tail-vein injection. After 2 weeks, when BMP4 can be detected in the circulation, fluorescently labelled MM cells were injected into the scaffolds. Tumor growth was examined by imaging every week. Strikingly, tumor growth was significantly reduced in AAV8-BMP4 mice compared with the AAV8-CTRL mice ($p < 0.01$), suggesting that increased circulating levels of BMP4 reduced tumor growth. However, bone formation was not increased in scaffolds of the AAV8-BMP4 mice. At end of the experiment, circulating serum levels of BMP4 in the AAV8-BMP4 mice were 50-200ng/ml.

To delineate the effects of BMP4 overexpression on bone per se, without direct influence from the cancer cells, we examined the femurs by μCT. Surprisingly, the AAV8-BMP4 mice had significantly reduced trabecular bone volume ($p = 0.017$), trabecular numbers ($p = 0.016$) as well as significantly increased trabecular separation ($p < 0.001$) compared with the AAV8-CTRL mice. Thus, high levels of circulating BMP4 seem to inhibit trabecular bone formation. There was no difference in cortical bone parameters.

Taken together, BMP4 gene therapy inhibited MM tumor growth, but also reduced trabecular bone formation in mice. Care should therefore be taken when considering using BMP4 as a therapeutic agent.

P14

ABSTRACT WITHDRAWN

P15

Myeloid Derived Suppressor Cells promote Multiple Myeloma cell survival by AMPK activation

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Multiple Myeloma (MM) is an incurable malignancy of terminally differentiated plasma cells, which are predominantly localized in the bone marrow. Myeloid derived suppressor cells (MDSC), a heterogeneous population of immature myeloid cells, are thought to promote MM progression by secretion of cytokines and growth factors, suppression of the immune system and induction of angiogenesis. The presence and activation of MDSC in MM patients has been well-documented, however their direct role in drug resistance and cell survival is still unclear. In this study, we performed coculture experiments of 5TMM derived MDSC with 5TMM cells *in vitro*, resulting in increased survival and proliferation of MM cells. These cocultures also resulted in a protection against drug-induced apoptosis of MM cells by bortezomib (2.5-5nM) or melphalan (15-30μM). To investigate underlying pathways, we performed a Pathscan® Intracellular Signaling Array demonstrating an upregulation of AMPK (Adenosine Monophosphate-activated Protein Kinase) phosphorylation in 5TMM cells after coculture with MDSC. As a cellular energy sensor, AMPK regulates lipid and glucose metabolism and could be essential in cancer cell survival during stress conditions. AMPK activation was confirmed by western blot and we could observe an increase in anti-apoptotic factors Mcl-1 and Bcl-2, and autophagy-marker LC3II. In addition, 5TMM cells were inoculated and showed a clear upregulation of AMPK phosphorylation *in vivo*, while 5TMM cells cultured *in vitro* had a reduced AMPK expression. AMPK targeting by BML-275 (Compound C) resulted in apoptosis and reduced viability of human myeloma cell lines (LP-1, RPMI-8226, U266), primary MM cells and 5TMM cells. Importantly, we observed that the tumor-promoting effect of MDSC was partially mediated by AMPK activation. More mature cell types like macrophages and fibroblasts were not able to induce AMPK phosphorylation. In conclusion, our data clearly demonstrate that MDSC directly increase the survival of MM cells and that this effect was partially mediated by AMPK phosphorylation, identifying this pathway as a new target in the treatment of MM patients.

P16

The role of serum vitamin-D and bone turnover markers in prognosis of bone metastasis and prediction of benefit from adjuvant zoledronic acid in patients with early breast cancer.

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40,000 women die from breast cancer (BC) annually in the US alone, mainly from distant relapse, which often occurs years after initial BC diagnosis. Because bone metastases ultimately affect more than two-thirds of patients with advanced disease, biomarkers related to bone metabolism such as vitamin-D and bone turnover markers may have value in identifying patients with early BC who are at high risk of relapse in bone.

The AZURE clinical trial recruited 3360 patients with early BC at moderate/high risk of recurrence, randomised 1:1 to receive standard adjuvant therapy +/- zoledronic acid (ZOL). Serum samples at study entry were collected from UK patients (441 control arm, 431 ZOL arm) for measurement of vitamin-D and bone turnover markers. Associations between biomarker levels and distant relapse events (median follow-up 84.2 months) were explored using Cox proportional hazards regression, adjusted for lymph node involvement, ER status, tumour stage and type/timing of systemic therapy.

The overwhelming majority of women in this study (91.2%, control; 87.7%, ZOL) had deficient levels of vitamin D (< 30ng/ml, a level considered necessary for good bone health). There was no difference in the proportion of patients with baseline values < 30ng/ml by menopausal status. In categorical analyses of normal (≥ 30ng/ml) vs low (< 30ng/ml), vitamin-D was prognostic for distant metastasis at any site ($p = 0.038$) and approached significance for bone metastasis at any time ($p = 0.067$).

In log transformed continuous analyses, the bone formation marker N-terminal propeptide of type-1 collagen (P1NP) and the bone resorption markers C-telopeptide of type-1 collagen (CTX) and pyridinoline cross-linked carboxy-terminal telopeptide of type-1 collagen (1-CTP) were each significantly associated with relapse in bone at any time ($p = 0.006$; $p = 0.009$; $p = 0.008$ respectively), but not with first relapse in bone only ($p = 0.411$; $p = 0.320$; $p = 0.190$ respectively). None of the bone turnover markers was prognostic for overall distant recurrence i.e. they were bone metastasis-specific and none were predictive of treatment benefit from ZOL.

Bone turnover markers in early BC are prognostic for future relapse in bone. Adequate vitamin-D at breast cancer diagnosis may signal protection against future distant relapse.

DISCLOSURE

Janet Brown. Speaker Bureaux and Advisory Boards with Amgen, Novartis, Bayer Robert Coleman. Research grants, Amgen, Bayer. Expert testimony, Novartis. Lecture fees, Amgen. Walter Gregory. Statistical Consultancy,

Cellgene, Honoraria, Janssen David Cameron. Non-financial support, Novartis.

P17

Lysyl oxidase promotes survival and outgrowth of cancer cells in the bone marrow, enabling bone metastasis formation.

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Lysyl oxidases LOX and LOXL2 are secreted copper-dependent amine oxidases whose primary function is to drive collagen crosslinking and extracellular matrix stiffness. LOX in colorectal cancer (CRC) synergizes with hypoxia-inducible factor-1 (HIF-1α)) to promote tumour progression. Here we investigated whether LOX/HIF1-α endows CRC cells with full competence for aggressive colonisation in bone. We show that a high LOX expression in primary tumours from CRC patients was associated with poor clinical outcome, irrespective of HIF-1α. Additionally, LOX was expressed by tumour cells in the bone marrow from CRC patients with bone metastases. In vivo experimental studies show that LOX overexpression in CRC cells or systemic delivery of the conditioned medium from LOX-overexpressing CRC cells promoted tumour cell dissemination in the bone marrow and enhanced osteolytic lesion formation, irrespective of HIF-1α. Conversely, silencing or pharmacological inhibition of LOX activity blocked dissemination of CRC cells in the bone marrow and tumour-driven osteolytic lesion formation. In vitro, tumour-secreted LOX supported the attachment and survival of CRC cells to and in the bone matrix, and inhibited osteoblast differentiation. LOX overexpression in CRC cells also induced a robust production of IL-6. In turn, both LOX and IL-6 were acting in concert to stimulate osteoclast differentiation, thereby creating an imbalance between bone resorption and bone formation. Collectively, our findings show that LOX supports CRC cell dissemination in the bone marrow and they reveal a novel mechanism through which LOX-driven IL-6 production by CRC cells impairs bone homeostasis. We are now conduing experiments in the breast cancer model, showing that both LOX and LOXL2 endow the tumor cells with the ability to thrive in the bone marrow microenvironment.

P18

Guidelines for the assessment and management of prostate cancer treatment-induced bone loss. A consensus position statement from a UK expert group.

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One in 8 men will receive a prostate cancer (PC) diagnosis during their lifetime. As a result of androgen deprivation therapy (ADT) and newer therapies, survival rates have improved considerably over the past four decades (current 5- year survival is 85% in all patients), so many patients now live with their disease for many years. However, cancer treatment induced bone loss (CTIBL) and the potential for osteoporosis has become a substantial issue and updated guidance on management of CTIBL is required.

This guidance was developed by a group of experts in the UK, identified from key opinion leaders in the management of PC and bone disorders and patient representatives. Input and endorsement was also sought from a range of specialist societies. A systematic literature search was undertaken using PubMed and Ovid MEDLINE databases. Randomised controlled trials, observational studies and meta- analyses were included for assessment.

Although national and international guidelines make recommendations that fracture risk is considered for all men with PC receiving ADT, they lack specific guidance for the management of bone health. Specialist societies have proposed useful guidelines suggesting that BMD assessment is undertaken prior to the initiation of long-term ADT, and that the FRAX tool should be used to estimate individual fracture risk. However, there is currently no guidance as to the intervention thresholds that should be used to initiate treatment, or the most appropriate bone targeted therapy or who should have overall responsibility for managing bone health in this group of patients.

Recommendations: All men starting long-term ADT for PC should:

1. Be provided with individualised and patient-centred information, including appropriate lifestyle advice regarding optimisation of bone health
2. Be referred to a supervised resistance and aerobic exercise programme of at least 12-weeks duration (in accordance with NICE guidelines)
3. Achieve or maintain adequate daily calcium (at least 1200mg) and vitamin D (800-1000 IU) intake through dietary intake, sunlight exposure, and supplementation
4. Have the 10-year probability of major osteoporotic and/or hip fracture assessed using FRAX® (without BMD initially) https://www.sheffield.ac.uk/FRAX/tool.jsp; including the presence of secondary osteoporosis as a risk factor.

P19

Computational modeling of macrophage polarization dynamics in skeletal malignancies. An integrated *in silico* and *in vivo* approach

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Tumor-associated macrophages are involved with the progression of various primary malignancies. Myeloid-derived monocytes and macrophages, that can polarize into pro- and anti-inflammatory phenotypes, are abundant in bone. Roles for these cell types in regulating bone injury repair have been described. Surprisingly, little is known about how polarization states and temporal dynamics impact osteoblast and osteoclast activity in skeletal malignancy; a gap in our knowledge that is difficult to address with traditional biological approaches. Previously, we have shown that data-driven computational modeling, combined with *in vivo* experimentation/validation, can be used to understand the dynamics of cancer cell-bone stroma (osteoblast/osteoclast) interaction. Here, we integrate empirical and published data on macrophage polarization states during bone remodeling into an ordinary differential equation (ODE)-based models. To this end, intratibial injuries were performed on C57BL/6 mice and bone marrow flushes were profiled by FACS analysis at days 0, 1, 2, 3, 7 and 14 (n = 5/timepoint) for pro- and anti-inflammatory myeloid content (CD11b, Ly6G, Ly6C, NOS2 and ARG1). Contralateral tibias collected at the same timepoints were histologically assessed for bone volume (μCT), osteoblast (Runx2), and osteoclast (TRAcP) numbers. The collated data was used to power ODE population-based models. The ODE model captures dynamic shifts in macrophage phenotypes during bone repair and their interaction with osteoclasts and osteoblasts that were in agreement with biological data. Model simulations and data fitting also revealed insights into inflammatory monocyte-macrophage sources and their viability/differentiation dynamics. Further, altering pro- or anti-inflammatory macrophages had profound effects on bone repair times. Having generated an ODE that takes into account macrophage

dynamics during normal bone repair, we are now assessing macrophage polarization phenotype and quantity over time in immunocompetent models of common skeletal malignancies such as multiple myeloma (5TGM1) and bone-metastatic prostate cancer (RM1). We are integrating these parameters to examine the effects of macrophage behavior on cancer-associated bone disease and response to applied therapeutics.

P20

ABSTRACT WITHDRAWN

P21

Host-derived matrix metalloproteinase-13 contributes to the progression of multiple myeloma

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Multiple myeloma promotes systemic skeletal bone disease that greatly impact the patient's quality of life. Processing of the type I collagen-rich bone matrix results in the release of sequestered growth factors that can drive progression of the disease. Matrix metalloproteinase 13 (MMP-13) is a type-I collagenase that is expressed predominantly in the skeleton by mesenchymal stromal cells (MSCs). Analysis of public datasets demonstrate that myeloma significantly induces the expression of MMP-13 in MSCs (1.81 LogFC, $p < 0.05$.) Consistent, with this observation, immunofluorescent analyses of human myeloma biopsies also demonstrated MMP-13 expression in bone-lining osteoblasts but interestingly, not in bone-resorbing osteoclasts. We therefore determined whether host-derived MMP-13 could contribute to myeloma progression and overall survival. Immunocompromised mice, wild type or null for MMP-13, were inoculated with luciferase-expressing 5TGM1 myeloma cells ($n = 8$/group). Using bioluminescence, IgG2b and immunohistochemistry as a readout for tumor burden, we detected no difference in myeloma growth rate. Surprisingly, we did observe a significant increase in overall survival in the MMP-13 null group (mean 39 vs. 43 days; $p < 0.05$.) *Ex vivo* analysis by high resolution μCT, normalized to age-matched control mice, demonstrated that myeloma-induced bone resorption was significantly reduced in the MMP-13 null mice (0.5 vs. 0.23 ratio; $p < 0.05$). Analysis of stromal co-cultures identified that MMP-13 null osteoclast formed more slowly than their wildtype counterparts with reduced functionality (24% reduction in resorption of a bone mimetic, $P < 0.05$.) Exploration of wildtype and MMP-13 null MSC-derived condition media indicate a number of potential substrates through which MMP-13 may be contributing to osteoclast formation and function. We further confirmed effects of MMP-13 ablation in multiple myeloma progression using a novel selective MMP-13 exosite inhibitor, RF-36. Mice ($n = 10$/group) were injected with 5TGM1 luciferase-expressing cells and received either vehicle or RF-36 (daily treatment at 2mg/kg). Our data demonstrate MMP-13 inhibition significantly delayed myeloma growth. Although recent reports have highlighted non-catatlytic roles for MMP-13 in myeloma mediated osteoclastogenesis, our data suggest that inhibition of MMP-13 activity remains a viable approach for the treatment of the disease.

P22

Imaging upregulated cell surface proteins, altered tumor metabolism and structural bone changes in multiple myeloma

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Multiple myeloma (MM) is a cancer of terminally differentiated plasma B-cells in the bone marrow (BM). The BM niche is a complex interplay of vasculature, bone remodeling cells, stroma, and other cells, including adipocytes and plasma cells. In the presence of myeloma cells, however, the highly regulated BM niche is disrupted. This leads to the increase in osteoclast activity, destruction of the cortical bone, reduction of constitutive marrow adipose tissue relative to the red marrow, increase of vascularization and altered metabolism within the BM. The changes in BM architecture and composition facilitate tumor survival, progression and drug resistance. Myeloma cells also differentially express cell surface proteins such as very late antigen-4 and CD38 to actively interact with the BM microenvironment for progression and survival. In this presentation, I will highlight the use of nuclear, optical and magnetic resonance imaging techniques with molecularly targeted agents for understanding myeloma progression in pre-clinical mouse models of MM. Specifically, imaging of very late antigen-4 and CD38 with positron emission tomography (PET) and near-infrared optical imaging will be presented. To demonstrate the unique metabolic signatures of MM, we will present our work on L-type amino acid transporter-1 (LAT1) targeted imaging with ^{18}F-FDOPA-PET and its complimentary role with the clinical standard ^{18}F-FDG-PET imaging. Lastly, the utility of targeted iron-oxide nanoparticles for magnetic resonance imaging of MM will be demonstrated.

P23

The effects of cathepsin K inhibition on osteocytes: its role in bone restoration in MM bone disease

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Multiple myeloma (MM) develops devastating bone destruction by enhanced osteoclastic bone resorption and impaired bone formation. The bone disease not only deteriorates QOL in MM patients but also aggravates MM tumor expansion. In order to improve the therapeutic efficacy for MM, we need to develop novel therapeutic anti-MM modalities to ameliorate the comorbidity associated with the skewed bone marrow microenvironment. In this regard, proteasome inhibitors have drawn considerable attention to their anabolic actions on bone formation in good responders. Cathepsin K (CatK) inhibitors potently suppress bone resorption while sparing cytotoxic damage in osteoclasts (OCs) to retain their coupling to osteoblastogenesis. We previously demonstrated that CatK inhibitor increased bone formation in mouse MM models partly through enhance coupling of OCs to osteoblastogenesis. Osteocytes, the most abundant cells in bone, are regarded as a major source of sclerostin, a potent Wnt inhibitor to suppress osteoblastogenesis. In the present study, we explored the therapeutic efficacy of CatK inhibition for MM bone diseases, focusing on its effect on osteocytes. We prepared mouse models with MM bone destruction by intra-tibial inoculation of 5TGM1 MM cells. OCs strongly expressed CatK in the tibiae with MM bone disease. Interestingly, osteocytes also expressed CatK along with screlostin. MLO-A5 and MLO-Y4, osteocytic cell lines, were found to express CatK and sclerostin, and further enhanced their expression in co-culture with MM cells. Intriguingly, the treatment with CatK shRNA or the CatK inhibitor ONO-5334 suppressed sclerostin expression and production by MLO-Y4 cells. Furthermore, sclerostin levels in sera were increased in the MM models. However, oral administration of ONO-5334 alone reduced serum sclerostin levels, and prevented bone destruction with an increase in BMD in the MM-bearing mice. Importantly, ONO-5334 was able to further reduce the sclerostin levels and increase bone formation while almost completely eradicating MM in combination with bortezomib. These results suggest

that downregulation of sclerostin production by osteocytes may contribute to the bone anabolic effects of CatK inhibition. CatK inhibitors and proteasome inhibitors appear to be a beneficial therapeutic combination for MM with bone destruction.

P24

Human osteoclasts generated from different individuals show a highly variable sensitivity to zoledronic acid *in vitro* – this sensitivity relates to *in vivo* characteristics of each individual

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Zoledronic acid (Zol) is used to treat cancer-induced bone disease. In general, Zol significantly reduces the risk of skeletal related events, but not for all patients. In our ongoing clinical trials, we observe that CTX levels after three months of Zol-treatment varies by 26- (from 0.034 to 0.899 ng/ml) and 25-fold (from 0.04 to 1.0 ng/ml) in two cohorts of breast and prostate cancer patients with bone metastases ($n = 22$, $n = 9$), respectively. In our present study we wish to investigate if part of this variable sensitivity may be due to intrinsic differences in Zol sensitivity of osteoclasts between individuals. In an ongoing protocol, we have isolated peripheral blood CD14+ monocytes from female blood donors ($n = 36$; ages 40 to 66) and have differentiated these cells into osteoclasts *in vitro*. Demographic data, CTX and PINP-levels *in vivo* were obtained. The resorptive activity of osteoclasts was quantified and their sensitivity to Zol determined. Multiple linear regression analyses show that the total resorptive activity of osteoclasts *in vitro* is positively correlated to donor characteristics (age $p = 0.015$; years since menopause $p = 0.028$; PINP $p = 0.045$). This suggests that characteristics of the donors can be traced in *in vitro* experiments. We also find that the sensitivity of osteoclasts to Zol *in vitro* varies by up to 140-fold (IC50: 0.09 to 12.6 μM). Multiple linear regression analyses show that some of this variation significantly correlates with *in vivo* characteristics of donors (PINP $p = 0.025$; smoking status $p = 0.015$) explaining up to 34% of the variation. The IC50 increases by 2.6% per unit increase of PINP (range: 23.01 to 100.3 μg/L) and by 284% from non-smoker to smoker. Furthermore, osteoclasts resorb bone in two modes - osteoclasts in trench-mode are more sensitive to Zol than those in pit-mode ($p = 0.001$) and it is only those in trench-mode that respond to Zol according to *in vivo* characteristics. We conclude that the sensitivity of osteoclasts to Zol *in vitro* is influenced by *in vivo* characteristics of the donors.

We raise the hypothesis that the variable sensitivity of cancer patients to Zol could partly be due to intrinsic (epigenetic?) differences between patients.

P25

Investigating the osteoblast-breast cancer cell interaction at early stages of bone metastasis

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Osteoclast activation is the cellular hallmark of breast cancer-induced osteolytic bone disease. However, based on the finding that osteoblast conditioned medium stimulates breast cancer cell (BCC) migration *in vitro* ($p < 0.01$), we hypothesize that osteoblasts are also playing a role during early stages of bone metastasis.

We identified the homeodomain protein TG-interacting factor-1 (Tgif1) as a strongly up-regulated molecule in osteoblasts upon stimulation by BCCs, suggesting a potential role of Tgif1 in the osteoblast-BCC interaction. Indeed, conditioned medium from osteoblasts of mice that have a germline deletion of Tgif1 (*Tgif1*$^{-/-}$) failed to induce BCC migration compared to control medium obtained from wild-type osteoblasts ($p < 0.05$), indicating that Tgif1 in osteoblasts might support the activation of BCC movement. To better understand this observation, we performed SILAC and RNA-seq analyses using *Tgif1*$^{-/-}$ osteoblasts. Interestingly, Semaphorin 3E (Sema3E), a negative regulator of cell migration, was abundantly secreted by *Tgif1*$^{-/-}$ osteoblasts. These findings suggest that in osteoblasts Tgif1 may suppress Sema3E expression, thereby supporting BCC migration.

To test the hypothesis that Tgif1 contributes to the initiation of metastatic bone disease *in vivo*, we employed a syngeneic metastasis model in Tgif1-deficient mice. In support of our hypothesis, ongoing studies revealed a decreased number of bone metastases in *Tgif1*$^{-/-}$ mice compared to littermate controls (33% vs. 64%, respectively) determined by bioluminescence imaging 7 days after intracardiac injection of 4T1-*GFP-Luc* BCCs. Consistently, the presence of single BCCs or micro-metastases in the tibiae was reduced by 25% in *Tgif1*$^{-/-}$ mice determined by confocal microscopy. Further histological analyses demonstrated that BCCs localize in close proximity to Endomucin-positive vascular cells as well as to osteoblasts. Although Tgif1-deficiency did not affect the bone marrow vasculature, the number and activity of osteoblasts were reduced compared to littermate controls ($p < 0.05$). This suggests that the protective effect on bone metastases might be mediated by the osteoblasts and not by the bone marrow vasculature.

In summary, we propose that lack of Tgif1 in osteoblasts is protective against cancer cell migration and metastasis formation, possibly through the suppression of Sema3E. Thus, our findings establish osteoblasts as important regulators during early stages of bone metastasis.

P26

Defining the role of P2X7 receptor in the dormant population of prostate cancer cells

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Prostate cancer (PCa) most commonly metastasizes to the bone, causing considerable morbidity and mortality. Bone metastases could occur several years after the removal of primary tumours, indicating a longer tumour dormancy during PCa metastasis. Our recent studies have suggested that the expression of P2X7 receptor (P2X7R) is transcriptionally up-regulated in the dormant metastasis initiating cells, while others suggested that P2X7R mediates the status of invasiveness/EMT of PCa cells. Therefore, we hypothesize that P2X7R plays a pivotal role in the induction of PCa dormancy.

To test whether the P2X7R is functionally up-regulated in dormant PCa cells and whether genetically knocking out P2X7R will affect tumour dormancy.

Dormant PCa cells (PC3 and C4 2B4 cells) were isolated using a method developed previously based on their ability to retain fluorescent lipophilic dyes when not dividing *in vitro*. The functional expression of P2X7R was assessed using an ethidium uptake assay (membrane pore formation) under stimuli of exogenous P2X7R agonist BzATP and/or antagonist A740003. After genetically depleting P2X7R in PCa cells with CRISPR/Cas9 technique (targeting exon 13, a C-terminal domain critical for function), alterations in frequency of dormant population, function of P2X7R and proliferation/viability of PCa cells were then examined *in vitro*, using FACS, ethidium uptake assay, MTS and Glo MT assay, respectively.

Dormant populations of both PCa cell lines showed increased pore formation in response to exogenous BzATP (Linear regression comparison of 30 minutes, $n = 3$, $p < 0.001$), suggesting enhanced functional expression of P2X7R in dormant PCa cells. Successfully disrupting the P2X7R by CRISPR/Cas9 led to complete abolishment of pore formation

in both cell lines and altered frequency of dormant tumour cells (t-test, $n=3$, $p<0.05$), without affecting the proliferation and viability (t-test, $n=3$, $p>0.05$).
Our data supports the hypothesis that the P2X7R plays an important role in PCa cell dormancy and warrants further studies to exploit its pharmaceutical potentials for fighting PCa bone metastasis.

P27

Characteristics of the stromal cells in the premetastatic and hematopoietic niches

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Dormant cancer cells in the bone marrow apparently use the same niche as the hematopoietic stem cells. Furthermore, stimulating stromal cells of osteoblastic origin increases the number of hematopoietic stem cells, and possibly their niches. We aimed to characterize the stromal constituents of the niche and determine whether they are similar in the hematopoietic and the premetastatic niche.
We used a murine model of homing, where cancer cells are injected intracardially and their number in the bone marrow is quantified (Cancer Research 2017). Decreasing the number of stromal cells in the bone marrow pharmacologically enhanced cancer cell homing to the bone marrow. In contrast, an increase in the number of stromal cells in transgenic mice (in which b1 integrin is deleted in the marrow) or by administering G-CSF diminished cancer cell homing. To establish causality between changes in stromal cells and homing we injected stromal cells expressing Sca1 intravenously and found a decrease in cancer cell homing. Taken together these data suggest an inverse relationship between stromal cells and cancer cell homing. Using multiple regression analysis we characterized a subpopulation expressing $Sca1^+CD31^+CD146^+CD44^-$ that diminishes whenever cancer cell homing is increased. In prostate and breast cancer patients, related subpopulations were lower in the presence of cancer cells in the bone marrow.
In order to determine whether the subpopulation identified is present in the hematopoietic stem niche, we diminished the subpopulation in mice pharmacologically and depleted hematopoietic stem cells using either radiation or chemotherapy. After bone marrow transplantation, survival improved and recovery from leukopenia was faster in mice in which stromal cells were diminished compared to untreated controls. Recovery of thrombocytes was even more pronounced than controls treated with parathyroid hormone after chemotherapy. This suggests that the stromal cell subpopulation also represents part of the hematopoietic stem cell niche.
Taken together, a subpopulation of stromal cells with stem cell characteristics and vascular potential in the bone marrow unequivocally constitutes part of the premetastatic niche, and is possibly shared with the hematopoietic niche. Furthermore, manipulating this niche might offer a new possibility for decreasing metastasis formation.

P28

Learning from osteoblasts how to fight cancer

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Osteoblasts lining the inner surface of bone are in proximity to the bone marrow and affect differentiation of several hematopoietic cell types. Osteoblasts also modify their own differentiation by producing various fibronectin isoforms. We therefore asked whether fibronectin from osteoblasts modifies the immune response against cancer, and whether the lessons learned could be applied to suppress cancer growth.
Deletion of osteoblast fibronectin in mice (conditional-Knockout: cKO) suppressed both myelopoiesis and growth of breast cancer bone lesions in two models (PLoS Biology 2016). Lower myeloid cell numbers were detected in cKO tumors. To establish a causal relationship between myeloid cells and cancer growth, adoptive transfer experiments were performed. Myeloid cells from cKO mice diminished cancer growth compared to myeloid cells from controls, suggesting a difference in their behavior towards cancer. Osteoblasts produce several fibronectin isoforms. Nevertheless, only exposure to EDA-containing fibronectin changes myeloid cell behavior in adoptive transfer experiments. In vitro experiments showed that only EDA-fibronectin increased arginase I (an inhibitor of the immune response to cancer) by acting on a5b1-integrin, while the classical ligand failed to affect a similar change. In vivo experiments confirmed that interfering with binding of EDA-fibronectin to its receptor diminishes cancer growth.
Tissue sections of breast cancer lesions showed high EDA-fibronectin expression. We therefore hypothesized that EDA-fibronectin production by breast cancer cells suppresses the immune response and increases cancer growth. Bone lesions induced in mice using a knockdown of EDA-fibronectin in MDA-MB-231 were smaller and contained less myeloid cells than controls. In these cells arginase I was decreased similarly to cKO mice. To test whether these myeloid cells not exposed to EDA locally were responsible for diminished cancer growth, they were depleted using liposomes containing clodronate. Cancer growth increased compared to mice receiving empty liposomes.
Thus, EDA-fibronectin both originating from the osteoblasts and locally produced in cancer inhibits the immune response against cancer. In its absence, cancer growth is diminished. Since this is mediated by a unique action of EDA-fibronectin on a5b1-integrin, developing a specific a5b1-integrin modulator offers a new possibility to enhance the immune response against cancer and diminish cancer growth.

P29

Myeloma-specific oncolytic adenovirus induces significant tumour oncolysis *in vitro* and *in vivo* and prevents cell line regrowth.

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Multiple myeloma is a largely incurable disease and despite current therapies achieving good initial responses, patients frequently relapse. Therefore, new approaches are required that not only reduce the tumour load, but also prevent the growth of residual disease. One such approach is the use of oncolytic viruses. We developed an oncolytic adenovirus that utilizes transcriptional control of E1A under the myeloma-specific promoter CS1 (ADCE1A). We hypothesised that ADCE1A would be myeloma-specific, inducing cell death and preventing tumour regrowth.
Myeloma cell lines (JJN3, U266 and 5TGM1) and a melanoma cell line (MDA-MD-435) were assessed for CS1 expression by flow cytometry (FC). Myeloma cell lines (JJN3, U266, OPM2 and 5TGM1), primary plasma cell leukaemic cells, MDA-MD-435 cells, and normal healthy cells were infected with ADCE1A (MOI 20) and cell death was monitored after 4 days using FC and propidium iodide staining. Apoptosis and autophagy were assessed following viral infection using qPCR and annexin V staining. Myeloma cell regrowth *in vitro* was assessed after bortezomib (0.56-2.81nM) or bortezomib and ADCE1A treatment. Viral efficacy was tested in a xenograft model of myeloma, where 5 weeks after tumour cell injection (10^6 U266 cells intravenously), mice were treated with ADCE1A ($1x10^7$ pfu, 2x/wk) or control (PBS) for 3 weeks. Tumour burden was measured *ex vivo* in the long bones by FC.
CS1 was expressed in all myeloma cell lines, but not in MDA-MD-435 cells. ADCE1A infection induced significant increases in cell death in all myeloma cell lines and primary cells, reaching greater than 90% ($p<0.001$), whilst not affecting MDA-MD-435 or normal healthy cells. There was no increase in expression of FASL (apoptotic marker), ATG5, LC3B (autophagy markers) or annexin V positivity at 8h and 24h post

infection compared to control cells. Interestingly in preliminary experiments, ADCE1A prevented regrowth of myeloma cell lines following treatment with bortezomib *in vitro*. In the U266 xenograft model, tumour load was significantly reduced ($p < 0.05$) compared to control treated mice.
In conclusion, ADCE1A has significant anti-myeloma effects that are not mediated via apoptosis. We are currently investigating.

P30

FACS-based isolation of primary and metastatic osteosarcoma cells in mice: a new tool to allow downstream molecular analysis and target identification

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The survival of patients with osteosarcoma (OSA) has not improved substantially over the last 20 years, despite the evolutions in surgical intervention and chemotherapeutics. Current knowledge of the genetic drivers of osteosarcoma progression is very limited. Lung metastasis is the main cause of morbidity and mortality, and over 80% of human and canine cases have clinically undetectable microscopic metastasis at diagnosis. Progression to metastatic disease limits the 5-year survival rates to only 60-75%. Murine models are invaluable tools to study disease progression and pathogenesis. A number of canine and human OSA cell lines have been characterized *in vitro* and *in vivo*, but a widely accepted orthotropic mouse model of OSA with reliable metastasis has not been established. We have re-evaluated and optimised a clinically relevant orthopic bioluminescent mouse model system that allows rapid isolation of key biological processes and effective evaluation of therapeutic agents in In Vivo. We have utilised the model to gain better understanding of the dynamics of metastatic spread by modelling the growth of the primary tumour, detecting circulating tumour cells and quantifying tumour burden in the lung by FACS sorting. Ultimately, this clinically relevant in-vivo model of osteosarcoma growth and metastasis will allow research into novel molecular drivers of disease which can potentially be used as both prognostic markers and as targets for biological and/or drug therapies.

P31

ABSTRACT WITHDRAWN

P32

ABSTRACT WITHDRAWN

P33

Inhibition of p62-ZZ Domain-Mediated Signaling in Myeloma Bone Disease Induces Osteoblast Differentiation and Overcomes Bortezomib Resistance.

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Multiple myeloma bone disease (MMBD) is characterized by bone lesions that do not heal even when the patients are in complete remission due to the persistent suppression of osteoblast differentiation. We previously reported that MM cells induce persistent changes in MM patient bone marrow stromal cells (BMSC) via epigenetic repression of *Runx2-P1* promoter induced by the transcriptional repressor Gfi1, thereby preventing osteoblast differentiation. We found that the p62-ZZ domain in BMSC was critical for the formation of MM-induced signaling complexes that mediate osteoblast suppression and identified XRK3F2 as an inhibitor of the p62-ZZ domain. ChIP-qPCR analyses showed that XRK3F2 both prevented and reversed MM-induced Gfi1 occupancy at the *Runx2-P1* promoter, thus preventing or reversing MM-induced decreases in the chromatin activation mark H3K9Ac at the *Runx2-P1* promoter. XRK3F2 partially relieved the block in differentiation of BMSC induced by MM cells allowing them to increase OB marker gene expression and to mineralize. Finally, the effects of XRK3F2 were potentiated when combined with the proteasome inhibitor (PI), Bortezomib (BTZ), a standard anti-MM drug with potential bone anabolic effects. XRK3F2 treatment of stromal cells also blocked TNFα-induced upregulation of Gfi1 and suppression of OB differentiation, effects that were further increased by BTZ.
We previously showed that XRK3F2 induced dramatic new cortical bone formation in mice with established MM but had no effect on tumor burden *in vivo*. We wonder if XRK3F2 could enhance BTZ-induced MM cell cytotoxicity and overcome BTZ resistance. We found that the XRK3F2-BTZ combination significantly reduced MM cell viability compared with either drug alone at the same doses, and increased MM cells sensitivity to BTZ, without affecting OB viability. Mechanistic studies revealed that XRK3F2-BTZ combination significantly increased annexin V in MM cells, reduced expression of the anti-apoptotic Bcl-2 protein, extensively activated caspase 3 and cleaved PARP1.
These data suggest that p62-ZZ antagonists in combination with PIs, have both anti-tumor and bone anabolic effects, and may overcome PI resistance in MM cells, making p62 an attractive target for the treatment of MMBD.

DISCLOSURE

GDR is a consultant for Amgen Denosumab Trial: Member on an entity's Board of Directors or advisory committees. XQX is a consultant for Oxis Biotech and ID4Pharma Founder

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The Role of Receptor Activity Modifying Protein 1 in Prostate Cancer.

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Prostate cancer (PCa) affects 1 in 8 men in the UK and metastasis to bone can lower prognosis by ~70%. Receptor activity modifying protein 1 (RAMP1) is a single transmembrane protein that interacts with G protein-coupled receptors (GPCRs) including the calcitonin receptor (CTR) and calcitonin-like receptor (CLR). Calcitonin gene related peptide (CGRP) binds to the RAMP1-CLR complex and has been shown to be increased in the serum of PCa patients. RAMP1 has also been identified as a potential biomarker of PCa as it is upregulated in prostate tumour tissue compared with healthy tissue. To determine the role of RAMP1 in PCa we have used CRISPR/Cas9 to generate RAMP1 knockouts in PC3 cells.
PC3 cells were co-transfected with a CRISPR/Cas9 and homology-directed repair (HDR) plasmid. Cas9 induced a double stranded break in the RAMP1 DNA, incorporating the HDR insert (containing an RFP marker and stop codon) stably into the gene. RFP labelled cells were sorted using FACS and successful RAMP1 knockouts were validated using PCR. RAMP1 knockouts were tested *in vitro* using assays for viability, migration, invasion, colony formation and apoptosis and then used *in vivo* in a xenograft mouse model.
Successful RAMP1 knockout was detected with endpoint and qPCR and confirmed with sanger sequencing and DD CT analysis. RAMP1 KO cells had 58% reduced viability compared with PC3 wildtype ($P = < 0.0001$) and caspase 3/7 activity was increased two-fold ($P = < 0.0001$). The ability of RAMP1 KO cells to adhere to ECM proteins

was reduced 33% (P = 0.01) and invasion through Matrigel membranes was decreased by 92% compared with PC3 WT (P = 0.005). Subcutaneous injection of RAMP1 knockout cells into nude mice resulted in a 90% reduction in tumour volume and mass compared with PC3 WT cells (P = <0.0001). IHC analysis showed structural differences between RAMP1 KO and WT tumours. However, no significant differences were seen in staining for the proliferative marker Ki67.
Deletion of RAMP1 reduced the tumorigenic ability of PC3 cells *in vitro* and *in vivo,* indicating an important role for RAMP1 in PCa and its inhibition may potentially prevent PCa metastasis to bone.

P35

Investigating the influence of bone marrow adipocytes on breast cancer metastasis

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Breast cancer (BC) is the most common cancer affecting women, with metastasis to the bone presenting the majority of secondary cancer development. Clinical consequences of BC metastasis to bone significantly impacts patient survival and quality of life. The microenvironment that the bone provides is an important factor in influencing metastasis, colonization, proliferation and dormancy/activity of breast cancer cells (BCCs) and response to treatment. However the molecular mechanisms underlying these processes, specifically the role of bone marrow adipose tissue (BMAT) are not fully understood. Here, we examined the effects of co-culturing BMAT with BCCs to study the influence of bone marrow adipocytes on BCCs activity and dormancy.
We used an indirect co-culture system either using conditioned media from adipocytes on BCCs or co-culturing adipocytes and BCCs in a transwell system. Adipocytes were derived from differentiated murine Bone Marrow Stromal (ST2) Cells and the BC cell lines cover the following subtypes: MCF7 (Estrogen receptor positive), BT-474 (Estrogen receptor and HER2 positive), MDA-MB-231 and SUM-159 (both triple negative).
Conditioned media from ST2 or adipocytes collected at increasing time points (24, 48 and 72h) applied to 2D cultured BCCs showed decreased proliferation in MCF7 and BT-474 cells, whereas MDA-MB-231 and SUM-159 showed increased proliferation with both ST2 and adipocyte media. We applied the mammosphere assay to investigate the effect of adipocytes media on cancer stem cell activity. Mammosphere formation also depended on BCC type, where only MCF7 and SUM-159 cells showed an increase in mammosphere formation efficiency, with SUM-159 cells showing a significant increase in mammosphere formation in adipocyte compared to ST2 media (72h). Initial experiments investigating cell migration show co-culture with adipocytes increased cell migration in MDA-MB-231 and SUM-159, but migration was decreased in MCF7 and BT-474 cells when compared to ST2 media.
Our preliminary investigations show that BMAT has distinct influences on BCCs, dependent on the type of BCCs. We need further studies to understand the different biological effects on the BC subtypes, including downstream signalling, such as EMT and WNT signaling. We are also developing a direct co-culture system to better understand the breast cancer metastatic niche.

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Leukaemia inhibitory factor: a novel mediator of prostate cancer bone metastasis?

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Bone metastasis is a frequent complication of advanced prostate cancer (PCa). Following metastasis, five-year survival rates plummet to just 30% and treatment options become limited. There is therefore an urgent need to elucidate the mechanisms that drive metastasis to identify novel therapeutics and biomarkers. Leukaemia inhibitory factor (LIF) is an IL-6 family cytokine known to be involved in a wide range of biological functions including haematopoiesis and bone formation. Moreover it has shown to be overexpressed in a number of tumour types including breast which also commonly metastases to bone. The aim of this study was to elucidate the role of LIF in PCa bone metatases.
We employed a powerful combination of *in vitro, in vivo* and *ex vivo* techniques including; cytokine array analysis of patient serum, murine models of PCa-bone metastasis, co-culture systems, qPCR and ELISAs.
Soluble LIF was elevated in patient serum before development of bone metastases vs non-metastatic patients (57.15% ± 17.06% increase, $p < 0.01$). LIF mRNA and protein expression was increased in bone metastatic PCa cell lines ($p < 0.001$). In a murine model of PCa bone metastasis, LIF mRNA expression strongly correlated with tumour burden (Pearson r = 0.9941). LIF serum levels correlated with late stage weight loss in tumour-bearing mice ($p < 0.01$) as well as leptin levels ($p < 0.05$). *In vitro,* chronic LIF treatment was found to prevent differentiation into adipocytes, but promote cells towards the osteoblastic lineage. Recombinant LIF treatment of PCa cells resulted in an increase in pSTAT3, P70 S6K and pMAPK. Furthermore PCa/stromal cell co-cultures demonstrated that PCa cells upregulate LIF expression in stromal cells ($p < 0.001$), accompanied by an upregulation in RANKL ($p < 0.001$) and a downregulation of osteoprotegerin (OPG) gene expression ($p < 0.0001$) indicating a mechanism by which LIF may be involved in PCa-induced bone disease.
Our results indicate LIF is associated with bone metastases in human PCa patients, is a potential biomarker of disease progression and induces functional effects in cells of the tumour-bone niche.

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Myeloma cell down-regulation of adiponectin in bone marrow adipose tissue promotes growth and survival via TNF-alpha

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Multiple myeloma (MM) is a destructive cancer of plasma cells which predominantly reside in the bone marrow. One of the most abundant cell types in the marrow is the bone marrow adipocyte (BMAT) which are implicated in MM progression. BMAT secretes numerous adipokines, including adiponectin, known to have anti-tumour properties. However, in MGUS and MM, adiponectin is down-regulated, creating a permissive environment for MM. Our goal was to understand how myeloma cells regulate BMAT and adiponectin to promote disease pathogenesis.
We have combined *in vivo* studies using the 5TGM1 MM model and BMAT imaging using osmium staining and microCT and IHC. Studies have used a panel of MM cell lines and primary MM cells, BMAT differentiated from ST2 bone marrow stromal cells (BMSCs) or patients with MM and white adipocytes differentiated from 3T3-L1 fibroblasts.
In vivo studies revealed that BMAT is elevated in early MM ($p = 0.042$) with low tumour burden ($p = 0.015$). Coculture of MM cells with BMAT increased MM cell viability by up to 95%, induced a 4-fold increase in migration and decreased apoptosis. MM cells were found to migrate towards and subsequently uptake lipids isolated from the bone marrow of patients with MM. Coculture of MM cells with BMAT significantly decreased adiponectin mRNA expression, protein expression and secretion ($p < 0.05$). Expression of adiponectin was lower in white adipocytes and in contrast to BMAT, coculture of MM cells with white adipocytes had no effect on adiponectin expression. TNF-α and IL-6 are cytokines secreted by MM cells and elevated during obesity. BMAT

treated with recombinant TNF-α or IL-6 exhibited a down-regulation of adiponectin. Addition of a TNF-α neutralizing antibody to MM-BMAT cocultures reversed the reduction in adiponectin.
Our studies show that BMAT is elevated during MM development in vivo. BMAT can support MM growth and survival, and we provide a novel mechanism by which MM-derived TNF-alpha down-regulates adiponectin, so avoiding the myeloma-suppressive effects of this key adipokine.

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3D Perfusion Bioreactor Model of Tumor-Induced Bone Disease

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Interactions between tumor and the bone microenvironment are known to play a critical role in the progression of tumor-induced bone disease (TIBD). However, there remains a limited understanding of those interactions and thus it is not possible to predict which tumors will induce bone disease or how tumors will respond to therapeutics. This is largely due to the inability to adequately model these cell-matrix interactions. The objective of this study is to develop an *in vitro* model of TIBD to predict the course of disease progression and response to drug treatments in individual patients. 3D-printed Tissue Engineered Bone Constructs (TEBCs) developed in our lab that recapitulate the mechanical and topological properties of trabecular bone have been employed in a perfusion bioreactor to simulate the *in vivo* bone microenvironment. Human mesenchymal stem cells (hMSCs), human monocytes (hMc), and MDA-MB-231 breast cancer cells were sequentially seeded onto the TEBCs in static culture and subsequently cultured under perfusion conditions for 30 days. The TEBC remodeling caused by the osteoblasts (OBs) and osteoclasts (OCs) was measured gravimetrically and by micro-computed tomography (μCT). The cohort of TEBCs containing only a co-culture of OBs and OCs exhibited no net change in mass or volume, suggesting a remodeling balance had been struck between the two cell types. In the tri-culture cohort of TEBCs containing OBs, OCs and tumor, a net loss in mass was observed, suggesting that the tumor disrupted the balance toward an osteolytic phenotype. Tartrate-resistant acid phosphatase (TRAP) staining and toluidine blue staining for resorption pitting indicated significant OC activity in 2D culture experiments conducted in parallel using the same cell types and TEBC material, reinforcing the hypothesis that the mass losses found in the 3D experiments are a direct result of increased OC activity. In summary, perfusion bioreactor culture of bone cells indicate TEBCs can be remodeled by OBs and OCs, these changes can be measured gravimetrically and by μCT, and tumor cells stimulate resorption of the TEBCs. Incorporation of patient tumor cells and therapeutics will make this *in vitro* model a powerful predictive tool for studying patient tumor progression and response to therapeutics.

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Unravelling the metabolic relationship in the prostate cancer-bone microenvironment; a novel role for the pentose phosphate pathway

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It is now widely accepted that metabolic changes are a hallmark of malignant transformation. To aid this cancer cells are thought to develop a metabolic relationship with surrounding stroma. Prostate cancer (PCa) has an unusually high propensity for metastasizing to bone, and interactions between PCa cells and the bone microenvironment promote tumour growth and survival, drug resistance and development of a destructive bone disease. These processes require energy, reducing power and a source of biosynthetic precursors. We hypothesized that identifying the metabolic changes that occur when PCa cells interact with bone would reveal new therapeutic approaches. In silico analysis of PCa cells isolated from patients with bone metastases as compared with primary tumours revealed that metabolic pathways were the most significantly altered gene sets. Comparing metastatic sites, specific changes were detected that were unique to bone metastases, including 7/19 genes involved in the pentose phosphate pathway (PPP) were significantly altered in bone metastases vs primary PCa, whereas 0/19 genes were altered in liver/lung metastases. The PPP is a metabolic pathway, parallel to glycolysis that generates NADPH, nucleotides and nucleic acids. As such, this pathway may play an important role in PCa bone metastases, however its contribution is unknown. In support of this, metabolic profiling of PCa cells following coculture with bone marrow stromal cells or presoteoblasts revealed changes in glycolysis, the TCA cycle and the PPP, with a significant increase in PPP metabolites including R5P and NADPH ($p < 0.001$). Expression of PPP proteins was higher in bone metastatic PCa cell lines (PC3, MDA-1a, MDA-1B) compared to non-bone metastatic cell lines (22RV1, LNCaP). Expression of the rate-limiting PPP enzyme G6PD was induced in a non-bone metastatic cell line by BMSC co-culture. This effect can be suppressed by inhibition of the PPP with 6-aminonicotinamide (6-AN). PPP inhibition reduced PCa cell viability, with a greater inhibition in bone –metastatic PCa cells. Our data suggests that the bone microenvironment promotes up-regulation of the PPP in PCa cells. Targeting this pathway alone, or in combination with current therapies, could have the potential to provide benefit to patients with incurable bone metastatic prostate cancer.

P40

Effect of tumour-derived extracellular vesicles on the molecular profile of osteoblasts and on endothelial functions

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Extracellular vesicles (EVs) are membrane-bound cargoes of biologically active molecules shed by cells, which are emerging as mediators of several pathological processes, including cancer. We investigated EV-mediated communication among MNNG osteosarcoma (MNNG-HOS) or osteotropic breast cancer cells (MDA-MB-231), osteoblasts and endothelial cells. In mouse osteoblasts, HOS- and MDA-EVs inhibited the expression of *Cyclin D1* (-75%, $p = 0.02$), *Alp* (-35%, $p = 0.002$) and *Osx* (-40%, $p = 0.06$), and enhanced the expression of *Nos2* (15-fold, $p = 0.015$). An increase of mRNA and protein expression of IL-1β (12-fold, $p < 0.001$), IL-6 (10-fold, $p = 0.05$), RANKL (1.5-fold, $p = 0.02$) and LCN2 (4-fold, $p = 0.01$) was also observed in tumour cell-EVs-treated osteoblasts. A cytokine protein array on conditioned media (CM) collected from osteoblasts treated with HOS EVs showed a decrease of IGFBP2 compared to CM from untreated osteoblasts, and an increase of Pentraxin 3, a positive modulator of tumour-associated inflammation. Moreover, the chemokines CXCL2, CCL2, CCL5, CCL20, Lix and MMP3, which were undetectable in untreated osteoblasts, were expressed by HOS EV-treated osteoblasts. Similarly, MDA-treated osteoblasts presented with an increase of CXCL1, CXCL2, CXCL10, CCL2, CCL3, CCL5 and of MMP2 and MMP3, thus suggesting shared pathways in the regulation of osteoblasts by the two tumour cells. HOS EV transcriptional profile showed the expression of genes involved in bone metabolism, such as *CLCN7*, *TGFβ1*, *Cathepsin K* and *VEGFA*. Consistent with this latter result, HOS EVs significantly increased endothelial cell chemotaxis ($p = 0.02$, AUC) and in vitro tube formation (3-fold, $p = 0.007$), evaluated by scratch assay and number of branching formed in HUVEC cell cultures. Moreover in vivo angiogenesis, evaluated by the matrigel plug assay, was significantly enhanced by HOS-EVs (3.5fold, $p = 0.001$;

n.mice = 5/group). In conclusion, these data allowed to elucidate i) the transcriptional profile of osteosarcoma and breast cancer derived EVs and ii) their impact on osteoblast and endothelial physiology.

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ABSTRACT WITHDRAWN

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LKB1 deficiency exhibits vulnerable mitochondrial defects by rapamycin on urothelial carcinoma cells

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Rapamycin, a Mammalian target of rapamycin (mTOR) inhibitor, has significant potential for the application of mTOR inhibitors in treatment of urothelial carcinoma (URCa) of the bladder. Previous studies have exhibited that regulation of the AMPK/mTOR signaling pathway enhances the apoptosis via induction of autophagy or mitophagy in bladder cancer. Alteration of LKB1-AMPK signaling leads to mitochondrial dysfunction and accumulation of autophagy-related proteins as a result of altered mitophagy, resulting in enhanced sensitivity to drug treatments. Therefore, we hypothesized that LKB1 deficiency on URCa cells could confer to sensitivity to rapamycin by mitochondrial defect-mediated mitophagy induction. Rapamycin enhanced growth inhibition and apoptosis in stable LKB1 knockdown URCa cells and xenograft mouse model. In spite of stably downregulated LKB1 expression, rapamycin induced activation of AMPK in URCa cells, which caused loss of mitochondrial membrane potential ATP depletion, and ROS accumulation, indicating alteration of mitochondrial biogenesis. Our findings suggest that that presence or absence of LKB1can be targeted to induce dysregulated mitochondrial biogenesis by rapamycin treatment as the design of novel therapeutic strategies on bladder cancer.

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Pretreatment with metformin alters the host microenvironment to increase myeloma tumour burden and bone disease in vivo

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Multiple myeloma (MM) is a B cell malignancy that develops almost exclusively in the bone marrow (BM) and causes osteolytic bone lesions due to a dysregulated bone turnover. It is widely believed that MM arises from an asymptomatic state referred to as monoclonal gammopathy of undetermined significance (MGUS), however the mechanisms that drive MGUS progression to MM are unknown. Metformin is universally used in the treatment of type 2 diabetes and recently it has been reported to have anti-cancer activities, including reducing the risk of progression from MGUS to myeloma. The goal of the current study was to determine whether pretreatment with metformin could alter the bone microenvironment and reduce subsequent development of MM in vivo.

C57Bl/KaLwRij mice were treated with metformin for 4 weeks prior to inoculation of MM cells, when metformin treatment was halted. In contrast to our hypothesis, we found that pretreatment with metformin induced a two-fold increase in tumour burden, evidenced by the proportion of MM cells in BM ($p < 0.001$). This was associated with an increase in osteolytic bone lesions ($p < 0.05$). During in vitro studies, 2T3 preosteoblasts were treated with metformin, prior to addition of MM cells. This resulted in an increase in MM cell adhesion to preosteoblasts ($p < 0.05$), an increase in non-dividing MM cells and increase in p21 expression. Metformin treatment increased the osteogenic capacity of osteoblasts and increased osteopontin (OPN) expression in osteoblasts in vitro. Silencing OPN expression in osteoblasts resulted in a reduction in MM cell attachment in response to metformin, suggesting that metformin-mediated OPN expression is at least partially responsible for the increase in adhesion. Furthermore, immunohistochemistry demonstrated an increase in OPN expression in osteoblasts following metformin treatment in vivo.

Altogether our results show that metformin pre-treatment induces changes in the bone microenvironment, increasing the capacity to harbour myeloma cells. This can be partially explained by the increase in osteoblastic OPN expression in vitro and in vivo which may act as chemoattractant for MM cells. In addition, metformin increases the osteogenic capacity of osteoblasts, potentially expanding the endosteal niche where MM dormant cells reside. Overall these results highlight the need for caution when considering the use of metformin in non-diabetic patients with MGUS as an approach to reduce progression towards MM.

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Marrow Adipose Tissue is Associated with Regions of Hypoxia During Metastatic Colonization of the Bone

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Marrow adipocytes can be found within close proximity to malignant cells in the bone microenvironment (BME) and increased marrow adiposity promotes metastatic colonization. These findings suggest marrow adipose tissue (MAT) may contribute to metastatic dissemination to the bone; however, the cellular and molecular mechanisms regulating MAT formation during metastatic bone colonization have not been fully elucidated. We show that upon intra-cardiac delivery, breast carcinoma and melanoma cell lines colonize to hypoxic regions in the bone microenvironment. Intriguingly, these hypoxic regions are also associated with marrow adipocytes and whole bone homogenates isolated from tumor bearing bones showed increased expression of *Pgc-1 alpha*, a gene which is coupled to both adipogenesis and the hypoxia inducible factor (HIF) signaling pathway. Given these observations, we hypothesized that cancer cells within the bone marrow secrete factors to induce MAT formation in a hypoxia dependent manner. To test this hypothesis, multipotent bone marrow stromal cells (BMSC) were isolated from 8 week old B6 mice and were cultured with either, adipogenic induction or conditioned media (CM) isolated from cancer cell (CC) lines. In the presence of induction media and under 20% O_2, BMSC underwent adipogenic differentiation as measured by the presence of lipid formation and Oil Red O staining. Interestingly, CM alone was also able to induce lipid formation in BMSC. Next, to examine the influence of hypoxia on BMSC lipid formation we cultured cells in 1% O_2. In hypoxia, adipogenic differentiation of BMSC with induction media was impaired; however, CM isolated from CC lines induced lipid formation even in the presence of low oxygen. Our data suggests that CC residing in the hypoxic BME may modulate MAT through the secretion of pro-adipogenic factors. Our studies have begun to elucidate the potential contribution of hypoxia in regulating MAT during bone colonization by metastatic tumor cells.

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Transcriptional regulators of oncogenesis and of osteoblastic differentiation revealed by microRNA profiling of osteosarcoma cell lines and primary human osteoblasts

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MicroRNAs (miRNAs) are 20-24 nucleotide non-coding RNAs which are increasingly recognised as regulators of aberrant gene expression in oncogenesis. In this study we performed miRNA profiling on three osteosarcoma cell lines, MG-63, TE85 and Saos2 and compared them with primary human osteoblasts (HOBS).

Human osteosarcoma cell lines were from extensively characterised lab stocks. HOBS were cultured from explants of bone from six donors. MiRNA was isolated and purified from cell culture homogenates using the miRNeasy Mini Kit (Qiagen). MiRNA profiling was performed with an Affymetrix GeneChip scanner 3000 7G on an Affymetrix miRNA 4.0 array.

In total 2587 miRNA were profiled across all samples. Principal components analysis (PCA) showed that the clearest difference in miRNA expression profiles was between osteosarcoma cell lines collectively and HOBS: 157 miRNAs were differentially expressed between these two cell types ($p < 0.05$ (FDR-adjusted), fold change > 2). Clustering analysis on these miRNAs identified three primary miRNA clusters, with similar expression profiles, that showed clear differences between osteosarcoma and HOBs: two clusters comprised miRNAs up-regulated in osteosarcoma cells compared to HOBS, whereas one cluster showed down-regulated miRNAs in osteosarcoma cells. miRNA annotation based on validated gene targets (odds:expected ratio > 1.5) found miRNA clusters up-regulated in osteosarcoma cells were involved in diverse pathways such as oncogenesis, immune response, VEGF signalling and glutathione metabolism. MiRNAs down-regulated in osteosarcoma cells were also associated with cancer pathways, but also arginine, proline and glutathione metabolism amongst other pathways. Interestingly, PCA analysis also identified profile differences between the three osteosarcoma cell lines: 85 miRNA were significantly different ($p < 0.05$ (FDR-adjusted), fold change > 2) between at least two cell lines. PCA component 3, which accounted for 9.8% of the variation, captured the sequential order of osteoblast differentiation between the cell lines. The miRNAs contributing most to this sequence of differentiation were hsa-miR-155-5p, hsa-miR-212-3p, hsa-miR-708-5p and hsa-miR-143-3p.

In conclusion, this study revealed major differences in miRNA profiles between HOBS and cell lines derived from human osteosarcoma. Several groups of miRNAs linked by expression profiles were identified as potential epigenetic regulators of the 'transformed' oncogenic state of osteosarcoma cells. Functional annotation of these miRNAs suggests diverse and complex regulatory roles particularly in cancer-related pathways but also in numerous other signalling and metabolic pathways.

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Vascular cell adhesion molecule (VCAM) 1 and α4β1 integrin interactions regulate myeloid-derived suppressor cells (MDSC) mobilization from the bone marrow of tumor hosts

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MDSCs are a subset of immature bone marrow-derived cells that play diverse pro-tumorigenic roles such as suppressing T cell-mediated anti-tumoral immunity. We have previously reported that tumor-derived parathyroid hormone-related peptide (PTHrP) increased MDSC recruitment in the tumor tissue. In contrast, little is known about how MDSCs are mobilized from the bone marrow of tumor hosts. In this study, we investigated whether interactions between α4β1 integrin expression in MDSCs and VCAM 1 expression in the bone marrow stromal cells (BMSC) regulate retention and release of MDSCs in the bone marrow. $CD11b^+Gr1^+$ cells (commonly defined as murine MDSCs) were isolated from the murine femurs by flow cytometry, and *in vitro* cell binding assays were performed. Briefly, MDCS were carboxyfluorescein succinimidyl ester (CFSE)-stained and co-cultured with MC3T3E1 pre-osteoblastic cells in the presence of anti-VCAM 1 and/or anti-β1 integrin neutralizing antibodies (0.5 to 2 μg/mL). After washing unbound cells, fluorescence intensity was measured to quantify MDSC-BMSC binding. VCAM-1 and/or β1 integrin neutralizing antibodies dose-dependently decreased MDSC-BMSC binding, suggesting that MDSCs bind to BMSC via VCAM-1 and β1 integrin. We further examined whether tumor-derived PTHrP disrupts the VCAM-1 and β1 integrin axis of MDSC-BMSC. PTHrP (1-34) administration rapidly increased MDSCs in the peripheral blood *in vivo*. In addition, PTHrP conditioned-media from calvarial osteoblasts reduced MDSC-BMSC binding *in vitro* compared with control conditioned media, and the effects were reversed by anti-interleukin (IL)-6 or anti-vascular endothelial growth factor (VEGF)-A antibodies, suggesting that PTHrP-induced IL-6 and VEGF-A expression in osteoblasts mobilize MDSCs. In addition, recombinant IL-6 and VEGF-A reduced MDSC-BMSC binding *in vitro*. Lastly, immunohistological analyses of the femurs of tumor-bearing mice showed that $CD11b^+Gr1^+$ cells localized adjacent to trabecular osteoblasts. In summary, our data demonstrate that bone marrow stromal cells regulate MDSC trafficking from the bone marrow to the peripheral blood, and also that VCAM-1 (expressed in BMSC) and α4β1 integrin (expressed in MDSC) interactions regulate MDSC retention and mobilization. Further studies on the PTHrP-induced expression of proteinases such as matrix metalloproteinases and A Disintegrin and Metalloproteinase (ADAM) in the bone microenvironment will further elucidate the mechanism of MDSC mobilization in the bone marrow of tumor hosts.

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Modelling the human bone-tumour niche *ex vivo*

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Bone is the most common site for tumour metastasis, particularly in breast and prostate cancers where ~70% of patients show evidence of metastatic bone disease. Consequently, a large field of research has evolved to specifically study cancer-bone disease, along with a large number of different animal models. These include inoculation of human or mouse tumour cells at extra-osseous sites e.g. heart, or implanted directly into skeletal sites. The limitations of *in vivo* bone-tumour models include a common mixed approach using human cells in mice, using immuno-compromised mice which do not clearly represent the contribution of the immune system within the bone-tumour niche, and the wide-ranging inherent differences between mice and humans.

To more accurately re-create the human bone-human tumour microenvironment, and importantly, replace the use of animals in unnecessary or poorly validated model systems, we aimed to develop an *ex vivo* assay mimicking the tumour-bone cell interactions which occur following cancer metastases to skeletal sites.

Fresh trabecular bone samples were collected following hip replacement surgery under approved Ethical and HTA procedures. Uniform areas of trabecular bone were created approx. $5mm^2$, and cultured with PC3-EGFP prostate cancer cells +/- HS-5-mCherry human bone marrow stromal cells. Cells were inoculated to the living bone by either direct plating upon the bone surface, insertion into small cores within the bone or by introducing the cells in a matrigel suspension. Following culture for 7-10 days the tumour-bone co-culture was processed for histomorphometric analysis.

Viable bone cells were clearly visible (osteoblasts, osteoclasts) and abundant fat deposits, common in ageing human bone. Importantly, tumour cells were observed adhered to the bone surface and occupying marrow spaces within the bone network when introduced within matrigel, suggesting the capacity to grow and colonise the bone niche. However, few GFP+ tumour cells were seen when inoculated by other means. Our assay has used human intact bone and human tumour cell lines to model the bone-tumour niche seen in patients with metastatic bone disease and suggests a potential alternative to the use of animal models in cancer-bone research.

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Contribution of marrow adipocytes to destructively lytic behavior of metastatic kidney tumors in bone

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Approximately 35% of patients with kidney cancer, most commonly renal cell carcinoma (RCC), develop bone metastases. Resulting skeletal involvement is not only painful and devastating, but also very costly to the patient. Despite the numerous advancements in systemic RCC therapies stemming from the elucidation of hypoxia-inducible factor (HIF) signaling and its regulation by von Hippel-Lindau (VHL) gene, the molecular mechanisms behind this highly lytic behavior of metastatic RCC tumors remain not understood. An important feature of metastatic RCC is that in contrast to other bone-trophic malignancies, which largely localize to the axial skeleton, RCC often involves peripheral sites, such as long bones. Notably, the peripheral skeleton is abundantly filled with marrow adipocytes, whose numbers increase exponentially with age and metabolic pathologies. Marrow fat cells play metabolically important functions, and have emerged as strong contributors to metastatic progression from several types of cancers. They are also known to express significant levels of receptor activator for NF-κB ligand (RANKL), one of the key regulators of osteoclastogenesis. Here we hypothesize that tumor-induced lipolysis and metabolic changes in marrow adipocytes promote osteoclastic activity and bone loss, and consequently support tumor growth and survival.

We demonstrate that adipocyte-supplied factors promote osteoclast differentiation and maturation *in vitro*. Transwell co-culture of RCC cell lines with adipocytes highly induces the levels of RANKL in fat cells, a process that is further potentiated by the stimulation of lipolysis. Both the interaction with RCC cells and the pharmacological induction of lipolysis promote thermogenesis in marrow adipocytes as indicated by the significantly elevated expression of uncoupled protein 1 (UCP1). Furthermore, two molecules with key roles in inflammatory bone resorption: cyclooxygenase (COX-2), a rate-limiting enzyme in prostaglandin synthesis, and a downstream effector of beta-adrenergic signaling in adipose tissue; and macrophage chemoattractant protein (MCP1), are highly induced in adipocytes interacting with tumor cells *in vitro* and in tumor-bearing tibia *in vivo*. Studies are currently underway to elucidate the precise molecular mechanisms behind the highly destructive RCC behavior in bone and its regulation by bone marrow adipocytes.

P49

Combined administration of a novel small-molecule inhibitor of TRAF6 and Docetaxel reduces breast cancer skeletal metastasis and osteolysis

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Tumour necrosis factor receptor-associated factor 6 (TRAF6) has been implicated in breast cancer and bone remodelling, but its role in the initiation and progression of breast cancer osteolytic metastasis remains unknown. Here we describe the effects of the verified small-molecule inhibitor of TRAF6, 6877002 on skeletal tumour growth, metastasis and osteolysis in a syngeneic mouse model of breast cancer. We observed that TRAF6 is highly expressed in a panel of human and mouse breast cancer cell lines including the parental human MDA-MB-231 and mouse 4T1 and their bone-tropic clones. Exposure of these cells to the TRAF6 inhibitor 6877002 suppressed breast cancer cell proliferation in a concentration-dependent manner (IC50, 24-31μM). 6877002 (3μM) also suppressed RANKL-stimulated migration (38.4%, $p < 0.05$) and invasion (57.3%, $p < 0.05$) of MDA-MB-231 cells in vitro and reduced skeletal tumour growth following the intra-cardiac injection of 4T1 in immuno-competent mice by 67.5% ($p < 0.05$). Furthermore, 6877002 exhibited synergism with a panel of chemotherapeutic agents including standard-of-care Docetaxel *in vitro*, and combined administration of 6877002 (20mg/kg/day) and Docetaxel (15mg/kg/week) reduced skeletal tumour growth (86.1% reduction, $p < 0.05$) and inhibited osteolytic bone damage (bone volume, 57.2% increase, $p < 0.05$ compared to vehicle treated) following the injection of syngeneic 4T1 in immunocompetent mice. Functional and mechanistic studies in bone and breast cancer cells revealed that 6877002 reduced RANKL-induced RANK/TRAF6 binding, IkB phosphorylation and osteoclast formation by up to 80%, whilst having no effect on osteoblast viability or differentiation. Collectively, our findings suggest that targeting of TRAF6, alone or in combination with conventional chemotherapies, shows promise for the treatment of advanced breast cancer.

DISCLOSURE

Dr Aymen I. Idris is an owner and major stockholder in a company established to develop and test TRAF/NFkB inhibitors as a novel class of anti-rheumatic and anti-metastatic drugs

P50

Altering glycosphingolipid composition to improve multiple myeloma bone disease

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Multiple myeloma (MM) is an incurable cancer of plasma cells (PC), with a median survival of 5-7 years. Osteolytic bone disease and skeletal complications occur in more than 80% of MM patients and significantly contribute to the morbidity and mortality of these patients. Glycosphingolipid (GSL) composition, constituents of the outer leaflet of the cellular membrane, is altered in multiple myeloma and other cancers with increased expression of GM3 and GM2. We previously reported that GM3 promotes osteoclastogenesis and that the GSL inhibitor N-butyl-deoxynojirimycin (NB-DNJ) reduced myeloma bone disease in the 5TGM1 mouse model of MM. NB-DNJ prevented OC development and activation by disrupting RANKL-induced localization of TRAF6 and c-SRC into lipid rafts and preventing nuclear accumulation of transcriptional activator NFATc1.

Using a new GSL inhibitor with less gastrointestinal side effect and weight loss, we now show in vitro dose dependent inhibition of both osteoclast formation ($p < 0.01$) and MM cell growth ($p < 0.0001$, thus GSL inhibition can potentially relieve two main features of MM - tumour burden and bone destruction. In vivo we found the GSL inhibitor can suppress germinal centre formation in mouse spleen induced by the injection of sheep red blood cells. Moreover, we determined that the GSL inhibitor decreased MM tumour burden, as evidenced by a reduction in the proportion of myeloma cells within bone marrow and spleen and bone loss in the 5TGM1 model of myeloma.

These studies elucidate mechanistic links between osteoclasts and increased susceptibility to MM. Moreover, as GM3 has been strongly implicated in adipocyte function in diabetes, we postulate that the GM3 rich environment created by the myeloma cells may create a supportive adipocyte niche in the bone marrow as well as providing the preclinical platform for GSL inhibition as a new tool against MM bone lesions and abnormalities in bone marrow adipocytes.

P51

Transcriptomic profiling of the *in vivo* myeloma bone-lining niche identifies BMP signalling as a therapeutic target for bone disease

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Multiple myeloma disrupts bone homeostasis causing lytic lesions, skeletal damage and pain. Mechanisms underlying myeloma-induced bone destruction are incompletely understood and current therapies do not restore lost bone mass. We have previously reported RNASeq transcriptomic profiling of isolated endosteal cell types from a murine myeloma model, not previously described in the cancer-bone niche. We found bone morphogenetic protein (BMP) signalling to be upregulated in mesenchymal progenitor cells of the bone lining niche in myeloma-bearing mice.

BMP signalling is not previously reported to be dysregulated in myeloma bone disease. In the current study, inhibition of BMP signalling in vivo using either a small molecule Type 1 BMP receptor antagonist (LDN193189) or a solubilized Alk3-Fc (Bmpr1a-Fc) receptor ligand trap prevented trabecular and cortical bone volume loss caused by myeloma, without altering tumour burden. These inhibitors directly reduced osteoclast formation and activity, increased osteoblast number and new bone formation, and suppressed bone marrow levels of sclerostin, a Wnt inhibitor implicated in mediating the failure of bone repair in myeloma sufferers. RNASeq of mesenchymal progenitor cells from myeloma-bearing mice showed upregulation of collagen matrix formation and vitamin D signalling gene sets with LDN193189 treatment, indicating enhancement of osteoblast maturation.

This work describes a novel technique in the investigation of myeloma-niche interactions, shows its use in revealing a novel pathway involved in the pathogenesis of myeloma bone disease, and demonstrates that the therapeutic targeting of the BMP pathway has both anti-resorptive and anabolic benefits to bone metabolism, which could have a role alongside anti-tumour treatments.

DISCLOSURE

Since completing this work, S Gooding's research fellowship at Oxford University is funded by Celgene.

P52

Bone-specific activation of a dietary polyphenol inhibiting TGFß-dependent breast cancer bone metastases

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The majority of women with advanced breast cancer develop osteolytic bone metastases, which are incurable. Secretion of osteolytic factors from bone metastatic tumor cells drives this process, which is fueled by tumoral effects of growth factors, such as TGFß, released from resorbed bone matrix. Smad signaling is an important mediator of TGFß-stimulated gene expression. In earlier studies using a human xenograft breast cancer bone metastases model dependent on TGFß-stimulated secretion of PTHrP ("MDA-SA" cells), treatment with turmeric-derived dietary polyphenols (curcuminoids) reduced breast cancer bone metastasis progression and inhibited Smad-dependent TGFß-stimulated secretion of PTHrP. However, the active metabolite *in vivo* was unclear since curcumin, analogous to many other dietary polyphenols, is rapidly glucuronidated (G-CURC), allowing for minimal systemic exposure to aglycone curcumin (CURC) in mice or humans. Studies were therefore undertaken: 1) to compare the ability of CURC vs G-CURC to inhibit Smad-dependent TGFß signaling in a panel of TGFß-dependent bone metastatic breast cancer cells, and 2) to examine bone-specific metabolism of orally ingested CURC in mice. In vitro, phosphorylated receptor Smad2 levels were significantly decreased in TGFß-stimulated human (MDA-MB-231-derived MDA-SA, 1833, and 2287 cells) and murine (4T1) bone tropic breast cancer cells in response to treatment with CURC (30 μM), while G-CURC was without effect. To query whether site-specific deconjugation of G-CURC could explain the *in vivo* protective effects of CURC in bone, systemic vs. site specific CURC metabolites were assessed following oral CURC administration. In serum, aglycone CURC accounted for 1.1% of total circulating curcumin [CURC + G-CURC]), while 39.6% was in the aglycone form (CURC) in bone ($p < 0.01$), where CURC levels were 10-fold higher as compared to serum ($p < 0.05$). Hemopoietic (but not tumor) cells in bone expressed glucuronidase (by Western analysis and enzyme activity assay), an enzyme with a Km of 72 μM that readily deconjugated G-CURC *ex vivo*, suggesting that site specific activation (deconjugation) of orally administered CURC within the bone microenvironment could contribute to its bone protective effects. Because many bone protective dietary polyphenols are similarly glucuronidated, these findings suggest that site-specific deconjugation of these "pro-drugs" may contribute to their bone-targeted bioactivity.

P53

Development of Novel Nanomedicines for Treatment of Primary and Metastatic Prostate Cancer

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Prostate cancer (PC) is the 2nd leading cause of cancer related deaths in U.S. men. The reason of prostate cancer mortality is not from the primary cancer but spread of the primary cancer cells to distant organs, especially bone. The current bone metastases therapy modalities are not efficient to cure bone metastasis because of the inability to deliver therapeutic concentrations of anticancer-agents (Cabazitaxel; CTX) to PC lesions in bone. To address the limitations, we synthesized a bone targeted amphiphilic triblock copolymer, pVTK-poly(ethylene)-*b*-poly(acrylic acid)-*b*-poly(methyl methacrylate), which self-assembles in aqueous medium forming nano-sized micelles that can encapsulate CTX in the hydrophobic core. Cross-linkage of the PAA blocks using a ketal-linker forms an acid-labile shell, which stabilizes the formed micelles at physiologic pH but allows selective release of the loaded cargo in acidic environments. The average size of the particles was around 100 nm. Further, we investigated the optimum bone binding mole percent of pVTK (0, 5, 10%) in the particles using HA-disc, bone powder, and bone-chip relying on FITC fluorescence of the peptide. The percent of bound of 5% and 10% pVTK micelles on HA-discs were 58 and 61, respectively, while the same formulations binding percentage on rat bone powder were 42 and 38, respectively. Furthermore, the CTX loading efficiency of 5 and 10% pVTK-micelles were around 26.0 and 18.8%, respectively. Based on the cytotoxicity results of the particles, pVTK-micelles (0, 5, 10%) did not have any cytotoxic effect on PC cells. The IC_{50} values of CTX-loaded non-targeted, 5%, and 10% pVTK-micelles were 0.76, 2.46, 0.37 nM, respectively with the control of 0.60 nM free CTX. These results indicate pH-sensitive, CTX-loaded, bone-targeted pVTK-micelles can preferentially bind to bone and achieve tunable CTX release in tumor lesion, which can selectively kill tumor cells while limiting systemic side effects.

DISCLOSURE
Mohamed El-Sayed works at Eli Lilly

P54

Mechanical signals retain musculoskeletal endpoints while suppressing adiposity in a murine model of complete estrogen deprivation

Gabriel M Pagnotti[1], Ryan Pattyn[1], Laura E Wright[1], Sutha Johns[1], Sreemala Murthy[1], Trupti Trivedi[1], Yun She[1], Clinton T Rubin[2], William R Thompson[1], Khalid S Mohammad[1], Theresa A Guise[1]
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Post-menopausal, estrogen-receptor-positive breast cancer patients treated with aromatase inhibitors to inhibit disease progression experience adverse musculoskeletal effects such as bone loss and muscle weakness. Mechanical signals, generated during physical activity and exercise, help maintain musculoskeletal homeostasis; however, many patients are unable to participate in regimented exercise. Low intensity vibrations(LIV), high-frequency low magnitude mechanical signals analogous to skeletal muscle contractions, were previously demonstrated as safe, deterring bone loss in two cancer models. We hypothesized similar effects in C57BL6 mice undergoing complete estrogen deprivation to model breast cancer therapy. Models were generated via ovariectomy at 8wo age and daily administration of aromatase inhibitor letrozole 24w. To test our hypothesis, mice were administered either LIV($n = 10$) or control-LIV(CTL; $n = 10$) for 4w prior to and 24w post-surgery. Whole-body DXA scanning was performed to measure lean and fatty tissue. LIV significantly increased lean mass over the 28w-treatment period relative to CTL. Total fat increased by 97% in CTL relative to baseline, while increasing by only 56%($p < 0.001$) in LIV-treated mice. Forelimb strength, from triplicate grip measurements, were significantly greater($p < 0.05$) in LIV-treated mice than in CTL, a 32% difference between groups at 28w. High-resolution ex vivo micro-computed tomography scanning was performed on lumbar vertebrae (resolution = 12microns). Bone volume fraction and connectivity density were both significantly($p < 0.05$ and $p < 0.01$, respectively) greater following LIV. Dynamic histomorphometry was performed on calcein-double-labeled PMMA-embedded femora and vertebrae. Bone formation rate and mineralizing surface normalized to bone surface were each significantly greater in distal femora($p < 0.01$) and L5 trabeculae ($p < 0.05$) in response to LIV. Additionally, as compared to non-LIV-CTL bones, mineral apposition rate was significantly greater($p < 0.05$) in LIV-treated L5 vertebrae, a site harboring greater metabolic response to mechanical signals. Von Kossa-stained LIV-treated lumbar vertebrae sections had significantly greater($p < 0.05$) osteoblast number per bone surface area than CTL. Conversely, TRAP-stained sections indicated significantly lower osteoclast surface area($p < 0.01$) and osteoclast number($p < 0.05$) normalized to bone surface in LIV-treated mice. A glucose tolerance test was performed indicating that the area-under-the-curve was significantly($p < 0.03$) lower in LIV-treated mice relative to CTL-treated mice. Together, these data highlight the effect of estrogen on musculoskeletal tissue and support the role of physical activity and mechanical signals in reducing fat while increasing lean tissue and bone.

DISCLOSURE
CTR has authored patents related to the mechanical regulation of metabolic diseases and is a founder of Marodyne Medical. The other authors declared no conflict of interest.

P55

Mechanical signals retain musculoskeletal endpoints while suppressing adiposity in a murine model of complete estrogen deprivation.

Gabriel M Pagnotti[1], Ryan Pattyn[1], Laura E Wright[1], Sutha K John[1], Sreemala Murthy[1], Yun She[1], Clinton T Rubin[2], William R Thompson[1], Khalid S Mohammad[1], Theresa A Guise[1]
[1]Indiana University, Indianapolis, USA. [2]Stony Brook University, Stony Brook, USA

Post-menopausal, estrogen-receptor-positive breast cancer patients treated with aromatase inhibitors to inhibit disease progression experience adverse musculoskeletal effects such as bone loss and muscle weakness. Mechanical signals, generated during physical activity and exercise, help maintain musculoskeletal homeostasis; however, many patients are unable to participate in regimented exercise. Low intensity vibrations(LIV), high-frequency low magnitude mechanical signals analogous to skeletal muscle contractions, were previously demonstrated as safe, deterring bone loss in two cancer models. We hypothesized similar effects in C57BL6 mice undergoing complete estrogen deprivation to model breast cancer therapy. Models were generated via ovariectomy at 8wo age and daily administration of aromatase inhibitor letrozole 24w. To test our hypothesis, mice were administered either LIV($n = 10$) or control-LIV(CTL; $n = 10$) for 4w prior to and 24w post-surgery. Whole-body DXA scanning was performed to measure lean and fatty tissue. LIV significantly increased lean mass over the 28w-treatment period relative to CTL. Total fat increased by 97% in CTL relative to baseline, while increasing by only 56%($p < 0.001$) in LIV-treated mice. Forelimb strength, from triplicate grip measurements, were significantly greater($p < 0.05$) in LIV-treated mice than in CTL, a 32% difference between groups at 28w. High-resolution ex vivo micro-computed tomography scanning was performed on lumbar vertebrae (resolution = 12microns). Bone volume fraction and connectivity density were both significantly($p < 0.05$ and $p < 0.01$, respectively) greater following LIV. Dynamic histomorphometry was performed on calcein-double-labeled PMMA-embedded femora and vertebrae. Bone formation rate and mineralizing surface normalized to bone surface were each significantly greater in distal femora($p < 0.01$) and L5 trabeculae ($p < 0.05$) in response to LIV. Additionally, as compared to non-LIV-CTL bones, mineral apposition rate was significantly greater($p < 0.05$) in LIV-treated L5 vertebrae, a site harboring greater metabolic response to mechanical signals. Von Kossa-stained LIV-treated lumbar vertebrae sections had significantly greater($p < 0.05$) osteoblast number per bone surface area than CTL. Conversely, TRAP-stained sections indicated significantly lower osteoclast surface area($p < 0.01$) and osteoclast number($p < 0.05$) normalized to bone surface in LIV-treated mice. A glucose tolerance test was performed indicating that the area-under-the-curve was significantly($p < 0.03$) lower in LIV-treated mice relative to CTL-treated mice. Together, these data highlight the effect of estrogen on musculoskeletal tissue and support the role of physical activity and mechanical signals in reducing fat while increasing lean tissue and bone.

DISCLOSURE
CTR has authored patents related to the mechanical regulation of metabolic diseases and is a founder of Marodyne Medical. The other authors declared no conflict of interest.

P56

Bone-sialoprotein (BSP), Dickkopf-1 (DKK1) and CXCR4 as potential biomarkers of breast cancer metastasis to bone: Analysis within the AZURE (BIG 01/04) study of adjuvant zoledronic acid.

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Biomarkers predicting the risk of breast cancer metastatic spread to bone (BCBM) are urgently required. Our previous in vitro studies have identified Wnt-family inhibitor Dickopf-1 (DKK1) as a potential such biomarker. Also, bone sialoprotein (BSP) has shown elevated expression within breast primary tumours that metastasize to bone and BSP levels are also increased within serum samples from BCBM patients. The receptor protein CXCR4 is another potential biomarker of bone metastasis shown to bind stromal-derived-factor-1 (SDF1 / CXCL12) released by bone. In the current study, we tested these proteins as predictors of bone-metastasis within tissue-microarrays from the AZURE trial in which 3360 patients with early breast cancer at moderate/high risk of recurrence, were randomised 1:1 to receive standard adjuvant therapy +/-zoledronic acid (ZOL).

In this pilot study, primary tumour cores from AZURE trial patients were stained with antibodies towards DKK1, BSP or CXCR4. Cytoplasmic staining was assessed independently by two trained operators, blinded to outcome data, under supervision of an experienced breast histopathologist. DKK1, BSP and CXCR4 levels were correlated with local and distant recurrences by multivariate analysis, adjusted for systemic therapy plan, ER status and lymph node involvement. Distant events examined included: first distant recurrence (metastasis to any site), first recurrence in bone (even if concurrent with relapse in other sites) and first recurrence in non-skeletal sites.

Pilot-scale analysis of 462 patients revealed statistically significant association of BSP levels with first distant recurrence (whole population, $p = 0.005$; ZOL arm, $p = 0.013$) and with first event in non-skeletal sites (whole population, $p = 0.008$; ZOL arm, $p = 0.046$).

- CXCR4 levels displayed statistically significant association with first distant recurrence (whole population $p = 0.031$) and first recurrence in bone (ZOL arm, $p = 0.030$).
- DKK1 was not significantly associated with distant events in this analysis.
- No significant associations were found for other distant events

Our pilot studies suggest that BSP and CXCR4, but not DKK1 may have potential as biomarkers predictive of metastasis to bone and/or non-bone sites. However these are initial data and we are currently extending these analyses within a larger patient group.

P57

Functionalized Rare earth-doped Nanoparticles for Breast Cancer Detection and Potential Bone-targeting Contrast Agents

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Breast cancer is the second leading cause of cancer death among women and represents 14% of death in women around the world. Breast cancer metastasizes to bone in more than 80% of patients with advanced disease, causing severe skeletal related events and bone metastases are incurable. The standard diagnosis method for breast tumor is mammography, which is often related with false-negative results leading to therapeutic delays and contributing indirectly to the development of metastasis to bone. Here, we have established a nanoparticle platform using fluorescence and CT imaging for the detection of breast cancer and bone metastasis by conjugating folic acid (FA) to rare-earth-doped luminescent nanoparticles (RE)

RE were synthesized using sucrose-assisted combustion synthesis and functionalized with FA using EDC-NHS coupling. Transmission electron microscopy analysis indicated an average nanoparticle diameter of 55 nm. FA-conjugated RE nanoparticles exhibited strong red emission at 613 nm with a 35% quantum yield. MTT analysis demonstrated that the nanoparticles had negligible cytotoxic effect on normal 293T cells and T47D breast cancer cells *in vitro*. Cellular uptake analysis showed significantly higher internalization of FA-conjugated RE nanoparticles into T47D cells ($Folr^{hi}$) compared to MDA-MB-231 breast cancer cells ($Folr^{lo}$).

In vivo studies in CD1 mice indicated that FA-conjugated RE nanoparticles are rapidly cleared from blood circulation after 2h post-injection thus minimizing the risk of toxic response. Additionally, these nanoparticles were well tolerated in mice even at doses as high as 300 mg/kg, after i.v. injection. We next evaluated the targeting ability of FA-conjugated RE nanoparticles in xenograft models of MDA-MB-231 and T47D breast cancer cells. Confocal and CT imaging studies indicated that FA-conjugated RE nanoparticles accumulated more efficiently in T-47D tumor xenograft compared to the MDA-MB-231 tumor. Overall, our results showed that our FA-conjugated RE nanoparticles have high tumor specificity and biocompatibility representing an advanced tool that can be applied for preclinical and clinical applications Further studies are going on to evaluate bone targeting ability of surface functionalized RE nanoparticles in a murine model of bone metastasis.

P58

An agent based model of the bone remodelling process and its disruption by multiple myeloma.

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Living bone tissue is constantly in a dynamic state of damage and repair. The bone remodelling process requires a delicate equilibrium between bone resorption and formation, removing damaged bone and replacing it with new tissue respectively. The mechanisms that regulate this cycle are disrupted by the presence of multiple myeloma, a cancer of plasma cells. Myeloma causes upregulation of osteoclastic activity, increasing resorption, while inhibiting osteoblastic bone reformation, resulting in bone lesions and associated risk of fracture in patients. By better understanding the nature of the bone remodelling process and how it is affected by multiple myeloma, we can better target future research into novel treatments for this debilitating disease.

A computational model has been developed in order to provide a deeper insight into the complex interplay of regulatory factors present within the bone marrow microenvironment. This agent-based model (ABM) allows each individual cell within the environment to be represented as a distinct entity, allowing for a level of granularity not possible in traditional mathematical models. Our approach currently simulates osteoclasts, osteoblasts, osteocytes, precursor osteoclast cells, myeloma cells and bone cells, along with a variety of associated chemokines and other factors facilitating intercellular interactions. This prototype model has produced emergent behaviour consisting of a cycle of bone remodelling, including trauma applied to bone, detection of and reaction to this trauma by osteocytes, and finally resorption and formation by osteoclasts and osteoblasts respectively.

Future iterations of this generic, cellular-based model will be further refined based on available clinical data and introduce new actors to the system to better understand individual mechanisms. Our primary interests for extensions include TGF-β and counteracting anti-TGF-β treatment, RANKL/OPG interactions affecting the differentiation of osteoclasts and osteoblasts, and contemporary treatments for myeloma such as Lenalidomide, Zoledronic Acid and Bortezomib. By performing this type of *in silico* experimentation it is hoped that clinically relevant novel avenues of treatment can be identified for future mouse model studies.

MolPharm Poster abstracts

MP1

ABSTRACT WITHDRAWN

MP2

ABSTRACT WITHDRAWN

MP3

Teriparatide use among post-menopausal women: A meta-analysis

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BACKGROUND: In the most recent guideline on the management of osteopenia and osteoporosis published by the American College of Physicians, teriparatide was excluded as a treatment option. This exclusion has sparked a controversy among specialty groups involved in the management of the disease.

METHODS: Published reports of randomized controlled trials (RCTs) in English from August 2007 to August 2017 were sought via PubMed and Google Scholar. Twenty-one studies were identified, of which 7 were included in the meta-analysis. Data from these studies were pooled to determine the efficacy of teriparatide in decreasing incident fractures and increasing bone mineral density.

RESULTS: Teriparatide reduced the risk of fractures and improved the bone mineral density at the lumbar spine, femoral neck and total hip. Pooled data from 1535 post-menopausal women showed that teriparatide reduces the risk of fractures [risk ratio = 0.35 (95% confidence interval [CI], 0.41, 0.80)] with an overall effect, Z, of 3.31 (P = 0.0009). Greatest improvement was seen at the lumbar spine with a mean difference of 6.03% (Z = 6.82; P < 0.00001). More modest improvements at the femoral neck of 2.79% (Z = 2.29; P = 0.02) and total hip 0.64% (Z = 16.77; P < 0.0001) were also noted.

CONCLUSION: Teriparatide may be considered as a pharmacologic option among patient with decreased bone mineral density, especially at the lumbar spine, and/or at high risk for fractures.

MP4

Denosumab-like anti-RANKL treatment reverts osteolytic and osteomalacic changes in a murine model of Fibrous Dysplasia.

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Fibrous Dysplasia (FD) is a rare genetic disease of the skeleton caused by activating mutations (R201C, R201H) of the stimulatory G protein α subunit (Gsα). We previously generated transgenic mice that express $Gs\alpha^{R201C}$ under the control of ubiquitous and constitutive promoters (EF1α-$Gs\alpha^{R201C}$ mice, PGK-$Gs\alpha^{R201C}$ mice) and reproduce a FD-like skeletal phenotype. Studies performed on these mouse models demonstrated that disregulated bone resorption plays a major role in the development and progression of FD lesions and allowed us to identify RANKL as one important molecular mediator of the abnormal osteoclastogenesis associated with the disease.

The aim of this work was to investigate the effects of RANKL-inhibition on the development and progression of skeletal lesions in EF1α-$Gs\alpha^{R201C}$ mice.

Based on radiographic analysis, we selected 2 month old EF1α-$Gs\alpha^{R201C}$ mice with early lytic intracortical lesions in the tail vertebrae and femurs. We generated multiple experimental groups, each of which was treated with an anti-mouse RANKL antibody according to a different regimen. Control groups were generated by treating transgenic mice with Rat IgG2a Isotype antibody.

The anti-RANKL treatment caused an overall increase in radiographic bone density, with disappearance of lytic areas. The radiographic changes were due to the replacement of the pathological fibrotic tissue with newly-formed bone, Of note, the new bone deposited in anti-RANKL treated mice was mineralized and, at least in part, lamellar, in contrast with the osteomalacic and woven FD bone observed in control mice. Furthermore, the anti- RANKL treatment also prevented the development of bone deformities as well as the appearance of new lytic areas.

The comparison of different treatment regimens demonstrated that a loading dose in the first week and a dose interval no longer than 3 weeks were required to effectively revert FD tissue changes and to prevent bone deformities.

This study demonstrates that RANKL inhibition may be an effective strategy to treat established FD lesions and to prevent the progression of the disease. Furthermore, by comparing different treatment regimens in our mouse model we paved the way to the development of effective therapeutic protocols for human patients.

MP5

Visualization of immune response during initiation and progression of heterotopic ossification in mouse model of fibrodysplasiaossificansprogressiva (FOP)

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Fibrodysplasia ossificans progressiva (FOP) is a rare debilitating genetic disorder characterized by progressive heterotopic endochondral ossification of skeletal muscle and tendons. FOP results from missense mutations in the type I BMP receptor *ACVR1*, the most common being R206H. FOP mutations alter the sensitivity of ACVR1 to Activin A from an antagonist to an agonist. We have previously shown that Activin A is necessary for driving heterotopic ossification (HO) in murine FOP (MGI:5763014, $Acvr1^{[R206H]FlEx/+}$; $Rosa\text{-}CreER^{T2}$ mice) in part by showing that prophylactic inhibition of Activin A blocks HO. Natural histories of FOP patients indicate that HO is often associated with flare-ups, which might be due to a soft tissue injury or other inflammatory stimuli. In order to explore the role of inflammation in the initiation of HO in FOP mice, we visualized the homing of phagocytes to the site of injury. Phagocytes were tracked by intravenous administration of Exitron nano - radio dense nanoparticles that are readily taken up by phagocytes and can be imaged by *in vivo* μCT. Phagocytes are recruited to the site of injury rapidly following the injury-inducing event, and remain in place for at least 4 days post-injury. Phagocytes can also be tracked *in vivo* using probes that target myeloperoxidase (a marker of activated phagocytes) with chemiluminescence. Using this imaging modality, we demonstrated a sustained increased inflammatory response correlating with HO lesion progression. Our findings demonstrate that HO formation is preceded by an inflammatory response at the site of injury and that inflammation is sustained during the early stages of mineralization of the lesions. This data is consistent with the proposed model of how HO arises in FOP, i.e. that it is precipitated by inflammation-inducing events that result in the recruitment of phagocytes, and which in turn express Activin A, the key HO-inducing factor in FOP. The ability to mark the site of early HO-arising events using imaging modalities that are compatible with life, enables investigation of these early events in a longitudinal manner that can be coupled with *ex vivo* interogations such as histology and gene expression profiling.

MP6

Paget's disease of bone: are we missing something?

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Paget's disease of bone (PDB) is the second commonest metabolic bone disorder in the UK, but is underdiagnosed and undertreated. X –rays are one of the commonest means by which the condition is identified. A retrospective audit of 68873 X-rays was carried out in a tertiary hospital for eighteen months (January 2015 to August 2016) to ascertain

the number of Paget's cases reported by radiologist and whether they had been referred to secondary care for treatment. The search was for the key word of Paget's in the report and included: hands, abdomen, hips, knee, pelvis and lumbo-sacral spine. There were 43 patients with Paget's identified, of whom 65% were males and 35% females. The mean age of the cohort was 86.7 (age ranging from 65-99). Of these 43 patients 77% had established Paget's changes on previous X-rays. Pelvic imaging gave the maximum diagnostic yield of 65% of the cases with 0.2% of pelvic films reporting PDB. Of the 43 patients 32 (74%) had not been referred to secondary care for treatment. Despite lack of therapy 12% patients had joint replacement and only 2 of these had prior bisphosphonate therapy with the risk of excessive blood loss. A further 21 % had fractures of involved bone and moderate to severe osteoarthritis of adjacent joints was found in 81%. This may partly explain the lack of response found to treatment in the PRISM study. In the next 8 months from the end of the study period 30% (13 patients) died possibly reflecting the mean age of the subjects.

The alkaline phosphatase (ALP) was also reviewed. The value closest to the time of imaging or the highest level pre-treatment was analysed. The mean ALP level was 189 (range being 47-804 units/litre); 35% of the patients had normal ALP. In conclusion only a quarter of the patients are being referred to rheumatology despite potential benefits from bisphosphonate therapy and specialist input.

MP7

Variation in *RIN3* is associated with the onset and severity of Paget's disease and bone.

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Paget's disease of bone (PDB) is the second most prevalent bone disorder and is characterized by late onset development of focal lesions affecting one or several bones. In ~40% of patients, mutations in the *SQSTM1* gene are found. Genomewide association studies involved 7 novel candidate loci. *Ras and rab interactor 3* (*RIN3*), located at the associated 14q32 locus, encodes for RIN3, a guanine-nucleotide exchange factor (GEF) which activates several small GTPases of the Rab5 subfamily.

Using molecular inversion probe technology, we screened 189 patients and 165 controls of Western-European descent for variation occurring in the coding regions and 5'UTR of the *RIN3* gene. We identified 22 distinct variants. Based on *in silico* functionality predictions, several interesting and potentially deleterious variants can be distinguished. We identified 2 rare variants in the N-terminal region and the SH2 domain, respectively, that occurred only in control individuals. Four more rare variants were exclusively found in patients. These variants are located in the RIN-homology and VPS9 domains of the protein that mediate Rab5 binding and activation. Finally, a functional effect was also predicted for a common variant (p.R279C) described by Vallet *et al.*.

In addition, we identified 6 common variants for which additive regression models were fitted looking for significant associations between genotype frequencies and disease state, disease extent, or age of onset. Associations were only significant for the abovementioned p.R279C variant, which confirms the association with PDB reported by Vallet *et al*. The alternate allele occurs in a significantly higher rate in control individuals ($p = 0.01$), decreasing the risk for PDB (OR $p.R279C^{+/-} = 0{,}562$; $p.R279C^{+/+} = 0.315$). Our statistical analyses also demonstrated a modifying effect of this variant on the age of onset, with an increased age of onset of 5,336 years per alternate allele.

In conclusion, in our patient cohort, we identified rare variants that potentially alter RIN3 GEF activity. This could alter vesicular translocation and intracellular transport and affect osteoclast maturation and activity. Furthermore, we confirmed the previously reported association of *RIN3* with PDB and demonstrated that the rs117068593 variant in *RIN3* is a potential modifier for PDB pathogenesis.

Paget's guidelines

Paget's Disease of Bone

Paget's Disease of Bone: a clinical guideline prepared on behalf of the Paget's Association, The European Calcified Tissues Society, and the International Osteoporosis foundation.

Paget's disease of bone (PDB) is a relatively common skeletal disease in people of European descent but the evidence base for diagnosis and treatment is limited. Here we provide an evidence based update on previous guidelines for the diagnosis and management of PDB published between 2002 and 2015 using GRADE methodology.

The remit of the guideline is to provide patient-centred, evidence-based recommendations for the diagnosis and management of classical PDB in adults. It addresses the tools for the diagnosis of PDB, the effects of bisphosphonates and other drug treatments on PDB, the predictors of treatment response and the effects of non-pharmacological treatments. The guideline focused on classical PDB and did not consider the diagnosis or management of rare PDB-like syndromes. This guideline is not intended to serve as a standard of care. Adherence to the recommendations will not ensure a successful outcome in every case, nor should they be construed as including all proper methods of care or excluding other acceptable methods of care aimed at achieving the same result. The ultimate judgement must be made by the appropriate healthcare professional(s) responsible for clinical decisions regarding a particular clinical procedure or treatment plan. This judgement should only be arrived at following discussion of the options with the patient, covering the diagnostic and treatment choices available.

A Guideline Development Group (GDG) was established in January 2016 by the UK Paget's Association, the European Calcified Tissues Society and the International Osteoporosis Foundation, which incorporated a multidisciplinary panel of medical practitioners, a non-clinical scientist, a specialist nurse and one lay member (a patient with PDB).

The GDG identified six relevant key questions and used GRADE methodology for defining diagnostic questions and management options. An extensive literature search was undertaken, which initially searched for systematic reviews that addressed the study question followed by randomised controlled trials if no systematic reviews were available. If no randomised controlled trials had been performed, we searched for observational studies and case series provided the number of individuals studied was greater than 10. Individual case reports and case series of less than 10 subjects were generally excluded, unless these provided significant insights into the question that were not addressed by larger studies or clinical trials.The PDB guidelines process was validated in accordance with the Appraisal of Guidelines for Research and Evaluation, using the AGREE reporting check list 2016. The guidelines are now open for external review.

Recommendations within this guideline are based on the best available clinical evidence.

The following recommendations were highlighted by the guideline development group as those that should be prioritised for implementation.

1. Radionuclide bone scans, in addition to targeted radiographs, are recommended as a means of fully and accurately defining the extent of metabolically active PDB. *[based on very low-quality evidence from observational studies and the opinion and clinical experience of the GDG]*
2. Serum total ALP is recommended as a first line biochemical screening test in combination with liver function tests in screening for the presence of metabolically active PDB. If total ALP values are normal and clinical suspicion of PDB is high, measurement of either BALP or PINP may be considered. *[based on very low-quality evidence from observational studies and the opinion and clinical experience of the GDG]*
3. Bisphosphonates are recommended for the treatment of bone pain associated with Paget's disease. Zoledronic acid is recommended as

the bisphosphonate most likely to give a favourable pain response. *[based on moderate quality evidence from randomised clinical trials and the opinion and clinical experience of the GDG]*

4. It is recommended that in patients with established PDB, the treatment goal should be to control bone pain with bisphosphonates and other drugs as opposed to giving bisphosphonates with the aim of maintaining ALP concentrations in the reference range. *[based on low quality evidence from randomised clinical trials and the opinion and clinical experience of the GDG]*
5. Total hip or knee replacements are recommended for patients with PDB who develop osteoarthritis in whom medical treatment is inadequate. There is insufficient information to recommend one type of surgical approach over another. *[based on low quality evidence from observational studies and the opinion and clinical experience of the GDG]*

Abstract written by Dr. Stephen Tuck chair of the writing group on behalf of:
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Paget's Association Student Presentation

The role of small RNAs in Paget's associated osteosarcoma

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Small RNAs (sRNAs) are a class of non-coding RNA molecules that are key regulators of gene expression. SRNAs are also specific biomarkers due to their dysregulation in disease. Next generation sequencing is the gold standard for sRNA discovery, profiling and expression analysis. Bias has been found in different platforms of sequencing due to RNA ligase preference for sequence complementarity between sRNA and adapters. We developed high definition (HD) adapters to overcome the bias. We applied the use of HD adapters and sequencing to our studies of bone cancer. One of these studies investigated sRNA expression in Paget's associated osteosarcoma, a rare complication of Paget's disease of bone that carries a poor prognosis. We found that expression of a microRNA, miR-16, was highly expressed in Paget's associated osteosarcoma tissue when compared to controls and Paget's disease of bone. Bioinformatics analysis revealed miR-16 directly targets the sequestosome 1 (*SQSTM1*) messenger RNA. SQSTM1 protein has long been associated with Paget's disease of bone development. SQSTM1 was hypothesised to be involved with transformation to osteosarcoma as *SQSTM1* variants are positively associated with disease severity. We speculated that negative regulation of *SQSTM1* by miR-16 incapacitates SQSTM1's role in the Kelch-like ECH-associated protein 1 (KEAP1)-nuclear factor erythroid 2-like 2 (NFE2L2) pathway, a major cellular defence mechanism against oxidative stress and cancer development. Molecular testing may help provide a robust diagnosis and is particularly useful in rare cancers such as Paget's associated osteosarcoma where transformation is often missed until late stage. We are now investigating this biological data further, using single cell simultaneous genome and transcriptome sequencing.

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