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MEETING REPORT

Second Herbert Fleisch Workshop, 2016

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Meeting Report from the second IBMS/KU Leuven Herbert Fleisch Workshop Brugge, Belgium, 28 February to 1 March 2016.

The second Herbert Fleisch Workshop was held in the beautiful city of Brugge, Belgium, on 28 February to 1 March, 2016. After the success of the first workshop, held also in Brugge in 2014, the IBMS board decided to support the repeat event as one that could be of great benefit to young investigators—PhD students, early postdoctoral researchers and early faculty members. This target audience was effectively reached, with 53% of the participants being younger than 35 and even up to 84% being below 45 years of age.

The same model of organisation was pursued, with six senior investigators ('mentors') being invited, each to give an in-depth review of their area of research in basic, applied and clinical and translational bone biology. This was with the aim of education of course but also of inspiring the young participants in their work. A further task of the mentors was to engage fully with the participants throughout the meeting, discussing their poster and oral presentations and career directions. The mentors carried out these various functions assiduously, and their undivided attention throughout the several days of the meeting was greatly appreciated by the participants.

The meeting programme was further built and driven by the young investigator attendees presenting their ongoing work. Posters were available for discussion at all times throughout the meeting and in the evenings, an aspect that was greatly appreciated. In addition to presenting their posters, as many as 34 of the participants were invited to give oral presentations. In these ways, the meeting design allowed those submitting abstracts maximum exposure and many possibilities for an in-depth discussion of their work.

A new feature that was added to this second edition of the meeting was the presentation of three methodological interactive workshops by some of the mentors. In her workshop on 'Getting mouse phenotypes right', Natalie Sims (Melbourne, VIC, Australia) discussed the essentials and the pitfalls that can be encountered in evaluating skeletal phenotypes in mice. Matt Warman (Boston, MA, USA) conducted a workshop on 'gene editing using CRISPR/Cas9 technology', pointing the participants to current resources and websites available in this new and rapidly advancing field, which is anticipated to be as revolutionary as polymerase chain reaction has proven to be. The workshop by Claire Edwards (Oxford) on 'Mouse models of bone invasion by cancer' took participants through the advantages and limitations of the *in vivo* approaches to studying how solid and haematological malignancies make their way in the bone microenvironment. These three workshops were so popular that the faculty members undertook generously to conduct each workshop twice, to increase exposure. They were enthusiastically appreciated by all.

The Magical, Multifunctional Osteocyte

The opening session of the workshop focused on the osteocyte, a cell revealed as a most remarkable center of control in bone. Osteocytes comprise virtually 95% of all bone cells and function to control the response of bone to mechanical loading, as well as regulating the levels of calcium and phosphorus, and finally by acting as an endocrine organ, affecting kidney, and possibly also muscle. The remarkable properties of osteocytes were discussed in the opening lecture by Lynda Bonewald (University of Missouri, Kansas City, MO, USA), who pointed out that they are the longest lived of the cells of bone, crucial in sensing and then mediating the skeletal response to loading, as with deficiency of osteocytes—for example, too many empty lacunae—the response to loading is lost.[1] She also demonstrated that with ageing there is a profound loss of connectivity between osteocytes.

One aspect of osteocyte function that received special attention in the lecture was the control of the perilacunar matrix, an important source of calcium. Nanoindentation has identified a hypomineralised area within 5 μm of the osteocyte lacunae and the perilacunar space appears to have a low pH, suggesting that osteocytes may actively acidify this space. Osteocytes have been shown to express genes that are necessary for bone resorption, including TRAP, cathepsin K, v-ATPase and carbonic anhydrase I, and lacunar size was found to be increased during lactation in mice.[2] Interestingly, such an increase was not found in mice in which the PTH receptor (PTHR1) was knocked out specifically in osteocytes, indicating

that lactation-induced perilacunar resorption represents a PTHR1 signalling effect.

The location of osteocytes embedded deeply in bone has made them naturally very difficult to study, but progress was reported with the MLO-Y4 cell line and a new cell culture model, IDG-SW3 cells. These latter cells seem to recapitulate the differentiation process of osteoblasts into osteocytes, showing increased expression of osteocyte marker genes over several days in culture and increased expression of PTHR1. *In vitro* experiments using these cell models suggest that osteocytes might also target muscle.[3] Some early data presented at the meeting indicated that culture media from the MLO-Y4 cell line contained activities that could either enhance or inhibit myocyte differentiation of the C2C12 myoblast cell line, with this communication between osteocytes and muscle cells being strongly affected by age. Recent work had also shown *in vivo* that osteocytes produce factors that contribute to the age-related loss of muscle mass and function, as conditional deletion of the protease Mbtps1 in osteocytes led to an age-related muscle phenotype.[4] There is, however, one caveat here, as it may well be that the DMP1-Cre is also expressed in the muscle.

Lynda Bonewald also discussed some of the roles of osteocytes as regulators of phosphate homoeostasis and as endocrine cells regulating kidney through their production of FGF23, a topic further elaborated on by Seiji Fukumoto (Tokushima University, Tokushima, Japan) (see below).

Indeed, osteocytes and their functions featured prominently in several of the presentations, both keynote lectures and short orals. Osteocyte biology was also the subject of a plenary presentation by Natalie Sims (St. Vincent's Institute of Medical Research, Melbourne, VIC, Australia), who described striking and different effects in bone of disruption of three different signalling pathways.

First, the actions of gp130 cytokines on the osteocyte had been indicated by the fact that oncostatin M and other members of the gp130 family rapidly decreased sclerostin production by osteocytes, in much the same way as did PTH and PTHrP. Investigating this further by osteocyte-specific knockout of gp130 revealed that gp130 was needed for normal bone formation and the anabolic response to PTH and particularly was needed for normal lamellar bone formation.[5,6]

A second important pathway identified was ephrinB2 signalling. Having found that ephrinB2 production is rapidly increased by PTH and PTHrP *in vitro* and *in vivo*, mice rendered null (Osx-Cre) for the ephrinB2 gene exhibited a block in mid-differentiation, with a substantial increase in apoptosis of osteoblasts, increased mineralisation lag-time and impairment of the PTH anabolic response.[7–9] With knockout of ephrinB2 in the osteocyte, however, there was no impairment of bone formation but a delay in mineralisation, which was shown to be due to the fact that ephrinB2 is needed to maintain collagen structure and mineralisation.[10]

The third pathway of interest was revealed through osteocyte-specific knockout of suppressor of cytokine signalling-3 (SOCS3). SOCS3 is an intracellular, ubiquitous, cytokine-inducible negative regulator of cytokine signalling, which functions to limit the action of gp130 cytokines in a negative feedback mechanism suppressing the JAK-STAT signalling pathway. The DMP1-Cre-driven SOCS3-knockout mice were found to have poor corticalisation of bone, an effect that presented in a sex-divergent way with much more marked findings in female mice. Oestrogen treatment of either orchidectomised males or ovariectomised females recapitulated the phenotype, suggesting that an oestrogen-dependent inhibition of the corticalization process resulted in marked improvement of cortex formation.[11]

These three signal pathways having different outcomes in earlier osteoblasts than in osteocytes (gp130 and ephrinB2) illustrated several aspects of osteocyte function, including a role for the osteocyte in the regulation of bone mass and collagen orientation, bone composition and mineralisation, and organisation of the trabecular and cortical compartments. Moreover, interesting physiological questions arise from the magnitude of the osteocyte network. Mathematical approaches have been used to estimate the size of the enormous network that comprises the osteocyte connections, with an analogy made between osteocytes and neurons.[12] There are estimated to be 42 billion osteocytes—comparedwith 86 billion neurons—with 175 000km of osteocyte processes and 150–180 000 km of dendrites. Clearly, the osteocyte network is a highly complex communication network, of the same order of magnitude as current estimates of the neural network in the brain, adding perspective to the many local and endocrine functions of the osteocytes.[12]

The osteocyte came up frequently for discussion throughout the meeting in varying contexts, and some of the short presentations had the osteocyte as a focus. Scott Youlten (Sydney, NSW, Australia) prepared bone marrow-deprived long bones and calvaria from mice to undertake transcriptome sequencing, identifying a 1126-gene osteocyte signature, which included the expected osteocyte genes, as well as 73 long non-coding RNAs that were actively expressed in every replicate of each bone type. Many genes had not previously been annotated with osteocyte biology. Although 87% of the osteocyte genes were found to be expressed in all bones examined, a small group of related transcription factors (mostly Hox-family genes) could delineate certain bones, for instance by a restricted expression in hind limb bones. The data from this work will provide a useful resource. Niloufar Ansari (Melbourne, VIC, Australia) reported on the phenotype of mice in which PTHrP was knocked out in the osteocyte, with the outcome indicating that osteocyte-derived PTHrP is required to maintain trabecular bone mass and the material strength of adult bones. She also reported findings with production and knockdown of PTHrP in the OCY454 osteocyte cell line, to show that PTHrP acts as an autocrine and a paracrine factor. If that is indeed the case in osteocytes *in vivo*, it could perhaps explain the PTHR1-dependent increase in perilacunar size described by Lynda Bonewald.

Kidney–Bone Communication

Another major function of the osteocyte is to act as an endocrine organ, as has been increasingly realised over the last several years. This is especially through production of FGF23, a phosphotrophic hormone, as discussed in detail by keynote speaker Seiji Fukumoto (Tokushima University, Tokushima, Japan). He outlined the several ways in which FGF23 decreased circulating levels of phosphate. It acts on the kidney to decrease NaPi IIa and IIc to decrease tubular reabsorption of phosphate, and to decrease the formation of 1,25-dihydroxyvitamin D, with both mechanisms contributing to the low circulating levels of phosphate.

Abnormalities of FGF23 structure, metabolism or secretion are associated with a number of disorders, particularly X-linked hypophosphatemic rickets (XLHR) and tumour-induced osteomalacia (TIO). In the former condition, in a mouse model, an antibody against FGF23 corrects the disturbed phosphate levels, improves the skeleton, and corrects the disorganised growth plate.[13] Efficacy has been shown in a clinical study of 28 patients with XLHR.[14] The higher the levels of circulating FGF23 in chronic kidney disease (CKD), the higher the mortality.[15,16] A humanised monoclonal antibody is effective in TIO.[17,18] Anti-FGF23 is also effective in early CKD in rats in increasing 1,25-dihydroxyvitamin D and decreasing serum phosphate and PTH levels but is ineffective in late-stage CKD.[19]

Some of the interesting unresolved questions with FGF23 were discussed: (1) how does it work in the kidney, with its co-receptor Klotho expressed in the distal convoluted tubule but the action on phosphate in the proximal tubule? (2) what triggers the increase in FGF23 in early CKD, and (3) why is FGF23 associated with certain adverse events, for example cardiovascular effects, fractures and mortality in kidney disease?

There was much interest in this area also among the short communications. Despina Sitara (New York, NY, USA) had recently reported that genetic inactivation of FGF23 in mice resulted in increased hematopoietic stem cell frequency and red blood cell production, and, inversely, infusion of FGF23 in wild-type mice reduced erythropoiesis.[20] Having shown that FGF23 decreases erythropoiesis, she now used a mouse model of CKD (5/6-nephrectomy) to show that the increased FGF23 was associated with reduced erythropoietin levels and erythroid cells, which could be restored by administration of an FGF23 blocking peptide. Inhibition of FGF23 in this CKD model was found to decrease apoptosis of erythroid cells, improve erythropoiesis, and rescue anaemia. Nina Bon (Nantes, France) studied the mechanism of the phosphaturic effect of FGF23, looking at the high affinity low capacity phosphate transporters, PiT1 and PiT2, producing evidence for a role of both in the phosphate-sensing mechanisms in the kidney. An interesting aspect of FGF23 regulation was reported by Yuichi Takashi (Tokushima, Japan). It was known that O-glycosylation of FGF23 prevents the cleavage of FGF23 to inactive fragments, an effect that is prevented by GalNac-T3, a product of the *GALNT3* gene. He showed that overexpression of GANLT3 *in vitro* increased the release of active FGF23. Most interesting though, a high phosphate diet in mice increased *GALNT3* expression in bone, as well as increasing circulating FGF23. A role for extracellular phosphate levels in post-translational processing of FGF23 would have important implications in CKD.

Energy Metabolism of Bone Cells

Each of the plenary speakers had new insights to offer, as well as review material relevant to their work. In the case of Fanxin Long (Washington University, St. Louis, MO, USA), we were taken to a new look at energy metabolism in bone cells, particularly osteoblasts. Quite some decades ago, there had been interest in the production of lactate in the metabolism of bone, and in those days it was wondered whether the lactate might contribute an acid environment that would favour the dissolution of bone. What Fanxin Long has done is to relate increased aerobic glycolysis to anabolic events in bone—both through PTH and through Wnt signalling.[21,22]

Increased metabolism of glucose through glycolysis has been recognised for a long time in cancer cells (the 'Warburg effect'), where the intermediates of glycolysis are used by the pentose phosphate pathway to increase cell proliferation. What is shown now is that this is not the case in osteogenic cells. Using the ST2 mesenchymal cell line, it was found that Wnt3a signalling suppresses the pentose phosphate pathway and produces energy by stimulating glutamine catabolism through the tricarboxylic acid (TCA) cycle, with the lower glutamine resulting in enhanced transcriptional activity of ATF4 and increased osteoblast differentiation. He showed that anabolic Wnts such as Wnt3a also increase aerobic glycolysis at the expense of TCA entry, resulting in a reduced availability of acetyl coenzyme A associated with reduced histone acetylation, including at the PPARγ gene promoter. This mechanism in fact could provide a link between the cell's metabolism and cell fate decisions and explain the preferred osteoblastic (versus adipogenic) differentiation of bipotent ST2 cells in response to Wnt signalling. Consistent with these findings, LRP5-mutated knock-in mice with high bone mass (HBM) showed a partial correction of the HBM phenotype in response to administration of either a glutaminase inhibitor[23] or a glycolysis inhibitor.[21] The signalling pathways for these Wnt-LRP5 effects were noted to be mTORc1 for glutaminolysis and the anabolic effects and mTORc2 for increasing aerobic glycolysis.

Of much interest is that the glycolysis pathway is used to similar effect with PTH. PTH increases glycolysis in osteoblasts, doing so through the mediation of IGF1 signalling. Notably, suppression of glycolysis inhibited the anabolic effect of PTH in mice.[22]

Overexpression of the key transcription factor that orchestrates the response of cells to low oxygen tension, the hypoxia-inducible factor (HIF)-1α, was also found to exert an anabolic effect on bone. Intriguingly, the increased bone mass in these mice expressing a stable form of HIF-1α appeared to be independent of VEGF but dependent upon increased glycolysis, as administration of the glycolysis inhibitor dichloroacetate (DCA) could reverse the phenotype.[24] Moreover, the physiological importance of glycolysis in osteoblast lineage cells was supported by new data showing that conditional inactivation of lactate dehydrogenase A (LDHA), the enzyme that catalyses the inter-conversion between pyruvate and lactate, results in reduced bone formation and bone mass.

The attention being drawn now to these biochemical pathways offers new approaches to the understanding of osteoblast function and bone formation, particularly with regard to oxygenation of tissue, where it has been known for some time that reduction in oxygenation leads to increased glycolysis.

Several short talks in this area of research explored the cellular and patho-physiological consequences of altered signalling through hypoxia-inducible pathways. Naomi Dirckx (Leuven) showed that stimulation of HIF activity in bone cells of genetically modified mice led to excessive glycolysis in osteoblast lineage cells, associated with a marked, progressive increase in bone volume and, intriguingly, with systemic alterations, particularly severely affecting their whole-body glucose metabolism. In the presentation of Claire-Sophie Devignes (Paris), we learned that changes in the local bone microenvironment elicited by excessive HIF activity, through deletion of the tumour suppressor and negative regulator of HIF activity Von Hippel Lindau (Vhl), could promote breast cancer

growth and lead to an increased susceptibility of mice to develop metastatic lesions. The mechanism underlying this effect was found to involve enhanced expression of CXCL12 in the bone and bone marrow environment, acting systemically to stimulate the growth of primary mammary tumour cells (expressing the CXCR4 receptor of CXCL12/SDF-1) and their metastatic dissemination to distant organs. The roles of HIF signalling in osteoclasts were investigated by Helen Knowles (Oxford, UK), by using *in vitro* osteoclastogenesis assays and mice lacking either of the key oxygen-sensing enzymes upstream of HIF, termed prolyl hydroxylases. She concluded that HIF-1a mainly affects osteoclast-mediated bone resorptive activity, while having less pronounced effects on osteoclast differentiation.

The Wnt- LPR5 Signalling Pathway in Bone

Of course the effects of Wnt signalling on bone, first noted only a little over 10 years ago, are so marked that they always have to remain a subject of interest. We were given further insights into this by Matthew Warman (Harvard Medical School, Boston, MA, USA), whose approach to this pathway comes through both human and mouse genetics. An important lesson is that, although mouse genetics can be useful in many ways, it is safer to think of its effects in parallel with what is known about human genetic mutation effects on the skeleton.

Matthew Warman pointed to the fact that a careful analysis of the expression of mRNAs for many Wnts in bone marrow, muscle and bone showed their relative expression levels. In bone, the most abundantly expressed was Wnt10b, with in decreasing order Wnt4, Wnt16, Wnt5b, Wnt5a, and then others.[25] Overexpression of Wnt10b or its knockout gave results that might have been predicted, with the former mice having increased bone and the latter decreased. On the other hand, human mutations of Wnt10b resulted in only split hand and split foot syndromes, suggesting quite significant differences in importance between mouse and human. Of considerable interest is the fact that, although Wnt1 showed a very low expression in mouse bone, human loss of function of Wnt1 results in a brittle bone disease, and, in mice in which Wnt1 is knocked out, there is a decrease in both trabecular and cortical bone.[26]

A mouse genetic experiment was described that illustrates a proof-of-concept that could be useful in human therapeutics. Mice with genetically induced HBM (by carrying the LRP5 HBM point mutation[27]) were crossed with osteogenesis imperfecta (OI) mice, with the result that enhanced LRP5 signalling increased the amount of bone in each of a few OI models, resulting in increased bone mass, skeletal strength and fracture resistance.[28] Although this is certainly promising, the question was asked why making more bone improves the outcome in OI models, even though the collagen abnormality remains? The thought was offered that perhaps this was the result of increased amount of collagen per unit of matrix.

Other Insights into Skeletal Abnormalities from Genetic Studies

The power of genetic approaches in mice and other animal systems for getting increased insights in human pathologies and developing potential therapeutic advances was also evident from many posters and short orals throughout the meeting. For example, Antonio Maurizi (L'Aquila) presented promising results in mice on the use of highly specific, mutation-targeting small-interfering RNAs as a novel experimental therapy for chloride channel type 7 (CLCN7)-dependent autosomal-dominant osteopetrosis type 2 (ADO2), a human disease caused by heterozygous dominant-negative mutations in the CCL7 gene that lead to osteoclast dysfunction. In the same research group, Mattia Capulli (L'Aquila) closely studied the behaviour and brain of the ADO2 mice, finding that the CCL7 mutation was associated with changes in the central nervous system (CNS). These important observations call for an increased awareness and evaluation of potential CNS involvement and neurological complications in ADO2 patients. In the presentation by Charlotte Gistelinck (Ghent), we were taken into the clinically relevant experimental possibilities beyond mice. She developed a zebrafish model to study the pathogenetic mechanisms leading to Bruck syndrome, a disorder reminiscent of OI and characterised by fractures. One of the causative genes is *PLOD2*, encoding a lysyl hydroxylase (LH2) that acts upon type I collagen telopeptides and thereby affects the stability and strength of the collagen fibrils. Here, a nonsense mutation in *PLOD2* was introduced in zebrafish, resulting in musculoskeletal abnormalities and a disturbed collagen organisation in bone, underscoring the significance of this model to study Bruck syndrome.

Cancer-Induced Bone Disease

Claire Edwards (University of Oxford, Oxford, UK) undertook the task of discussing how tumour cells interact with the bone microenvironment, beginning by pointing out that, in the case of solid tumours, these may be predominantly osteoclastic, osteoblastic or mixed in nature. Whichever is the case, interactions between the tumour cells and the microenvironment of bone are of the utmost importance. That environment is now recognised to consist not only of osteoblasts and osteoclasts but also stromal cells, adipocytes and lymphocytes.[29]

The earliest work on establishment of tumours in bone took place with solid tumorus, particularly breast cancer, where the role of the osteoclast was thought to be dominant. In that case, the tumours were shown to produce factors that were capable of promoting osteoclast formation by acting upon the host osteoblasts to generate the osteoclastogenic signal RANKL. The responsible tumour products were thought to be PTHrP and certain other cytokines such as IL-11. It is increasingly realised that cellular components of the bone are important in both establishment of tumour in bone and its growth and advancement.

The condition of multiple myeloma (MM) was discussed in some detail, with a major question being: how do changes in the bone microenvironment favour MM establishment and progression? MM is a major cause of blood cancer deaths in many countries, and recent data indicate that it is consistently preceded by its precursor condition, monoclonal gammopathy of unknown significance (MGUS). Although MGUS can clearly be recognised, not all patients with MGUS will progress to MM—over a period of 20 years about 16% will do so. MM is a condition that profoundly affects the skeleton. It does so by causing multiple lytic lesions in the skeleton but also by causing a generalised bone loss, which is recognised as a cause of

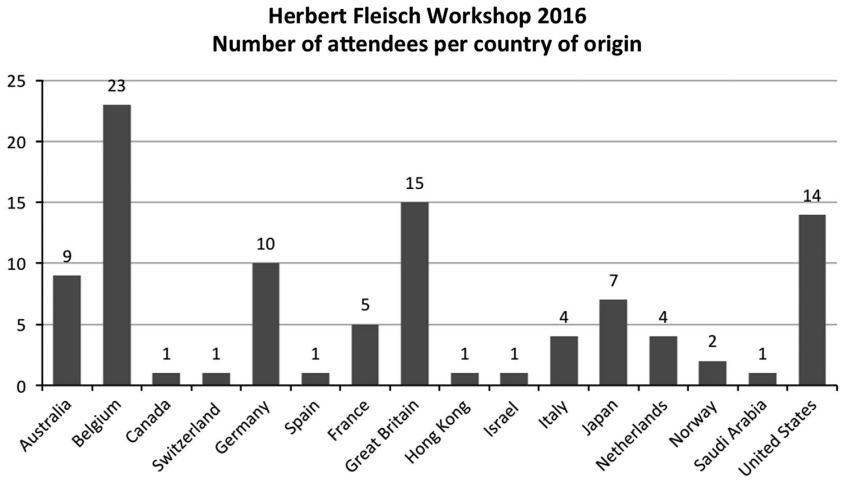

Figure 1 International distribution of participants in Workshop.

'secondary' osteoporosis. It is also important to note that there is clear evidence of bone loss in MGUS.[30]

Much useful information has come from the use of a mouse model of MM, known as the 5T myeloma model, in which 100% of the mice develop MM, with bone disease and paraprotein evident. These MM cells need to be grown and propagated in a sub-strain of C57Bl6 mice, known as KaLwRij mice. Interestingly, although 5T cells alone do not induce MM in C57Bl6 mice, co-injection of bone marrow stromal cells from KaLwRij mice together with the MM cells did lead C57Bl6 mice to grow this tumour and develop MM.[31] This effect was found to be dependent on the Wnt inhibitor Dkk1, which is increased in MGUS. This important observation illustrates how the bone marrow environment can profoundly influence MM development and, consequently, how pharmacologically manipulating the tumour's interactions with this niche is a major driving force in current myeloma research.[32]

As a next step, micro-array analysis comparing the bone marrow of non-permissive C57Bl6 mice and permissive KaLwRij mice was performed, indicating that the latter had decreased the levels of adiponectin. The possibility was examined that a decrease in adiponectin in a host might increase MM progression. In support of this, increased burden of MM was found when the cells were injected into adiponectin-null mice, and the conclusion from these and other experiments was that increasing adiponectin pharmacologically reduces MM growth and advance.[33] Adiponectin is inversely related to obesity, and diet-induced obesity in KaLwRij mice promoted the growth of MM and its advance in bone with the development of lytic lesions.[34] A clinical observation relevant to this is that obesity has been found to be associated with increased MGUS.[35]

The links between tumour growth and the cellular components of bone are such that a number of specific pathways are being identified that either enhance or oppose tumour growth; hence,the area is one that merits increasing attention in bone biology research. A variety of related questions were addressed in short presentations at the workshop. Hanna Taipaleenmaki (Hamburg) investigated a microRNA (miR-218) that they had previously shown to be highly expressed in osteoblasts and to promote osteogenic differentiation. It was found to be highly expressed also in bone-metastasizing breast cancer cells, MDA-MB231. When miR-218 was delivered to the breast cancer cells, it promoted tumour growth in bone with increased osteoclast activity and bone resorption, whereas antagonising miR-218 prevented the development of osteolytic lesions. An inhibitory effect of Wnt5a has been reported, and Stefanie Thiele (Dresden, Germany) investigated the mechanism in three human prostate cancer cell lines, showing that Frizzled 5 and RYK mediate the anti-proliferative and pro-apoptotic effects of Wnt5a in prostate cancer cells. In two short presentations, tumour-derived exosomes were found to enhance osteoclast formation. Muhammad Zahoor (Trondheim, Norway) found this in MM cells where the responsible agent was Il-32 in exosomes. Irene Bijnsdorp (Amsterdam, The Netherlands) found that exosomes from three human prostate cancer cell lines were taken up by monocytes *in vitro*, thereby enhancing osteoclast formation in response to RANKL stimulation. Such mechanisms influenced by cancer-derived exosomes clearly are worth exploring further.

Although the emphasis was on the tumour invasion by bone in these presentations, as is usually the case, it was interesting to have work presented concerning the major primary tumour of bone, osteosarcoma. Agi Grigoriadis (London, UK) presented results with the c-fos oncogene-induced osteosarcoma in the mouse that results in a highly malignant, metastatic tumour that expresses high levels of Fgfr1 mRNA and FGFR1 protein, associated with sustained activation of MAPKs and increased anchorage-independent growth in response to FGF2. Orthotopic injection of osteosarcoma with knockdown of FGFR1 resulted in a marked decrease in lung metastases, and a similar effect was obtained *in vivo* with the administration of a small molecule inhibitor, AZD457. The prospect of using antiFGR therapies was therefore raised.

Concluding Remarks

In the above not every presentation is mentioned, the range of topics was large and also included stem cell biology, imaging techniques for bone and insights into pathologies that are less frequently discussed, such as osteophytes and periodontal disease. There was robust discussion after every presentation, which continued during the excellent lunches organised by our hosts in the Novotel Brugge. Overall, the meeting allowed participants to find new collaborators, learn from the invited

Figure 2 Group photograph of Workshop participants.

mentors and from each other, get feedback on their work and generally network. The meeting was complemented by a social programme including a wonderful evening with dinner in the Brewery 'De Halve Maan', where we were welcomed by a glass (or more) of 'de Brugse Zot', their trademark beer. We were also very warmly welcomed in the impressive City Hall of Brugge where the chief of protocol introduced us on the long history of Brugge and especially the important role of the medieval city hall for the protection of the city rights of 'het Brugse vrije' (fee state of Brugge). He also emphasised that the city now attracts more than 5 million visitors per year and invited all participants to take time to enjoy the richness of the diversity of cultural and culinary aspects of the city. Needless to say that this was followed by a reception in the historic building.

On the final day, a short guided tour of the city was a fitting finale to this meeting. Undeterred by the rain, many participants joined our two knowledgeable local guides who took us to key sites such as the Begijnhof, the Belfry and the Church of our Lady, where we saw the statue 'the Madonna of Bruges' created by Michelangelo.

The survey at the end of the meeting was completed by 80+ % of the participants, and, they scored the science of the meeting as excellent (68%) or good (29%), whereas 99% 'loved or liked' the city of Brugge. The meeting was attended by about 100 people coming from around the world (see **Figures 1** and **2**). The broad international attendance was enormously facilitated by the generous support of sponsors (see acknowledgements). In addition, apart from IBMS itself, the American Society of Bone and Mineral Research (ASBMR), the European Calcified Tissue Society (ECTS), the Japanese Society for Bone and Mineral Research (JSBMR), the Australian and New Zealand Bone and Mineral Society (ANZBMR) and the Belgian Bone Club all provided travel grants, resulting in an impressive 36 travel awards in total, and such that, effectively, this last IBMS meeting already functioned as one organised by the International Federation of Musculoskeletal Research Societies (IFMRS).

What next?

IBMS was the organiser of the last meeting in Davos (2009) and of two H Fleisch workshops in Brugge (2014 and 2016). However, IBMS has been merged in 2016, together with ECTS, ASBMR, ANZBMS, JSBMR, SIBOMM, ORS and ICMRS, into the IFMRS. The IFMRS purpose is to advance musculoskeletal research globally in order to prevent and treat musculoskeletal diseases by collaborating with international societies to share resources, raise public awareness and provide education.

The organisers of the meeting as well as most of the attendees hope that the strengthened global society will maintain the spirit of the Davos/Brugge meetings and, while learning from experience, will continue to provide a forum for young bone scientists to attend a meeting that fulfills their needs for active interaction with their young colleagues and learn from the rich experience of a few international leaders in this field.

Conflict of Interest

The authors declare no conflict of interest.

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