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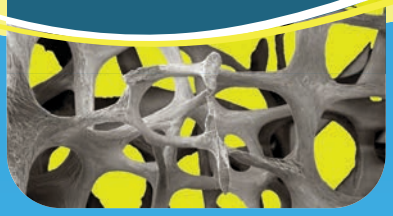

44th European Calcified Tissue Society Congress
13-16 May 2017, Salzburg, Austria

Congress Report

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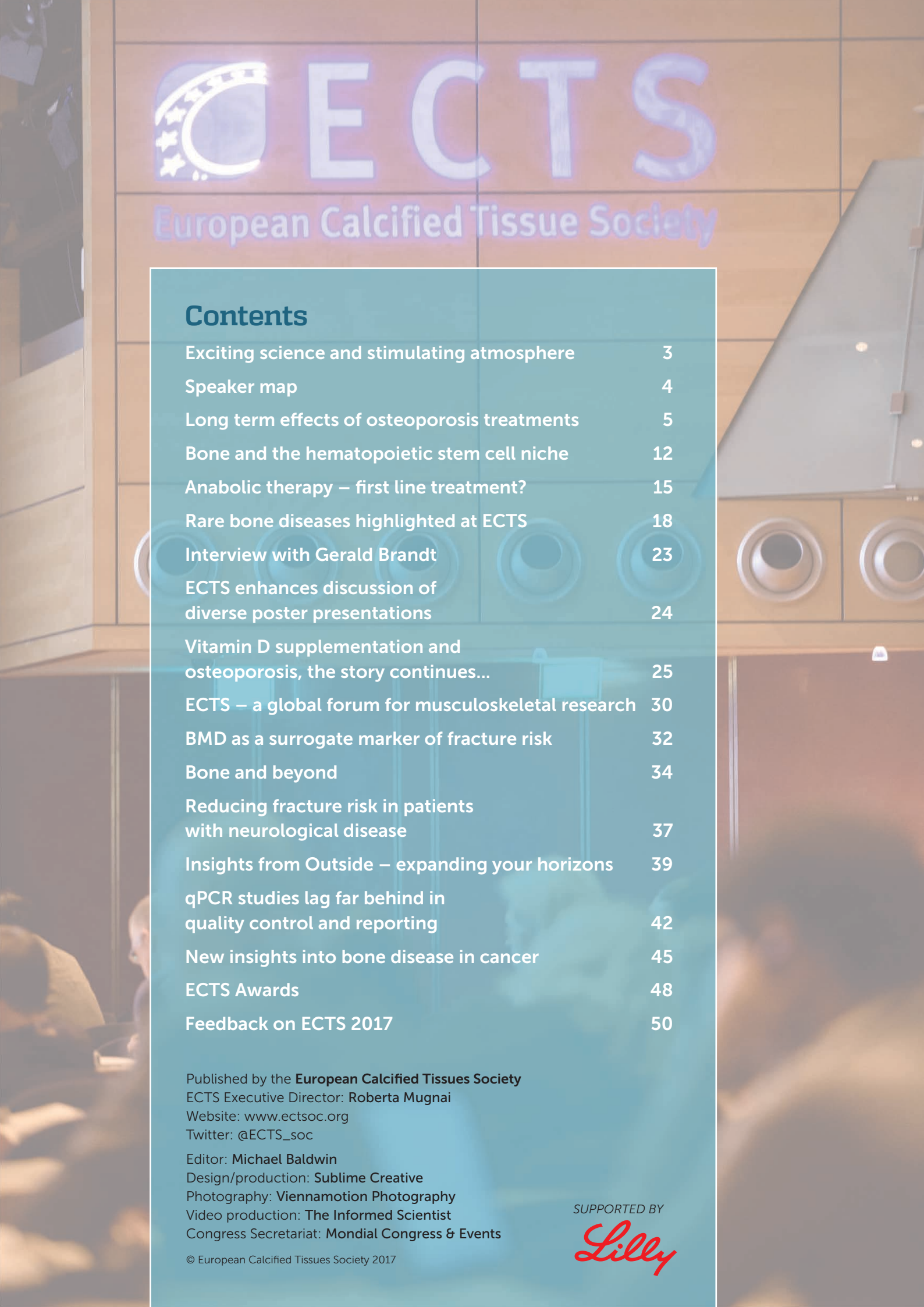
European Calcified Tissue Society



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Exciting Science and Stimulating Atmosphere

ECTS Past President Claus-C. Glüer looks back at the ECTS 2017 Congress, and forward to an exciting future

Thank you to all 1000+ congress participants, speakers and sponsors of ECTS 2017 – you made this a truly outstanding event! It was a pleasure and honour to chair this meeting as the final activity of my three years as president of ECTS.

We are looking back at a most successful congress with four and a half days packed with superb science. The meeting was characterized by a truly collegial and highly stimulating scientific atmosphere, and Salzburg proved to be the perfect venue for our annual convention. During and after the meeting we received a lot of positive feedback and we take this as encouragement for continued development and diversification of ECTS activities in the future.

Only at ECTS!

What makes ECTS a favourite among bone specialists, including opinion leaders, clinical and basic science researchers, as well as new investigators?

Reason one: Top quality of the speakers and a choice selection of up to date traditional and innovative topics. To phrase it differently: *Be there and you know where the field stands and where it is going to move in the future!*

Reason two: ECTS' reputation for objective and unbiased science.

Reason three: Great combination of basic and clinical science, enabling translation of lab science to improvement of patient care.

Reason four: Communicate in a stimulating atmosphere with colleagues from all over the world

Beyond this there are a number of distinctive programme features that make the ECTS Annual Congresses truly special and unique. In 2017 this included special training courses on biomarkers, highly interactive formats of the poster session (Ask your peers), five working groups for special interests, and exceptional face-to-face interaction at Meet-The-Expert sessions. And finally, the typical style of an ECTS congress with plenty of possibilities for networking.

I was impressed to see so many new investigators presenting their excellent research with great dedication and enthusiasm. No doubt, ECTS is the home of the next generation researchers in the musculoskeletal area! Just before Salzburg the third generation of ECTS Academy members was selected from a very strong group of applicants. While Academy membership is clearly a special honour, importantly it entails the responsibility to contribute to the society. And the members of the ECTS Academy delivered in a very strong way! They put together a very attractive programme for new investigators, they were notably present in the discussion of the lectures, and they contributed a lot to the great spirit of the meeting. Thank you very much for your dedication!

These programmes and the top international speakers presenting also attracted several external groups to come to Salzburg, to make ECTS their home this year: we again hosted the European Research Consortia Summit with 10 consortia in attendance, the East-Meets-West programme was again carried out with our prominent partners from the leading bone societies in China, Japan, and Korea, the Central and Eastern Europe Osteoporosis Summit group organized a workshop at ECTS with representatives from 10 countries present, and a wonderful and inspirational Fishbone Workshop was held the day before ECTS started.

Reading this Congress Report will provide insight into the great science that was presented and discussed in Salzburg. But nothing beats the real thing – being at the Congress, meeting the top experts face to face and enjoying stimulation interaction with fellow researchers and clinicians. So mark your calendar for ECTS 2018 in Valencia, with arrival suggested on Friday 25th May, 2018 (for participation in the fascinating pre-congress programme on Friday afternoon and the educational programme on Saturday morning), and departure on Tuesday 29th May. Valencia is going to be a great venue, and ECTS President Anna Teti and her team will bring the world's top experts to ECTS 2018, a meeting not to miss next year. Hope to see you there.

And in the meantime, watch out what ECTS has to offer in addition to the congress: find out by subscribing to our ECTS Newsletter, watch the ECTS Webinars, participate in ECTS Training Courses and educational activities and more to come... Stay connected throughout the year because our research field is dynamic and exciting!

Claus-C. Glüer, *ECTS Past President*

ECTS SALZBURG AUSTRIA 13-16 MAY 2017

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14 European countries

17 speakers from
4 additional continents

CEE Osteoporosis Summit
Delegates from 10 countries in Central & Eastern Europe

CEE Osteoporosis Summit Working Group

ECTS 2017, Salzburg

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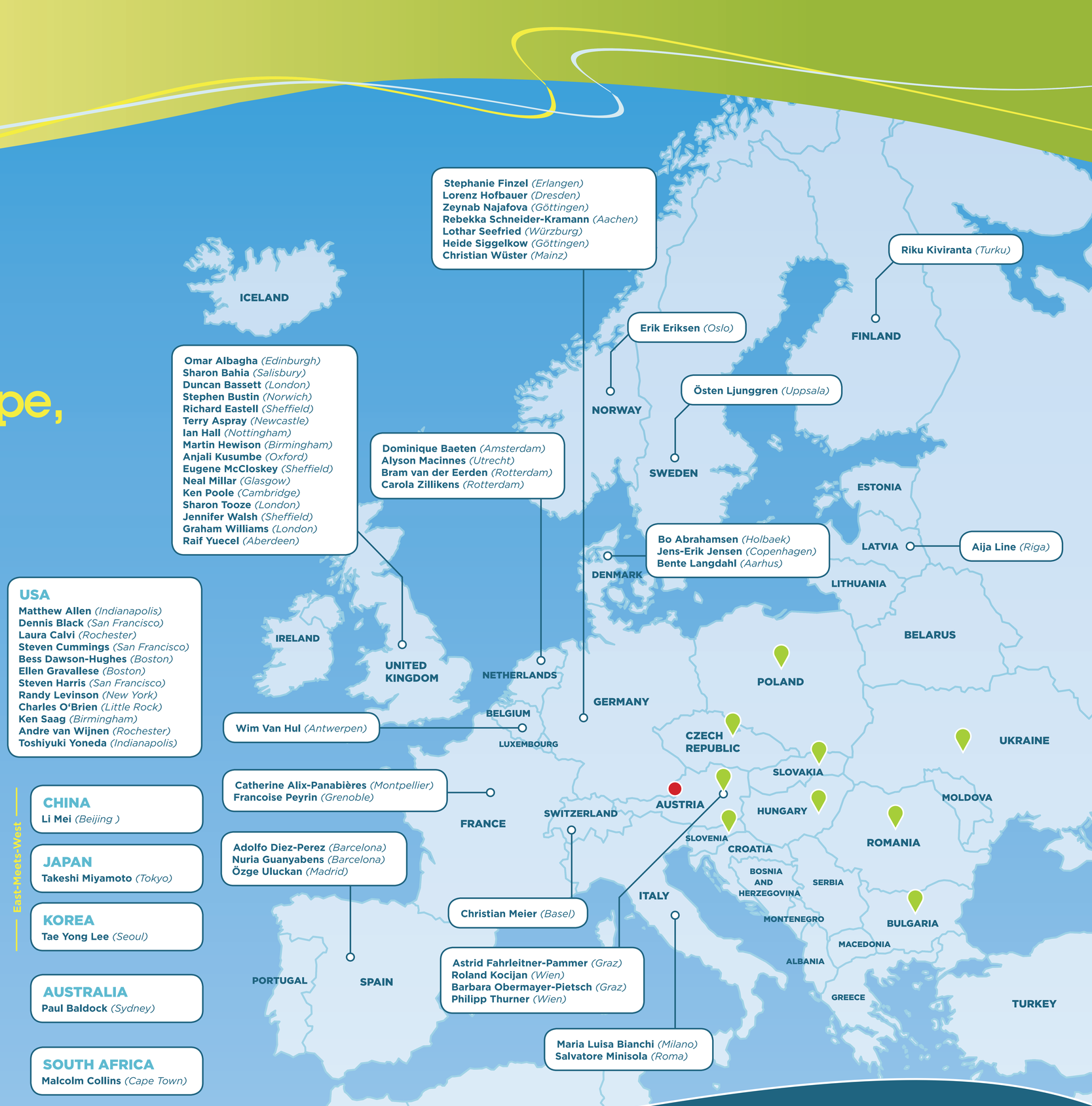

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Long term effects of osteoporosis treatments

At the ECTS 2017 symposium "Osteoporosis – a chronic disease that needs long term treatment" we heard the latest clinical evidence concerning long term efficacy and safety of available treatments for osteoporosis.

Long term management of osteoporosis

The first symposium speaker, **Astrid Fahrleitner-Pammer** (Austria) reviewed results from recent clinical trials to discuss the long term efficacy and safety of the main classes of osteoporosis treatment.

Bisphosphonates

The FLEX trial investigated postmenopausal women treated with alendronate for 5 years, who were then either moved to placebo or continued with alendronate for a further 5 years. While a moderate decline in BMD was recorded along those that transferred to placebo, no significant difference in fracture risk was found between the two groups, with the exception of clinical vertebral fractures. The study concluded that continuation of alendronate may be beneficial for those at higher risk of clinical vertebral fractures.

The multi-centre, double blind HORIZON-Pivotal Fracture Trial studied long term treatment with zoledronic acid. Already, a decrease in vertebral fractures was observed as a result of continuing therapy up to 6 years. An extension of the study of 190 women for a further three years found no significant difference in hip BMD or fractures between those who moved to placebo after six years, and those that continued with zoledronic acid up to nine years. A small increase in cardiac arrhythmias was noted in the zoledronic acid group, but no other differences in adverse effects. The study therefore concluded that almost all patients treated for six years may stop treatment for a further three years without additional significant risk of fracture.

Denosumab

The effects of denosumab on BMD are reversible, so in principle treatment should be maintained. A 7 year extension of the international phase 3 FREEDOM trial of denosumab enabled the collection of up to 10 years of data in postmenopausal women with osteoporosis. A total of 2626 women completed the extension period. BMD was found to increase progressively in all women receiving treatment, but those who had been treated with placebo prior to the extension period had lower BMD at every point of the extension. Rates of fracture and adverse events remained low during the extension, similar to those during the first three years. Participants in the FREEDOM trial that discontinued use of denosumab after three to five years were also studied, and found to have no additional risk of fracture for up to 24 months after ceasing their treatment, compared to those on placebo during the trial.

However, at ASBMR in 2016, Brown et al. reported that new vertebral fracture risk was increased among the FREEDOM participants that had discontinued denosnumab, if they had experienced a previous vertebral fracture. Therefore, continuation of denosumab was recommended in cases of prior vertebral fracture and those otherwise at higher risk of vertebral fracture, while other patients discontinuing denosumab should consider a transition to another therapy.

Teriparatide

The EFOS prospective, multinational study investigated postmenopausal women receiving 18 months' treatment with teriparatide, mostly after previous treatment with

In other news...

Our educational programme reviewed the latest developments in the bone field from around the world. In this report, look out for the **In other news...** sections highlighting some of the exciting papers selected by our speakers **Duncan Bassett** (UK) and **Salvatore Minisola** (Italy) (www.informed-scientist.org/presentation/what-is-new-in-clinical-research) in their preclinical and clinical "What is New?" Talks, as well as by **Bram van der Eerden** (Netherlands) in his preclinical update "Bone and beyond" focusing on novel developments in bone biology.

Concerning preclinical research, Duncan Basset said "*Papers published this year seem to be high quality and diverse, presenting exciting and innovative work. All use multiple, complementary genetic mouse models, mostly with loss of function mutations. Neglected areas are now coming to the fore. The endocrine role of bone and the circadian clock, and advances in skeletal imaging including 3D confocal microscopy and synchrotron microCT are having a big impact.*"

bisphosphonates, with 18 months follow up. Risk of fracture reduced by 74% in months 30-36, compared to the first 6 months of the study period. The reduction in back pain also continued during the follow up period. The recently published follow-up study to the DATA-Switch study of denosumab and teriparatide, performed at a mean 15 months after the study in 50 women, found that the gains in BMD obtained by women treated with teriparatide (and denosumab) during the four years of the study were maintained in those who subsequently received antiresorptive therapy, while BMD decreased in the untreated women.

Further evidence for long term effects of osteoporosis treatment is becoming available as new treatments are approved and enter into long term use. More data is expected soon on hormone replacement therapy, raloxifene, bisphosphonates, denosumab, and teriparatide, as well as on the effects of various sequences treatment with different therapies during the progression of osteoporosis.

Balancing risks and benefits of long term use of bisphosphonates

Bo Abrahamsen (University of Southern Denmark) is a consultant endocrinologist at Glostrup Hospital, Copenhagen, and a leading contributor to Danish and international epidemiological studies of fracture risk and the long term safety of osteoporosis treatments. In the symposium he gave a highly anticipated update on the latest research concerning the risks and benefits of long term antiresorptive treatment with bisphosphonates.

The benefits of bisphosponates therapy for BMD and reduced fracture risk are quite well defined from RCTs, up to and beyond 5 years (see above). In particular, treatment up to 10 years was suggested by the ASBMR Task Force to be mainly beneficial for protection against vertebral fractures.

However, announcements by FDA in past years have warned of adverse effects of bisphosphonates: osteonecrosis of the jaw (2005), atrial fibrillation (2007), and atypical femur fracture (2010). Analysis of US healthcare claims data has shown that these announcements had a measurable impact on the use of bisphosphonates after hip fracture. Overall use in the USA decreased almost 50% between 2004 and 2013.

Strong, conclusive evidence associating these risks to bisphosphonate use has been lacking, particularly during long term use of up to 10 years. As most RCTs run for < 5 years, we have to rely on observational studies to identify any longer term risks. So far, data from these observational studies is still limited, and the analysis is complicated by confounders and individual patient decisions about their treatment.

A recent meta-analysis of cardiovascular events in RCTs of bisphosphonates found no significant risk with oral bisphosphonates. The data is close to identifying a risk with i.v. treatments, but this is not statistically significant. Neither was any association found with myocardial infarction, stroke or CVD death. The association of atrial fibrillation with bisphosphonates in long term observational data was examined in the UK CPRD database and by the Abrahamsen group in Danish data. In both studies, a possible higher risk was identified in the first few weeks of treatment, possibly due to patients at higher risk discontinuing treatment after experiencing some discomfort. Concerning risk of heart failure in observational settings, the analysis is complicated because low BMD is also a known risk factor in heart failure. However, the Danish data suggests that alendronate use may reduce this risk in a dose-dependent manner.

Benefits and risks in relation to developing cancer have also been studied. Neither alendronate and zoledronic acid have been found to decrease the risk of developing break cancer, as was hoped. There are several case-control studies suggesting an association between bisphosphonate use and developing oesophageal cancer, but the evidence is not strong. There is some evidence from combined studies for a plausible protective effect of bisphosphonates against colorectal cancer.

Atypical femur fractures (AFF) are rare, transverse fractures of the subtrochanteric femur (more common in Asia) or femoral shaft (more common in Europe). They are diagnosed by radiology according to the ASBMR methodology. A recent communication to NEJM has reported a mutation affecting the mevalonate pathway (GGPPS1) in three sisters who all experienced AFF after receiving bisphosphonate therapy for 6 years. This mutation has not been found so far in other AFF patients but this result shows that genetic determinants of AFF risk may exist, at least in some patients.

A Canadian study published last year aimed to define the profile of patients at higher risk of AFFs. In Quebec City, 36 cases of AFF were reported during 19 months, giving a low mean incidence of 7.0 cases per 100,000 person-years. Within these patients a significant association with use of bisphosphonates was found, along with some notable differences in proximal femur geometry - excessive femoral offset, proximal femoral neck angle in varus, and a greater proximal cortical thickness, compared to controls. Morin et al. investigated bone geometry of 16 women with AFF, matched with 16 controls for age, weight, ethnicity and cumulative

bisphosphonates use. These results suggested that the women with AFF had distinctive femoral geometry parameters, including lateral femur bowing, that results in a higher tensile mechanical load on the lateral femur.

Bo Abrahamsen's group has conducted a study in the Danish prescription database (n=61,990) aiming to define how the incidence of AFF compares with the number of hip fractures prevented during extended use of alendronate. It is not possible to discriminate in this data between subtrochanteric and femoral shaft fractures. The first part of the study focused on highly adherent bisphosphonate users with over 10 dose-years of treatment, and the second part was a nested case-control study to characterise those at more risk of AFFs.

This study found no increase in the total incidence of subtrochanteric and femoral shaft fractures as a result of alendronate use, not even in the highly adherent population or those being treated for more than 10 years. If there is any increased risk of subtrochanteric fractures, this is offset by reduced risk of femoral shaft fracture. The data also reproduces the expected reduction in risk of hip fracture, and has been analysed for incidence of ONJ surgery, which is low overall but higher in participants continuing treatment for more than 5 years.

Swedish data shows overall frequencies of AFF and hip fracture among bisphosphonate users to be 11 and 155 respectively per 10,000 patient-years of use, and also that there is no association of excess mortality with AFF. It is important to consider this result in the context of the one year mortality rate after hip fracture, which in Sweden is 22%. The Danish data shows overall incidence of AFF and femoral shaft fractures at 30-40 per 10,000 patient years, which is not incompatible with the Swedish incidence of AFF, and clinical observations from the USA estimating AFFs to be 3-38% of all femur shaft fractures.

Other studies have confirmed other long term benefits of treatment, especially for prevention of vertebral fractures which further outweigh the risk and

After the congress, we contacted Bo Abrahamsen to find out more about his research and the issues raised in his talk.

How did you get into studying fracture risk?

I've been working with osteoporosis in the past 25 years, and for most of that time Denmark has had the unique advantage of national healthcare data for both fracture outcomes and for prescriptions filled. I think it is important to use this long term data source as extensively as possible, as we need a coherent long term strategy for the best management of patients with osteoporosis.

Has the level of concern about bisphosphonates in Denmark and in Europe been similar to the USA, among clinicians or patients? Has there also been an actual drop in bisphosphonate use observed here?

Overall there has not been a decline in the uptake of osteoporosis drugs in Denmark. The most widely used class of drugs are still the bisphosphonates, as there is a good safety record and as the cost is lower than for newer osteoporosis drugs. For many European countries there does appear to have been a drop in bisphosphonate use.

Are there similar studies in other countries prescription data apart from Denmark and Sweden?

There are also studies from areas of the United States, from Austria, Korea and Taiwan. There are some differences in methodology and in the nature of the databases. In many countries prescription information is still so new that the registries are non-informative as regards long term risks.

Are you planning further studies to build on these results?

Yes, we have ongoing studies in this field with our collaborators in Oxford, Barcelona and San Francisco.

Your data suggests that the concern over AFFs is unfounded, at least compared to the risk of hip fracture and actual impact of AFFs. Has the FDA responded to these findings yet? How do you think we should proceed to restore confidence among patients and clinicians?

I don't think concerns over AFFs are unfounded, but we must make sure not to lose sight of the more important task, i.e. to prevent major osteoporotic fractures. It is exactly because AFFs are very rare that it is taking so long to come up with precise risk estimates. We need more knowledge on the risk factors for AFFs so that we can identify in advance the probably small number of patients who are likely to sustain such fractures if treated with antiresorptives. It may be that certain mechanical factors matter, such as femur geometry, or it may be down to genes modifying bisphosphonates action, or both. This will take time because these fractures are rare. Meanwhile our data suggest that current clinical management including informal treat-to-target practices succeed in maintaining a low likelihood of fracture in those on long term bisphosphonate treatment.

Any other comments?

There has been a lot of resources wasted by unclear reporting and poorly chosen control groups of observational studies in this area. Funding bodies and journals need to do a better job at ensuring transparent reporting including making sure that papers are STROBE compliant, so that readers can assess the existence of confounding and make their own inferences about the strength of the observations.

impact of AFF. However, these conclusions are based on studies in Europe and the risk-benefit balance may be different in other populations, especially in Asia. In any case, the problem of a lack of confidence in osteoporosis therapy remains. A recent JBMR paper has suggested some strategies for addressing patient concerns, highlighting better education, monitoring and identifying risk markers for AFFs.

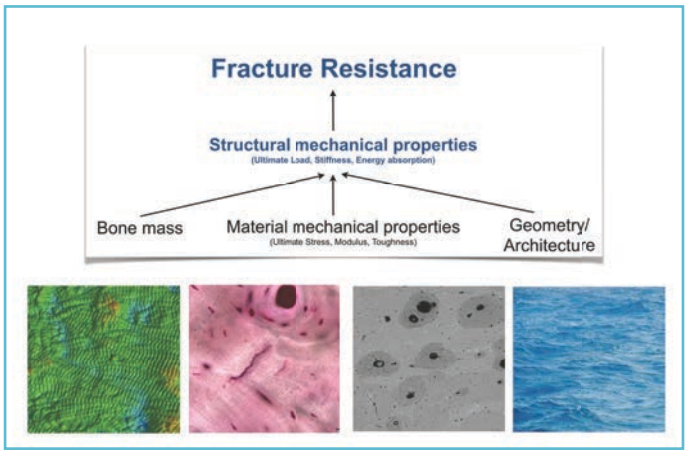
In conclusion, the Danish data concurs with findings in Sweden, that the incidence of AFFs as a result of long term use of bisphosphonates does not offset the benefits in hip fracture reduction resulting from long term treatment. Bo Abrahamsen concluded "Treatment pauses may be considered safe and justified in low risk patients, but there really is limited evidence that this is so".

Changes in bone quality and mechanical properties during treatment of osteoporosis

In what could well be the world's first Sound of Music-themed presentation on bone quality, **Matthew Allen** (Indiana University, USA) discussed how four factors contributing to bone quality – collagen, micro-damage, bone mineralisation and the role of water – are affected by osteoporosis therapy using bisphosphonates, denosumab, raloxifene and PTH.

The structural mechanical properties of bone, when exceeded, will cause fractures. Alongside BMD, bone quality is an important contributor to mechanical properties, but has only recently started to be characterised in detail.

Bisphosphonates have well documented effects on bone quality, including changes to mineralisation and increased microdamage. The more homogeneous mineralisation associated with use of bisphosphonates can allow cracks to propagate. However, improved BMD generally counteracts these effects in maintaining energy absorbance and reducing overall fracture risk. Data on how denosumab affects bone quality are limited.



Collagen, micro-damage, bone mineralisation and the role of water contribute to bone quality (Indiana University)

Increased BMD is known to account for only 4% of fracture risk reduction from raloxifene treatment. The main impact on bone quality appears to be in the water content, with higher levels of bound water at the mineral-collagen interface contributing to increased toughness. In contrast, PTH appears to generate a more heterogenous matrix distribution and reduces pre-existing microdamage, enhancing tissue-level mechanical properties.

Finally, there is as yet no data available on what happens to these four aspects of bone quality after osteoporosis treatment is stopped, for example in drug holidays. Modelling of the effects of drug holidays by Hernandez et al. suggests that BMD can be lost after suspending antiresorptive treatments, but the increase in the mean tissue age as a result of treatment is retained, and thus effects on bone quality may not be reversible.

Choice of treatment for osteoporosis

In the ECTS 2017 clinical update on osteoporosis treatment, **Adolfo Diez-Perez** (Spain) discussed clinical decision-making for the treatment of idiopathic osteoporosis, combining evidence, clinical experience and opinion.

He first emphasised that a patient diagnosed with osteoporosis should always be offered a treatment, in addition to calcium and vitamin D supplementation. All therapeutic trials have shown an effect on fracture risk reduction versus the control group, even if not always statistically significant, and it is worthwhile to explore the various treatment options.

More than 200 guidelines for osteoporosis treatment exist worldwide, but most are not very precise, and therefore limited when considering choice of treatment for an individual patient. This year the ACP published its latest recommendations, giving a strong recommendation for alendronate, risedronate, zoledronic acid OR denosumab in women, but with no comparison between these therapies. Bisphosphonates are recommended for men but only as a weak recommendation, while there is a strong recommendation against estrogen, estrogen plus

progesterone and raloxifene for women. No recommendation is made for treatment duration but high risk patients may benefit from longer treatments.

AACE/ACE guidelines (2016) have been slightly more targeted, recommending alendronate, denosumab, risedronate and zoledronic acid for patients with no prior fragility fracture or moderate fracture risk. For patients with prior fragility fracture or at higher fracture risk (e.g. the elderly, frail, glucocorticoids users), they recommend denosumab, teriparatide and zoledronic acid.

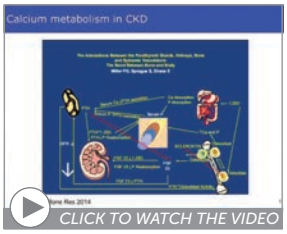
Ideally there would be data from head-to-head trials of different treatments but this is rarely done. Instead, indirect and mixed comparisons can identify the strongest therapies (anabolics, denosumab, zoledronic acid), therapies with intermediate potency (oral bisphosphonates) and less potent therapies (raloxifene, bazedoxifene, ibandronate).

Developing a more precise treatment strategy for an individual patient should consider the stage of osteoporosis disease (not necessarily age-related) and fracture risk factors. Treatment with oral antiresorptive treatments is currently considered more relevant for early menopause, potent antiresorptives for established osteoporosis, and anabolic treatments in the advanced stages. However, there is currently some debate about whether anabolic treatment should in fact be a first line therapy (see our report on the ECTS 2017 debate).

Following selection of the therapy, there are still choices to be made about dosage form and frequency, and the preferences of the patient must also be an important consideration.

The Philippe Bordier Clinical Award was given to Bente Langdahl (Denmark) for her significant clinical contribution to the field of bone and calcified tissue. Her scientific work has included important studies in the genetics and treatment of osteoporosis, osteogenesis imperfecta, and interactions between adipose and bone tissues. She is a past president of ECTS and has contributed greatly to the development of ECTS training courses.

Treatment of osteoporosis in patients with reduced kidney function



Osteoporosis with fragility fractures is a common complication of renal failure. In our clinical update on osteoporosis treatment, **Erik Eriksen** (Norway) discussed the treatment options available for osteoporosis in renal patients, while **Östen Ljunggren** (Sweden) presented clinical experience and case studies of individual patients.

The disruption of calcium metabolism in chronic kidney disease (CKD) involves complex interactions between kidney, bone, vasculature and the parathyroid glands, and circulating hormones including PTH, vitamin D metabolites and FGF23. CKD is associated with hypercalcemia leading to secondary hyperparathyroidism, hyperphosphatemia and a higher risk of cardiovascular disease. CKD patients also develop osteoporosis, combined with a general disruption of mineral and bone metabolism known as CKD-MBD.

CKD patients are treated with the normal range of antiresorptive and anabolic treatments for osteoporosis, but as yet there are no clear guidelines for diagnosis and treatment. Patients at each stage of treatment – preuremic, dialysis and transplant, have differing needs. During the first two stages, osteoporosis is associated with CKD-MDB, while after transplant this condition may be mostly resolved but the hyperparathyroidism persists, and long term glucocorticoid use is a further risk factor.

Most data on anti-fracture efficacy of osteoporosis treatments relates to CKD stages 1-3 (glomerular filtration rate GFR > 30 ml/min). These generally show similar responses to treatment between patients at these three stages. Clinical trials have mostly excluded patients at CKD stages 4 and 5 (GFR < 30 ml/min) so very little is known about their response to treatment. Denosumab is being used off-label for treatment of patients with GFR < 30 ml/min and one study has examined a small group

of stage 4 patients, which suggested similar outcomes to patients at stages 1-3. However, with denosumab the risk of hypercalcaemia is increased and careful monitoring is important. Teriparatide is effective at GFR< 30 ml/min, but its use is limited in cases of secondary hyperthyroidism. FGF23 inhibitors are becoming available in the near future, but their role in the treatment of CKD-MDB needs to be established.

Hypothetically, vascular calcification in CKD might be treated by modification of bone turnover, modulating levels of circulating osteopontin, matrix-GLA-protein and other calcification promoting agents A trial of alendronate in 2010 showed no significant effect on vascular calcification, but further studies are certainly merited to address this major aspect of CKD.

To limit progression of osteoporosis in CKD, the most important preventative measure is to limit the development of secondary hyperparathyroidism in patients in all stages of the disease. This can be done with vitamin D and calcimimetic treatment. Care should be taken to avoid overtreatment, which could lead to adynamic bone disease (very low bone turnover) and vascular calcifications. HRT may be an option for some specific groups.

The occurrence of adynamic bone disease in CKD patients has been much debated. Some have linked this disease to the presence of aluminium in dialysis fluid. Now that removal of aluminium has become standard, a marked reduction in prevalence of adynamic bone disease occurred. In any case, it is still essential to rule out adynamic bone disease, if an antiresorptive therapy is considered. However, a recent clinical study performed by Östen Ljunggren's group has tested denosumab for six months in six dialysis patients with CKD, fractures and varying bone turnover. All six patients have responded with a higher PTH level, and bone biopsies showed no evidence of adynamic bone disease. Follow-up over the next 12 months should provide complete efficacy data.

Denosumab a new alternative to risedronate in patients receiving glucocorticoid therapy

Patients receiving long-term glucocorticoid therapy have increased levels of RANKL and are at risk of osteoporosis. Denosumab, as a RANKL antibody already approved for osteoporosis treatment, is clearly of interest to investigate as a possible treatment. In his plenary oral presentation at ECTS 2017, **Kenneth Saag** (USA) reported on an international, multi-centre phase 3 study to determine the safety and efficacy of denosumab for treatment of patients receiving glucocorticoids.

The study involved both adult women and men, and compared denosumab with the bisphosphonate risedronate. Two groups of patients were studied, 505 receiving glucocorticoids for more than 3 months (GC-C) and 295 with less than 3 months' treatment (GC-I). At baseline, the GC-C group had more fractures, and a higher proportion of women, than the GC-I group.

BMD measurements at 12 months after initiation of treatment showed significantly higher gains in total hip and lumber spine BMDs for those treated with denosumab, compared to those on risedronate, for both GC-C and GC-I groups. Therefore, denosumab is shown to be both non-inferior and superior to risedronate in terms of BMD gained after 12 months, and there are so far no significant differences in fracture rates between treatments. The safety profile of denosumab was found to be similar to risedronate in both groups.

The study remains blinded and is ongoing, but the results so far clearly show that denosumab has potential as a new treatment option for glucocorticoid users at risk of osteoporosis.

In other news...

New guidelines on the use of anti-osteoporosis therapies in England and Wales were provided by NICE in 2005, coinciding with the market authorization of a generic form of alendronic acid in the UK. Using primary care data from 1999 to 2013, a study has correlated prescription of anti-osteoporosis therapies directly to reductions in fracture risk. They found an absolute 14.9% increase of anti-osteoporosis prescriptions and reductions in incidence of major and subsequent hip fractures of 14% and 22% respectively, at 3 years after intervention, relative to the pre-intervention levels and trend. Hawley S et al., 2016

An international, multi-centre trial in post-menopausal women with osteoporosis has compared the transition from oral bisphosphonates to densumab or zoledronic acid. A total of 643 women participated in the study, which measured changes in BMD and bone turnover markers. After 12 months treatment, denosumab was found associated with significantly higher BMD at the lumbar spine, hip and femoral neck, and also a greater inhibition of bone remodelling, in comparison with zoledronic acid. Miller PD et al., 2016

In a phase III trial, monthly injections of romosozumab or placebo were given to 7180 post-menopausal women for 12 months, followed by 6-monthly doses of denosumab to both groups for the following 12 months. Romosozumab was associated with a lower risk of vertebral facture than placebo at 12 months, and also after transition to denosumab at 24 months. Cosman F et al., 2016

An Australian multi-centre trial has tested the effectiveness of vertebroplasty performed for analgesia within 6 weeks in 120 patients with acute vertebral fractures, with a placebo group receiving a simulated vertebroplasty. Among the vertebroplasty group, 44% had NRS pain score below 4/10 at 14 days, compared to 21% in the control group. An early intervention of this kind is unusual, but these results show early vertebroplasty could be a useful additional approach for pain management in acute, painful vertebral fractures. Clark W et al., 2016

Bone and the hematopoietic stem cell niche

Laura Calvi (USA) discovered the role of osteoblasts in regulating the hematopoietic stem cell (HSC) niche.

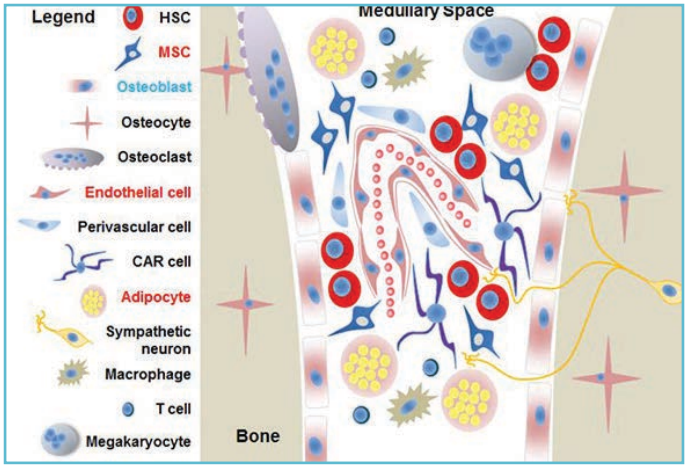
At ECTS 2017 she presented the latest developments in our understanding of the HSC niche and the techniques important for these investigations.

Heterogenity of the niche

The skeleton is an essential site for blood formation through differentiation of HSCs, that are an important target in cell-based therapies for many hematopoietic, skeletal and malignant diseases. A particular challenge is to manipulate HSC differentiation without increasing malignancy.

It appears there is no equivalent of complete pluripotency among HSC sub-populations, as is found in embryonic stem cells. Epigenetic memory appears to pre-determine HSC behaviour and they cannot differentiate to all cell types. HSC differentiation and function is also strongly influenced by the niche – physical interactions with other cells nearby and local microenvironment factors. The niche can now be observed directly through intravital 2 photon microscopy of cell engraftments, revealing niche heterogeneity - how various cell types localise to different niche sites. Perivascular mesenchymal stem cells are most important in supporting differentiating HSCs in bone marrow, while interactions can also involve osteoblasts at various stages of differentiation, adipocytes, macrophages, T cells, megakaryocytes and senescent neutrophils. In fact, through study of genetic models, interactions within the niche are now known to involve support from multiple sub-populations of these cells.

It is important to define exactly which sub-populations of cells are involved in the HSC niche. This is challenging, as cells may express the same markers, but behave



Cell types involved in niche interactions within the HSC niche (University of Rochester)

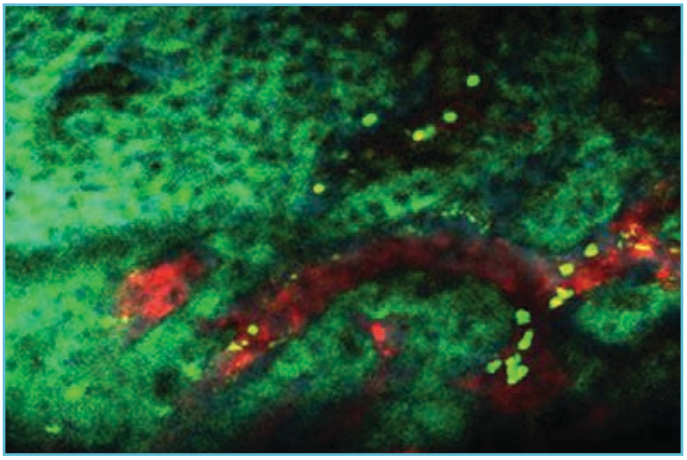
differently when compartmentalised at different niche locations. New advances in flow cytometry are allowing us to isolate different cell progenitors (see below).

The effects of ageing, therapeutic intervention and other factors on the functions of the niche are being investigated. New therapeutic strategies to target expansion of specific niches can help direct support to specific HSC lineages, rather than directly act on the HSCs themselves. All in vivo studies of the hematopoietic stem cell niche are conducted so far in animal models – studies in humans are not yet possible. However, investigations in stored human bone marrow samples have started to provide evidence that the HSC niche is indeed relevant to human disease.

Current and future directions

Current efforts are focused on understanding more completely how all the cell sub-populations active within the HSC niche interact and work together. For example, recent data obtained by Laura Calvi's group suggest that PTH affects HSC indirectly via stimulation of osteoblasts to produce pro-angiogenic factors, which also remodel the arterial and sinusoidal cell populations.

Paolo Bianco and collaborators have revealed in a study of mesenchymal stem cell (MSC) sorting and analysis that there is no single, uniform MSC population in all tissues. Rather there are distinct tissue-specific sub-populations clustering around distinctive transcriptomes and in vivo differentiation properties. These populations can be identified through various methods. Flow cytometry markers for MSC populations have been identified such as αV, CD51 which is expressed in mesenchymal to osteoblastic cell populations, and PDGFRα which is generally accepted as a MSC marker and is more highly expressed in mesenchymal populations compared to osteoblastic cells. Cell isolation and preparation steps such as digestion are also important to control and standardise, as MSC may cling



Intravital 2-photon imaging of mouse calvaria. Blue: Collagen; Green: CFSE labeled Cells; Red: Dextran-Texas Red labeled blood vessels.
(Laura Calvi/University of Rochester)

tightly to dissolved tissues. It is helpful if authors publish these methodological details in their papers and more discussion and consensus is needed around these questions.

Reporter systems to discriminate MSC populations have included Nestin-GFP developed by Paul Frenette's lab, though this approach has been further refined by use of antibodies. Single cell 'omics should help define the heterogeneity and hierarchy of MSC sub-populations even more clearly. Hopefully a consensus can soon be built to define the minimum criteria for an osteoprogenitor cell.

Ageing impacts the skeleton, fracture healing and the function of HSCs. HSC develop myeloid skewing in their differentiation, which is associated with increased risk of leukaemia and myeloid deficiencies. Increased senescence and mutational burden with ageing both contribute to a reduced numbers of clones and increased malignancy risk.

Laura Calvi discussed her recent data from intravital imaging of bone marrow microenvironment changes in mice as they age to two years, showing bone and collagen loss, expanded marrow cavities and endothelial structures, and a loss of osteoblasts, but this was specific to the bone surface. Analysis of human bone marrow samples from healthy volunteers shows a decrease of functional osteoprogenitor populations with age, but this was more marked in the bone associated populations versus those located in the central marrow. This highlights the need to study these different heterogenous populations separately.

Hartmut Geiger's group have elegantly shown how the HSC niche can be affected by ageing, focusing on the reduced osteopontin (OPN) expression that occurs in ageing mice. They implanted young HSCs into an OPN knockout mouse as a model of an ageing microenvironment, and the cells developed signs of ageing, including decreased engraftment and increased polarity. In vivo treatment with osteopontin reversed these effects. Later this year, the Calvi group and others also working on the effects of ageing on HSCs are expected to disclose multiple further mechanisms for rejuvenation of haematopoiesis through targeting of the niche.

Concerning the role of the niche in malignancy, Laura Calvi discussed myelodysplastic syndrome (MDS), a pre-maliginant condition of the bone marrow affecting mostly elderly people, which can progress to acute myeloid leukemia and is not easy to treat. It is thought to result from mutations in HSCs, and is characterised by reorganisation of the bone marrow. The Calvi group has identified in a MDS mouse model an increase in endothelial cells and dysfunction of osteoblast and MSC populations in MDS, suggesting an abnormal microenvironment. MDS, as many cancers, is genetically heterogeneous and may benefit from targeted therapy. However, interactions of malignant cells with the microenvironment transcends this heterogeneity and targeting of the niche could be a promising alternative approach for MDS treatment.

The question of whether HSCs are programmed to be completely autonomous in function, or are heavily influenced by the microenvironment has been controversial for over 30 years. Laura Calvi concluded, "We now think that the HSC population is composed of dissimilar clones varying in function, and the niche contributes to clonal selection rather than changing directly the fate of HSCs. This is a change in thinking, but it does not diminish or change the importance of the niche".

Anabolic therapy – first line treatment?

A highlight of ECTS 2017 was the Saturday evening debate on the motion "This house believes that anabolic therapy should be the first line therapy for all patients with established osteoporosis".

Before the debate started, electronic voting showed an evenly balanced opinion in the audience:
FOR: 41.6% AGAINST: 42.4% UNDECIDED: 16.0%

THE ARGUMENT FOR – Bente Langdahl

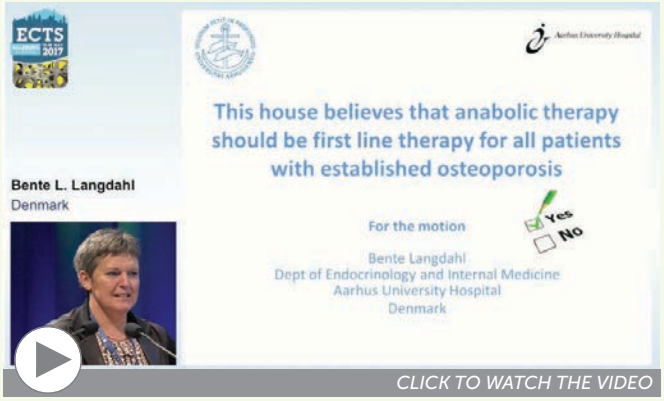
Bente Langdahl is a consultant at the department of Endocrinology and Internal Medicine at Aarhus University Hospital and Professor at Aarhus University. Her recent clinical research has focused on development of new therapies for osteoporosis and studying the mechanisms of osteogenesis imperfecta.

- Anabolic therapies for bone disease include teriparatide (TPTD), abaloparatide and romosozumab, which is still being evaluated.
- Anabolic therapies work by stimulating bone formation on all bone surfaces to increase BMD: increasing the number of trabeculae or by making them thicker – a distinctly different mechanism to antiresorptive therapies.
- Evidence available from clinical trials showing that TPTD has several advantages over antiresorptive therapies:

  The SHOTZ study – a head to head comparison of TPTD with zoledronate - found that TPTD stimulated new bone formation that was not observed with zoledronate, and the bone mineral distribution was modified with zoledronate but remained normal with TPTD

  QCT and HRQCT bone structure data from the EuroGIOPs trial in 2013, conducted in men using glucocorticoid medication or with osteopenia, found that TPTD gave significantly higher improvement in integral and trabecular BMD compared to risedronate, also better bone microarchitecture and estimated bone strength.

  The Back Pain study compared back pain in women with vertebral fractures receiving either TPTD or risedronate. They found a similar efficacy in reducing back pain, but a much lower risk of new vertebral fractures or worsening of the existing fracture in the women receiving TPTD, and any fractures experienced by these women were generally milder.



The GIOP study evaluated TPTD or alendronate patients in osteoporosis patients receiving glucocorticoid therapy, showing a significant reduction in vertebral fractures with TPTD compared to alendronate.

The VERO study is not yet published but results presented at a recent conference show significant reductions in vertebral and clinical fractures after two years with TPTD in comparison to risedronate.

The DATA-Switch study and a recent review by Cosman et al. show that treatment sequence matters – in total hip BMD a clear benefit has been found for total hip BMD by treating with TPTD first and then an antiresorptive, compared with the other way round.

Clinical trials of the latest anabolic treatments abalaparatide and romosozumab indicate an even greater reduction of fracture risk than TPTD.

- Evidence is still needed on long term efficacy of anabolic versus antiresorptive therapies, and large head-to-head study over 5 years would provide a definitive answer on this debate

In conclusion:

Anabolic therapies have a distinctly different mechanism of action that in clinical trials is showing better bone formation, bone microarchitecture, bone strength, BMD and fracture risk reduction.

THE ARGUMENT AGAINST – Steve Harris

Steve Harris is an endocrinologist and Clinical Professor of Medicine at the University of California, San Francisco, specialists in osteoporosis, metabolic bone disease and disorders of mineral metabolism. His research has involved many studies on osteoporosis prevention and treatment and he maintains an active clinical practice.

- The motion states that anabolic therapies should be used as first line therapy (initial treatment) in ALL osteoporosis patients, not just those with severe osteoporosis. Do we really agree with that?
- The mechanism of action of anabolic therapies is important, but this is not the point. We also need to consider:

  Efficacy – Clinical trials have shown antiresorptive therapy to be effective in reducing risk of ALL fractures, particularly hip fractures. There is robust evidence that anabolic therapies reduce fracture risk, but evidence does not yet show this per se for hip fracture. The DATA-Switch study showed an important difference in outcomes for hip fracture depending on the sequence of denosumab and TPTD treatment. Steve Harris agrees with Cosman et al. that the optimum utilization of anabolic treatment may indeed be to apply it first, followed by antiresorptive therapy. But, this is not relevant in our current clinical treatment environment, especially when considering:

  Safety – What level of risk is acceptable for our patients? No treatment modality is absolutely safe, including anabolic therapies.

  Patient preference – Patients often prefer therapies for which there are more years of clinical experience, also have preferences for route and frequency of administration, acceptable out-of-pocket costs, perceived risk/benefit ratio. These are important considerations. Healthcare providers and clinicians may have a similar set of preferences.

  Cost effectiveness – Steve Harris recommended the recent ICER report which has modelled the cost-effectiveness of anabolic therapies, calculating a much higher cost in comparison with most therapies for each 1 QALY obtained by anabolic therapies.

In conclusion:

Low cost generic antiresorptive therapies should remain the first line therapy for relatively low risk patients on a cost-effectiveness basis.

In rebuttal, Bente Langdahl made several criticisms of the ICER report including their consideration only of clinical trials comparing anabolic therapies with placebo, rather than head-to-head comparison of therapies, and that some trials included were only run for 12-18 months. She said "As a scientific society we need to stand up for science and the best possible treatment for patients, not just taking into account the views of economists". She also explained that in Denmark, the responsible use of TPTD as a first line treatment is already established.

Steve Harris replied that he was not against anabolic therapy in principle, and it makes sense particularly for high risk patients. However, many won't consider a new treatment, as a result of concern about relatively rare side effects and general cynicism. He explained "In my clinic, there are many patients who will not consider a new treatment until it has been available for a considerable period of time". A big question is how to position new therapies in the context of the world of cost-effectiveness. In the USA, anabolic therapy is recommended already for patients at high risk of fractures, but insurers are so far generally not supportive of these treatments. Economics clearly matters in clinical judgements, especially when alternative, lower cost generic treatments are available to provide effective reduction of fracture risk.

After the debate, the final electronic vote was taken **FOR: 52.5% AGAINST: 38.9% UNDECIDED: 8.6%**

Congratulations to Bente Langdahl, the winner of the debate who shifted the audience's opinion her way by over ten percentage points, and was awarded the ECTS Golden Femur!

After the debate, Prof **Salvatore Minisola** (Italy) said "*We can consider this debate from point of view of principle and in practice. In principle everyone now agrees that it's better to prescribe an anabolic agent first and then an antiresorptive agent. However, there are two points to consider. Firstly, this has to be decided considering what will be best outcome for the individual patient, and it's not appropriate to apply the same approach for ALL patients. Also in most European countries, applying this approach to all osteoporosis patients would not be affordable by the health system, as the price of anabolic treatments is very high.*"

Prevention and healing of bone erosions in rheumatoid arthritis

Articular bone erosion is a central feature of rheumatoid arthritis and is associated with a higher level of disability. Over 15 years ago, **Ellen Gravallese** (University of Massachusetts Medical School, USA) discovered the essential involvement of osteoclasts in articular bone erosion in rheumatoid arthritis, and the stimulation of osteoclast differentiation by RANKL secreted by synovial fibroblasts and activated CD3+ T lymphocytes. As erosions typically do not heal in rheumatoid arthritis patients even when inflammation is controlled, the group also investigated the role of synovial inflammation in rheumatoid arthritis. They have shown in mouse models that inflammation impairs osteoblast activity via changes to Wnt signalling, thus contributing further to focal bone loss. Other recent research in her laboratory has investigated the role of innate and adaptive immune mechanisms in bone in arthritis, and the regulatory role of miRNAs.

In the clinical workshop "Bone and Rheumatic Diseases", Ellen Gravellese discussed the prevention and healing of articular erosions in rheumatoid arthritis. Therapies currently being investigated for prevention of bone erosion include biologic, small molecule and cell therapies targeting cytokines, including IL-1 and TNF, or immune cells. But, is healing of existing erosion possible?

Bone anabolic therapies are already available or in advanced development for treatment of osteoporosis and other bone diseases, that may also be effective for treatment of articular erosions in rheumatoid arthritis. Antibody therapies blocking sclerostin as a negative regulator of bone formation are likely to be approved over the next few years for osteoporosis and other diseases involving bone loss. However, Wehmeyer et al. reported last year their observations that sclerostin inhibition accelerated RA-like disease, including joint destruction in a human TNFα transgenic (hTNFtg) mouse model. They have therefore expressed concern about use of anti-sclerostin antibody therapies in rheumatoid arthritis patients.

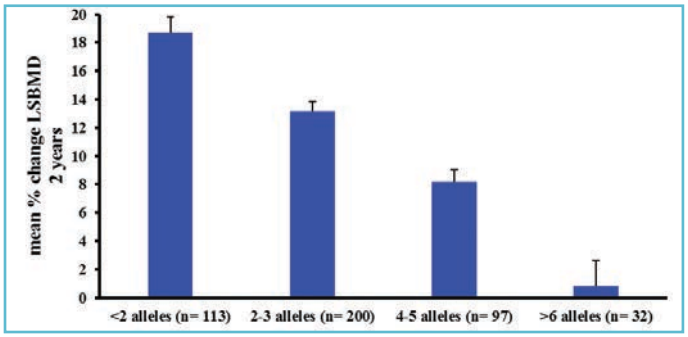
Ellen Gravallese's group initiated a RCT, the TERA trial in 24 patients of teriparatide combined with an anti-TNF

biologic agent, results of which have just been published. Changes in erosion volume were measured by 3-dimensional CT at six anatomical sites over 12 months. At the end of the study, while BMD was found increased in the spine and femoral neck among those on the combined treatment compared to the controls, there were no significant differences in erosion volume.

Ellen Gravallese told us "The trial did demonstrate that teriparatide was safe for rheumatoid arthritis patients. We feel that this is still early days for anabolic therapy in rheumatoid arthritis. Our trial enrolled a small number of patients with longstanding disease. It may be that if teriparatide were used earlier in the disease, or for a longer period of time (2 years), the results may have been different. It will also certainly be worthwhile to examine other anabolic agents for systemic osteoporosis and erosion healing in rheumatoid arthritis and other inflammatory arthritides."

CXCR4 identified as predictor of response to teriparatide in osteoporosis patients

Nerea Alonso reported on the results of a genome-wide association study in 442 patients with osteoporosis in UK, Slovenia and Denmark, investigating response to teriparatide treatment with change in the lumbar spine BMD as the primary outcome. The top hit was a e-QTL for CXCR4 expression in peripheral blood cells, and an allelic score has been developed that identifies patients with a 10-fold difference in treatment response. The next step will be to functionally validate this result, which if confirmed will represent a significant step towards personalised therapy using teriparatide.



Allele score showing the increase in change of BMD at lumbar spine after 24 months of TPTD treatment, considering the number of carried SNPs (University of Sheffield)

In other news...

Results have been published from the AVA Osteoporosis Study comparing the effects of daily teriparatide treatment for 6 months, with a single treatment of denosumab, on intact PTH and bone formation indices. The aim has been to determine if denosumab-induced changes in intact PTH have an early anabolic effect, in comparison with teriparatide. Analysis of histopmorphometry and bone turnover markers in 69 post-menopausal treated women have shown that a single denusumab treatment increased intact PTH, peaking at 1 month. However, bone formation indices were significantly lower than teriparatide at 3 months. This suggests that the observed increased in BMD as a result of denosumab treatment are not obtained through increases in endogenous PTH, and other mechanisms must be responsible. Dempster DW et al., 2016

Abaloparatide, a selective activator of PTH type 1 receptor, was evaluated in phase III in the 10 country, 28 site ACTIVE trial, in comparison with teriparatide. Among 2463 women, 1901 completed the study, and those receiving each treatment experienced a reduced risk of new vertebral and non-vertebral fractures after 18 months of treatment, compared to placebo. Incidence of hypercalcemia with abaloparatide was 3.4%, lower than found with teriparatide (6.4%). Miller PD et al., 2016

Rare bone diseases highlighted at ECTS

Over 450 rare inherited bone disorders have been described, and are now a very important area of research, both to improve outcomes for rare disease patients and aid our understanding of bone biology and more common bone disorders.

ECTS 2017 received an impressive number of abstracts on rare bone disorders, and several sessions and presentations were specifically dedicated to this area of research. Here we highlight some of the important new results presented at congress, and also the inaugural session of the ECTS Working Group on Rare Bone Disease, and the involvement of the Germany hypophosphatasia group in ECTS 2017.

Osteopetrosis – too much bone

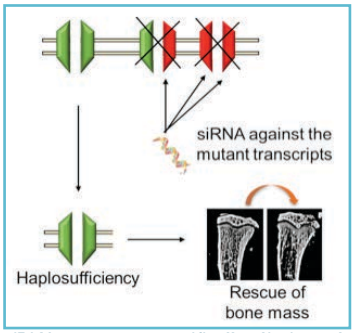
Several forms of inherited osteopetrosis have been classified, all due to mutations causing osteoclast dysfunction, leading to reduced bone resorption and increased bone fragility.

Autosomal recessive osteopetrosis (ARO) is a very rare heterogeneous disease and a molecular diagnosis is very important to help treat individual patients and families. Eleonora Palagano (Milan, Italy) presented results from whole exome sequencing of 16 unrelated families affected by ARO, including 21 ARO patients. The study obtained a molecular diagnosis in 10 of these families, with mutations identified in CLCN7, TCIRG1, OSTM1, CTSK and CA2 genes. Two unusual cases of individual ARO patients were discussed. The first, with osteoclast-rich osteopetrosis required bone marrow transplant at 2 months of age, but had no other classical ARO features. Exome sequencing in this individual identified a novel homozygous mutation in FERMT3, which is essential for integrin mediated cell adhesion especially in hematopoietic cells. Kindlin3 knockout mice have osteopetrotic bone. A second patient was diagnosed aged one month to display osteopetrosis and Poikiloderma with Neutropenia, which has only been reported in one other individual in the literature. Here, exome sequencing has revealed a homozygous mutation in the C16orf57 (USB1) gene, which is believed to interact with Smad4 involved in osteoclast differentiation, but further investigation is required to clarify the pathophysiological mechanism. Overall, this study has demonstrated how whole exome sequencing can enable a precise molecular diagnosis for individual patients and contribute to the classification of heterogeneous rare diseases.

Autosomal dominant osteopetrosis type II (ADO2) is a form of osteopetrosis resulting from dominant negative mutations of the proton/chloride antiporter ClC7 encoded by the CLCN7 gene. Researchers at University of L'Aquila have previously described the effects of this mutation in osteoclasts, which results in impaired bone resportion. Antonio Maurizi presented results from their latest investigations into the pathophysiology and treatment of ADO2.

In addition to osteoclasts, CLCN7 is also expressed in other cells, so it is important to investigate if its mutation has additional effects beyond bone. The study involved an ADO2 mouse model carrying the Clcn7G213R mutation. These mice experience increased levels of anxiety and depression, as well as osteopetrotic bone. CLCN7 expression was confirmed in the brain by immunohistochemistry, as well as an increased β-amyloid accumulation in the hippocampus thalamus and amygdala. Expression of CLCN7 was also found in some kidney and lung tissues, and in alveolar and spleen macrophages. In muscle cells, no expression was found, but an increased muscle fibrosis was identified, as well as CLCN7-positive macrophages. These results suggest that ADO2 is not just a rare bone disease but is a systemic disease with effects in other organs, including some indirect effects in muscle via macrophage activation.

Antonio Maurizi also reported on the latest progress towards a siRNA gene therapy for ADO2, where currently patients only receive palliative treatments. The therapeutic approach aims to silence expression of the mutated gene, leaving the normal copy active to restore normal functions. Previous experiments with siRNA targeted to specific gene mutations have shown a reversal of phenotype in ADO2 mice, and in osteoclasts from a patient. The team are now progressing through preclinical development with further studies of efficacy and safety in mice of different ages.



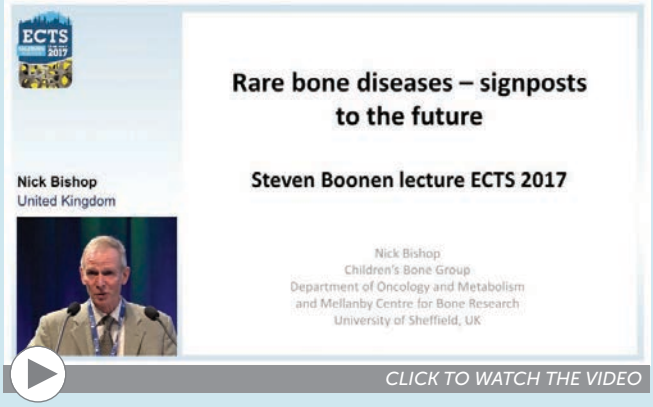
siRNA treatment specifically eliminated only the mutant proteins (red) and returned the ADO2 bone phenotype to normal (University of L'Aquila)

Results were presented from 3-months old adult mice that closely model human ADO2 and are an important model for assessing safety. Treatment over 12 weeks with intraperitoneal injection was found to successfully downregulate the mutated CLCN7 gene in femur bone marrow cells and in multiple organs, and a reduction in

Rare bone diseases – signposts to the future

The annual Steve Boonen clinical lecture was given by pediatrician Professor **Nick Bishop** (Sheffield University, UK). Nick Bishop trained in Manchester, Cambridge and Montreal, and has contributed significantly to the field of rare bone diseases, the development of guidelines and collaborative groupings within Europe.

In his lecture, Nick Bishop discussed examples of rare bone diseases and how these have contributed to our understanding of bone biology, highlighting disorders of low bone or high bone mass, or inadequate bone mineralisation. He also reported on latest impressive results of several clinical trials in rare bone disease patients. His talk was one of the highlights of the ECTS 2017 congress and can be viewed HERE. The ECTS Steven Boonen award is supported by Amgen Europe.



cellular mislocalisation of ClC7 was observed. This was accompanied by improvements in the osteopetrotic bone phenotype, and lung, kidney and muscle fibrosis. No indications of adverse effects were found in histopathology or inflammatory markers. Preliminary experiments with subcutaneous injection of siRNA also showed treatment efficacies, and options are being considered for how to cross the blood-brain barrier.

Osteogenesis imperfecta – bone fragility

Osteogenesis Imperfecta (OI) is a rare bone disorder caused by mutations in mostly type 1 collagen, resulting in bone fragility and a very high risk of fracture.

Fang Lv (Beijing), one of our East meets West award recipients, reported on a study to characterise mutations in Chinese OI patients and investigate genotype-phenotype relationships, employing next generation sequencing. A total of 103 patients from 101 unrelated OI families were studied with targeted sequencing of a panel of 14 known OI genes. A definite molecular diagnosis was obtained in 90 patients - 75 with autosomal dominant OI, and 15 with autosomal recessive OI. More than 70% of these patients had mutations in type 1 collagen, while others were found with mutations in IFITM5, SERPINF1, WNT1, FKBP10, TMEM38B and PLOD2. Overall, 79 mutations were found in the 90 patients, including 43 novel mutations. Preliminary data from clinical measurements were shown, identifying some genotype-phenotype relationships, though further analysis especially for the autosomal recessive forms of OI will require a larger sample size. The results of this study should contribute to new molecular diagnostic approaches to improve the care of OI patients in China.

Elena Makareeva (NIH, USA) is studying the most common forms of OI, which involve glycine substitutions in the triple helix of type I collagen. In a heterozygous G610C mouse model with moderate to severe OI, it was previously found that misfolded collagen accumulated within osteoblast cells is a major factor in their malfunction, and that cells degrade this protein through autophagy. The aim of this study was to determine if genetic modulation of autophagy to alter osteogenesis and severity of the bone phenotype in this mouse model. Cross breeding was used to obtain G610C mice lacking Atg5, which is essential for autophagy. Resulting newborn mouse pups experienced a significant drop in autophagy, an increase in skeletal deformities and 30-50% reduced survival compared to their wild type littermates. The skeletal effects were more severe in G610C mice with a conditional Atg5 knockout in osteoblasts. The study suggests that the variations in autophagy may contribute to the overall phenotypic variation in this form of OI and that modulation of autophagy could provide a basis for new OI therapeutic interventions. See also our Insights from Outside report for more on autophagy.

Imke Fiedler (UKE-Hamburg, Germany) is working on the development of new zebrafish models of OI. The Chihuahua (chi/+) zebrafish, which she investigates in collaboration with the University of Pavia in Italy, features an alteration in the α1 chain of collagen type I resulting in deformations and fractures in the ribs and vertebrae, heterogeneous mineralisation and a smaller body. She reported on the latest characterisation of bone morphology of this model, in direct comparison with wild type zebrafish.


Wild type zebrafish

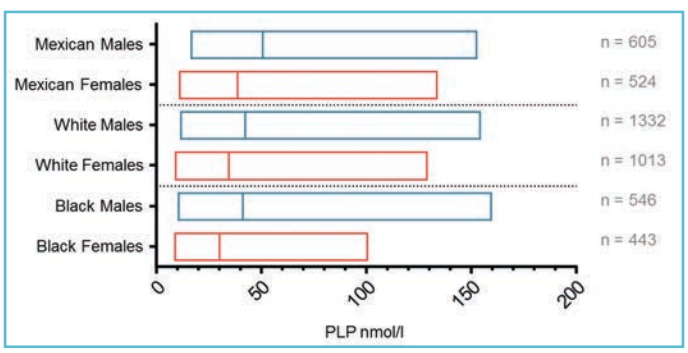
Imaging with high resolution micro-CT showed that the vertebrae in chi/+ zebrafish had a smaller overall size and thinner cortical shell. Tissue composition in bones was also investigated with quantitative backscattered electron microscopy and FTIR, showing reduced mineral crystallinity and collagen maturity. These results provide further confirmation of the suitability of the chi/+ zebrafish as a model for OI (I-IV) at the whole bone and tissue level. Further work is now planned to characterise the bone mineral phase and mechanical properties.

Hypophosphatasia and XLH

Hypophosphatasia is a rare metabolic bone disease caused by impaired activity of tissue non-specific alkaline phosphatase, resulting in rickets or osteomalacia.

Hypophosphatasia is difficult to diagnose, especially in adults. Along with low alkaline phosphatase (ALP), raised serum levels of pyridoxal 5′-phosphate (PLP) are characteristic of hypophosphatasia, and PLP is a useful diagnostic marker. However, while there is good agreement for reference intervals for ALP, there is poor agreement concerning reliable reference levels for PLP. **Philip Nicklin** from the University of Sheffield reported on their recent work to define race- and gender-specific reference intervals for PLP in adults, through analysis of samples obtained from two years of the US National Health and Nutrition Examination Survey.

Four confounders were identified that themselves modified PLP levels: inflammation, reduced kidney function, low ALP and vitamin B6 supplementation. A remaining reference population of n=4463 was analysed. Statistically significant race and gender differences were found in PLP levels, for example with males having higher levels than females, but the mechanisms behind these differences are not yet known.



95% Reference Intervals in NHANES 2007-2010 (Nicklin P, et al. ECTS 2017)

X-linked hypophosphatemia is a rare inherited form of rickets with a prevalence of around 1 in 20,000, associated with mutations in PHEX, and resulting in FGF23-induced phosphate wasting, bone deformities and bone pain. Most adults with the disease have increased lumbar spine BMD, but there is little data on the phenotype in the peripheral skeleton. Thomas Funck-Brentano (Paris) reported on a study to characterise volumetric BMD, geometry and microarchitecture of the peripheral skeleton of XLH patients. Bone densitometry and high-resolution peripheral quantitative computed tomography (HRpQCT) was carried out in a diverse group of 14 male and female patients (ages 28-70), and in 34 age- and sex-matched controls. Biochemical assays were also performed. The results showed a higher lumber spine BMD in patients, and a higher total bone area in the periphery, suggestive of higher trabecular mineralisation, but no changes in cortical bone. Further investigation is needed to understand the mechanism of these differences between axial and peripheral bone in XLH patients, and how these develop with age.

Other rare gene mutations causing bone loss

Maja Vujic Spasic (Ulm, Germany) presented an investigation of bone loss in the rare disorder of iron accumulation, hereditary hemochromatosis, caused by mutations in the HFE gene. The exact mechanism by which osteoporosis occurs in this disorder is not known, and whether it is solely due to iron overload or other effects of HFE mutation in bone cells. The Ulm group examined mouse models with constitutive, liver-specific and osteoblasts-specific deletions of Hfe. The constitutive ($Hfe^{-/-}$) mice developed osteoporosis with changes to bone mass and microarchitecture, but this phenotype was not found in the liver-specific deletion – suggesting that iron is not primarily responsible for bone loss. Osteoblast cells obtained from mice with constitutive and osteoblast-specific Hfe deletion showed a large decrease in alkaline phosphatase activity and increased expression of several osteoblast markers.

Timur Yorgan (UKE-Hamburg, Germany) is investigating Hajdu-Cheney syndrome (HCS), an extremely rare autosomal dominant disorder resulting from mutations in the NOTCH2 gene, featuring severe osteoporosis and other symptoms. Worldwide, only around 70 cases have been reported since 1948. A study at UKE has generated a knock-in mouse model with a pathogenic mutation in the Notch2 gene and investigated its bone phenotype at 12, 24 and 52 weeks. A marked decrease in trabecular bone was found at all three ages and an increase in osteoclast and osteoblast numbers, implying that increased bone turnover is present. Expression analysis of osteoblasts showed increases in RANKL and IL-6 suggesting that coupling

between osteoblasts and osteoclasts is involved. Treatment of the mice with weekly injections of alendronate for 6 weeks showed a normalization of bone mass and bone turnover, indicating that bisphosphonates therapy is a promising option for HCS patients.

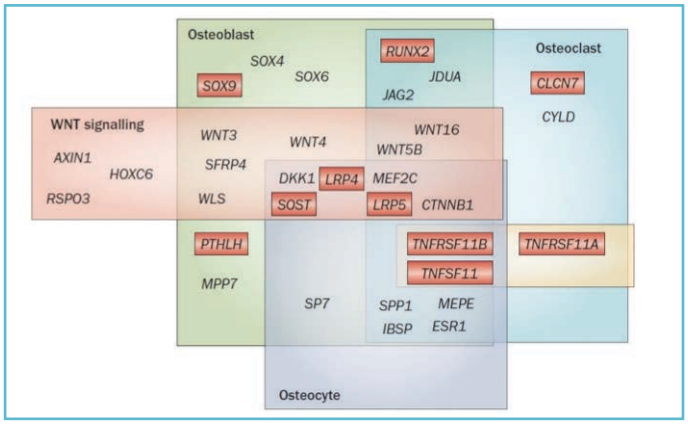
Working Group on Rare Bone Disorders

The ECTS Working Group on Rare Bone Diseases was assembled for the first time at ECTS 2017, with view to it becoming a permanent Working Group within ECTS. Its first meeting, chaired by **Uwe Kornak** (Berlin) explored how research into rare bone disorders can also help develop our understanding of common bone disorders.

The first presentation of the workshop focused on Paget's disease, which involves increased and disorganized bone turnover, often localized and leading to bone pain, bone deformities, and increased risk of fractures. The common form of Paget's disease affects around 2% of the over 55s. Around 40% of cases have a family history of the disease and it mainly affects populations of European descent. While there is clearly a genetic component, a strong environmental influence is also involved. Incidence of the disease has in fact decreased over the past 25 years. The reasons for this trend are not yet determined, but suggested environmental triggers have included viral infection, poor calcium intake and mechanical loading.

Omar Albagha (Edinburgh) explained how collaborations involving his group have investigated the genetic determinants of common Paget's disease over recent years. It was already established that SQSTM1 mutations are involved in around 10% of sporadic cases of common Paget's disease. Genome wide association studies, published in 2010 and 2011 identified 7 susceptibility loci, accounting for a further 15% of hereditability. The involvement of OPTN was quite surprising and has been the focus of functional studies to investigate its role in the NFkβ pathway and osteoclast differentiation.

Several rare inherited forms of Paget's disease all involve mutations affecting the RANK/RANKL/OPG pathway, essential for osteoclast differentiation. For example, Juvenile Paget's disease is an autosomal recessive disease resulting from a loss of function mutation in OPG, while Inclusion body myopathy with early-onset Paget disease and frontotemporal dementia (IBMPFD) is an autosomal dominant degenerative disorder featuring muscle weakness, early onset Paget's disease and dementia. Whole exome sequencing is now being used to investigate whether mutations found in the rare forms of Paget's disease are associated with the risk of developing common Paget's disease. The results obtained by other groups have so far been inconclusive. The integration of transcriptomic, epigenetic and microbiome factors may also help explain more of the hereditability of the disease.



Overlap between genes associated with BMD and genes involved in monogenic bone disorders (in red) (from Hendrickx et al. *Nature Rev Reumat, 2015*)

"Father of sclerostin" **Wim Van Hul** (Antwerp) then presented some latest results from work on rare disorders of high bone mass that may give new insight into the genetics of common osteoporosis. So far, mutations in over 240 genes are characterised in monogenic rare bone disorders, including differences in promoter expression, regulatory effects, and copy number variations. Genetic variability in baseline BMD is an important factor in fracture risk in common osteoporosis. European projects GENOMOS and GEFOS conducted a meta-analysis published in 2012 identifying 56 BMD loci associated with BMD, with several clustering to the RANK/RANKL/OPG and Wnt signalling pathways. Studying these loci also for mutations in rare bone disorders can provide new insights, especially as the effect size is bigger, and assist development of new therapeutic targets for common osteoporosis.

In fact, the study confirms the relevance of genes such as LRP5, SOST and CLCN7 previously studied in monogenic rare disorders as potential contributors to natural variations in BMD. However, some Wnt ligands and frizzled genes found by Estrada et al. have not so far been identified in any rare bone disease. Even so, this study has clearly identified that variations in genes encoding RANK/RANKL/OPG, along with LRP5, LRP4 and SOST can eventually explain much of the variation in BMD.

In the third presentation, **Lothar Seefried** (Würzburg, Germany) discussed clinical treatment options for several rare bone disorders, including hypophosphatasia, X-linked hypophosphatemia, tumor-induced osteomalacia, hereditary hypophosphatemic rickets with hypercalciuria, McCune–Albright syndrome, osteogenesis imperfecta, X-linked osteoporosis and autosomal dominant osteopetrosis type 2.

An important issue for treatment of rare bone disorders is the common misdiagnosis of patients, confusing symptoms with those of common bone disorders such as osteoporosis, other rare bone disorders, or nutritional deficiencies.

In other news...

Cortical bone fragility is common in osteoporosis linked to non-vertebral fractures, but little is yet know about how this occurs, and the mechanisms regulating homeostasis of cortical bone. A multinational collaboration examined four patients with the rare skeletal disorder Pyle's disease, characterised by thinning of cortical bone, limb deformity, and fractures. In all four patients they found mutations in the gene SFRP4, which encodes a soluble Wnt inhibitor frizzled-related protein 4. A Sfrp4-knockout mouse model replicated the features of Pyle's disease with thinner cortical bone, but increased amounts of trabecular bone. Treatment of these mice with a Bmp2 receptor or with antibodies to sclerostin corrected the defect in cortical bone. These results show that cortical and trabecular bone homeostasis in Pyle's involve different mechanisms, sFRP4 plays a key role in regulating BMP and Wnt signalling, and that these changes may be treatable by pharmacological interventions. Simsek Kiper PO et al., 2016

Studies of cherubism, arising from missense mutations in the SH3BP2 gene, have led to the discovery of a new feedback loop for osteoblast differentiation and proliferation involved in embryonic skeletal development. Studies in knockout mice have identified that tyrosine kinase ABL activates the RUNX2-TAZ complex required for osteoblastogenesis, and also enhances TAZ nuclear localization and the formation of the TAZ-TEAD complex required for osteoblast expansion. ABL is therefore essential for osteoblast differentiation and skeletal development. Matsumoto Y et al., 2016

One form of osteopetrosis is caused by mutations in PLEKHM1, which is involved in lysosomal trafficking, but the precise mechanisms are unknown. Researchers in Arkansas have investigated the function of PLEKHM1 in osteoclast-specific knockout mice that have increased trabecular bone, resulting from impaired osteoclasts. Examination of the mouse osteoclasts revealed an absence of the ruffled border, and clustering of the lysosomes at the perinuclear area rather than at the cell periphery. The group also established that DEF8, FAM98A, and NDEL1 all interact with PLEKHM1 in the regulation of lysosome positioning and secretion through RAB7. Fujiwara T et al., 2016

An interview with Gerald Grant

Gerald Brandt is president of the German hypophasphatasia patients' association Hypophosphatasie Deutschland (HPP) e.V and represented the association at ECTS 2017. He found time to give us an interview.

How is hypophosphatasia diagnosed?

I was diagnosed with HPP as an infant, which was not typical at that time. Most people with HPP are diagnosed much later, in adulthood. The older you get, the more difficult it is to diagnose as the symptoms are also found in other diseases, such as rheumatoid arthritis or osteoporosis. Awareness of the condition among clinicians could be improved. A combination of the clinical symptoms and low ALP results should confirm a diagnosis.

How did HPP e.V. come about?

HPP e.V. was formed in 2006, as the second national hypophosphatasia patient's association after the first one formed in France in 2004. There are now also associations in the USA, Japan, Canada, Poland, and the UK. We have currently 127 members, most of whom are adults with HPP, plus some parents with children who have HPP. When we started we came into contact with the group at the Wuerzburg University Hospital supporting children with HPP, so immediately joined forces.

We work closely with the clinicians in Würzburg, who now form the biggest European clinical center for hypophosphatasia. We also collaborate with other rare bone disease groups, especially OIFE for people with osteogenesis imperfecta.

Charity run at ECTS 2017. ECTS 2017 delegates raised more than €1750 in donations for HPP research.

Gerald Brandt with ECTS Executive Director Roberta Mugnai

What are the aims of HPP e.V?

We act as a grassroots patient organisation, providing advice to newly diagnosed people or those that suspect they have the disorder. Raising awareness of the disorder amount the medical community is our second aim. We also promote research into hypophosphatasia.

How does the association interact with researchers?

We collect knowledge and experience of our members which is useful for research, we interact regularly with researchers and connect with different centres. We run an annual meeting as a unique forum for discussion between patients, scientists and clinicians. And we directly promote research with grants.

What do you find useful about coming to ECTS 2017?

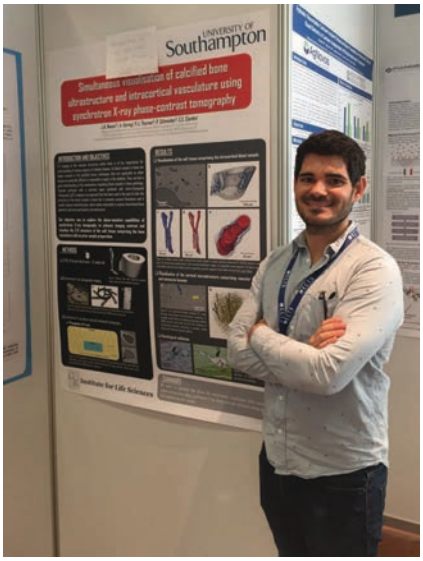
Firstly, we can raise awareness of hypophosphatasia and the charity run* is part of that. I can get myself updated on the latest scientific results that may be relevant, not only for hypophosphatasia itself, but also other diseases that may share some common factors. For example, I've been very interested in results related to Wnt signalling that seems involved in many disorders. Reports on new therapies, imaging and surgical techniques are also very interesting - anything related to osteoporosis may also be useful for treatment of hypophosphatasia.

**HPP e.V. donated ten copies of new book "Diagnostik und Management der Hypophosphatasie" as prizes for the ECTS 2017 charity run.*

ECTS enhances discussion of diverse poster presentations

In addition to 39 oral presentations, a total of 362 posters were presented at ECTS 2017, grouped by subject:

- Cell biology: osteoblasts and bone formation (31 posters)
- Cell biology osteoclasts and bone resorption (18 posters)
- Osteocytes, mechanobiology, and bone matrix (7 posters)
- Bone biomechanics and quality (11 posters)
- Preclinical and ex-vivo imaging (3 posters)
- Biochemical testing (10 posters)
- Osteoporosis: evaluation and imaging (23 posters)
- Osteoporosis: treatment (31 posters)
- Osteoporosis: pathophysiology and epidemiology (27 posters)
- Genetics & Epigenetics (16 posters)
- Hormones and mineral metabolism (14 posters)
- Energy metabolism and bone, fat and bone, diabetes (14 posters)
- Nutrition (12 posters)
- Muscle, clinical studies, physical activity and bone (21 posters)
- Bone development/growth and fracture repair (22 posters)
- Paediatric bone disease (9 posters)
- Other diseases of bone and mineral metabolism (48 posters)
- Chondrocytes and cartilage (7 posters)
- Arthritis and other joint diseases: translational and clinical (14 posters)
- Cancer and bone (17 posters)
- Zebrafish (7 posters)



Juan A. Nunez (Southampton, UK) presenting his poster "Simultaneous visualisation of calcified bone ultrastructure and intracortical vasculature using synchrotron X-ray phase-contrast tomography"

The congress also provided further opportunities to present and discuss results, including some innovative, new approaches:

Oral poster presentations

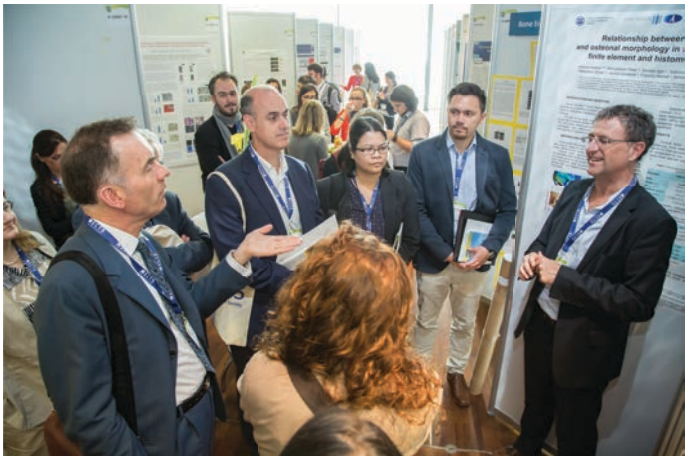
Offering more delegates the opportunity to make a short oral presentation of three minutes, with discussion after each group of three presentations

SNAPs

One-minute elevator pitch style presentations with a single slide to highlight key messages and invite a further look at your poster

Ask your peers

A short guided presentation of your poster followed by a chance for the presenter to ask questions to their audience



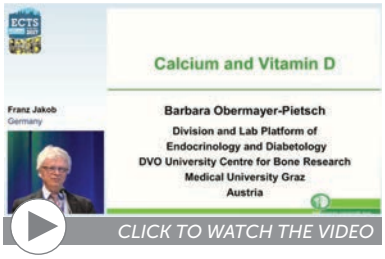
Ask your peers session: From pediatric bone disease to cancer

Vitamin D supplementation and osteoporosis, the story continues

Vitamin D supplementation was a hot topic discussed at ECTS 2017. In our clinical update on osteoporosis treatment, we heard the latest evidence on vitamin D and calcium supplementation. Later in the congress, clinical workshop "Vitamin D – back to the future" discussed the resurgence of research into vitamin D and osteoporosis, particularly concerning the biological functions of different forms of vitamin D, and the possible benefits of vitamin D for reducing the risk of falls.

Vitamin D and calcium

Franz Jakob gave a presentation prepared by **Barbara Obermayer-Pietsch** (Austria), discussing the evidence on the efficacy and safety of vitamin D and calcium (Ca) supplementation for reducing fracture risk.



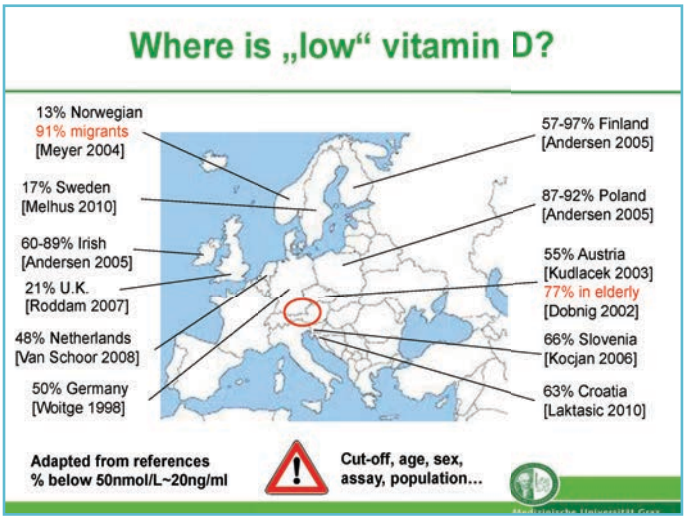
The prevalence of Vitamin D deficiency is high in almost all European countries, and within populations both younger and older age groups are affected, reaching more than 75% among the elderly in institutional care. Adequate vitamin D levels are essential for bone health, and vitamin D supplementation, along with calcium, is standard for patients with osteoporosis. However, evidence concerning the efficacy and safety of supplementation is conflicting. Appropriate targeting of vitamin D measurement and supplementation among various groups is a major current debate. Clinical studies are helpful to develop strategies taking into account the benefits, safety and cost-effectiveness.

Recommendations for adult vitamin D supplementation are generally around 20 µg per day (~800 IU). Supplementation with an extra 1000 IU of vitamin D should generally increase 25(OH)D by 10 ng/ml, and EFSA have defined the tolerable upper limit for vitamin D supplementation as 100 µg (4000 IU) for adults. However, serum levels of vitamin D3 that exceed 50 µg/ml have been associated with increased mortality, falls, fractures and other risks.

Study design should take account of the factors that can independently affect vitamin D absorption and metabolism. Weight loss is known to affect levels of serum 25(OH)D, as vitamin D is sequestered into adipocytes, conversely people gaining weight have lowered serum 25(OH)D. Many factors influence vitamin D levels, including geographical location and time spent outdoors, and it is challenging to measure these environmental and lifestyle factors in clinical studies. In general, significant effects of vitamin D supplementation have only been observed in individuals with very low vitamin D levels, and studies must take this into account in recruiting participants. As many studies include patients with relatively high vitamin D levels, results on a large range of outcome parameters may be affected and remain therefore sometimes conflicting.

An individual's response to vitamin D supplementation is also influenced by complex genetic factors, affecting the functioning of skin, liver, kidney, and efficiency of 25(OH)D uptake into different cells around the body. Various studies have identified SNPs and epigenetic changes related to vitamin D responsiveness, but as yet there is no tool to effectively target more appropriate supplementation to specific patients. Potential avenues for the future include miRNA markers of vitamin D status, and investigating the interactions of vitamin D with the gut microbiome.

Vitamin D metabolism is closely connected with Ca metabolism but is often studied separately. Europeans have among the highest dietary Ca intakes in the world, and Ca supplementation is very common especially for treatment of bone disorders, but also to promote general health in the population. Most dietary recommendations suggest around 1000 mg Ca per day is sufficient with maximum dose of 1.5 g per day. Also they recommend that Ca supplementation is only given where there are problems in absorbing sufficient Ca from the diet.



Several studies have suggested an increased risk of cardiovascular events (including myocardial infarction) with Ca supplementation, but data is inconsistent with contrasting results obtained from different parts of the world. The NIH-AARP study found a significant effect from Ca supplementation in men, with no significant effects in women, but only with actual Ca supplementation, not from dietary Ca intake. A prospective longitudinal cohort study in women in Uppsala found a narrow window for efficacy and safety of long term Ca supplementation, optimally around 1000 mg/day, and concluding that Ca supplementation is not helpful in women with an already high dietary Ca intake.

Where Ca supplementation clearly has value is for people that have problems achieving a normal dietary intake. Lactose intolerance is quite common especially in central and southern European countries, forcing low dairy consumption and a lower dietary Ca intake.

It is still unclear whether it is beneficial for everyone to receive Ca and vitamin D supplementation, separately or in combination. A Ca rich diet appears to be safe, and Ca supplementation should be advised together with meals for individuals who have insufficient dietary Ca intake to mimic physiologic intake. There are many factors affecting vitamin D levels in both serum and cells that we need to understand better in order to design optimal vitamin D supplementation. Many large trials on vitamin D supplementation are currently ongoing but there is as yet not enough data to give clinicians clear recommendations. Nevertheless, recommendations are already established for vitamin D supplementation for some specific groups.

Vitamin D and BMD

A sub-study of the New Zealand VIDA trial (see also below) has investigated whether vitamin D supplementation can influence BMD in older adults. Results were reported in clinical oral presentations at ECTS 2017 by **Ian Reid**.

The study participants received an initial dose of 200,000 IU of vitamin D3, followed by monthly doses of 100,000 IU, or placebo for two years. 418 participants completed the study and were evaluated for changes in lumbar spine BMD as well as BMD at other sites. No significant treatment effect was found on the lumbar spine BMD, however a significant effect of BMD changes in the spine and femoral neck of around 2% were found in those participants with baseline 25(OH)D ≤ 30 nmol/L.

Further trials of targeted intervention of vitamin D supplementation towards this group therefore appear justified.

Total versus free vitamin D – a new twist?

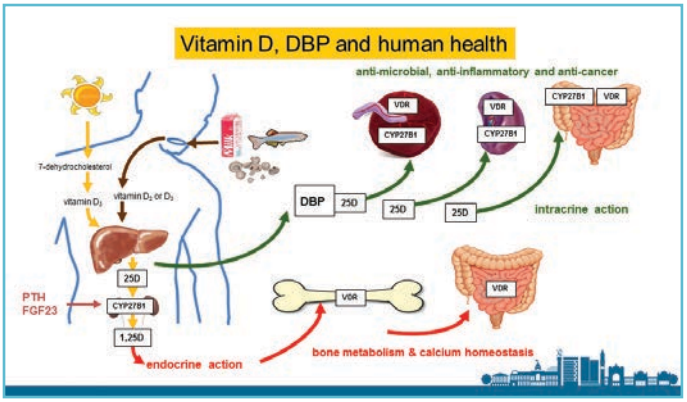
Martin Hewison (Birmingham, UK) discussed which forms of vitamin D should be measured in studies of vitamin D. This question has been investigated more intensively in the past few years.

In an average healthy person, around 90% of their typical total 50 nM of 25(OH)D is bound to vitamin D binding protein (DBP). The remainder is bioavailable, but mostly bound to other proteins such as albumin, with a very small amount of free 25(OH)D. Its established that the amount of free 25(OH)D can be reliably estimated from the measured total 25(OH)D and levels of serum DBP and albumin.

Expression of the main vitamin D binding protein (DBP) does vary between individuals. A GWAS study conducted by Wang et al. identified several SNPs within the gene for DBP (Gc) were associated with low serum DBP and low vitamin D status. In fact, there are some differences in distribution of the various forms of DBP proteins between ethnic groups. DBP has also been identified as a macrophage activation factor and as an actin binding agent.

All 25(OH)D is formed in the liver from vitamin D2 or D3. Active 1,25(OH)D is then generated in the kidney from 25(OH)D, with the involvement of DBP, and has important functions in the bone and colon where there are vitamin D receptors. 25(OH)D also exerts extraskeletal effects in the colon, kidney and placenta, but is completely dependent on transport by DBP to these organs, and then on localised conversion to 1,25(OH)D by CYP27B1.

It is usually assumed that there is a linear relationship between the levels of bound and free vitamin D. This was found to be the case in a recent clinical trial of vitamin D supplementation. This study also found that PTH was significantly suppressed by free 25(OH)D, but not total 25(OH)D. However, other studies have come to the opposite conclusion. For example, a poster P-HORM-4 at ECTS 2017 by Peris et al. presents results from a study in Barcelona of 173 postmenopausal women, which finds



similar relationships between PTH levels and the different forms of 25(OH)D.

Powe et al. found that the combined total of free and bioavailable vitamin D is a better marker of BMD, compared to 25(OH)D alone. In an later study, they also found significantly lower 25(OH)D and DBP levels in black versus white people in Boston, but total bioavailable and free vitamin D levels were similar between the two populations. The suggested explanation was that differences in DBP phenotype between ethnic groups could be responsible.

However, Martin Hewison and colleagues identified a methodological flaw in the Powe study. In two of their own studies in the USA, UK and Gambia, they found no significant difference in DBP expression between black and white men. They concluded that the use of monoclonal antibody-based ELISA was not allowing for detection of all the forms of DBP, which would be measured by an assay using polyclonal antibodies. When this artefact is corrected, there is a good correlation found between free and total 25(OH)D in both black and white populations.

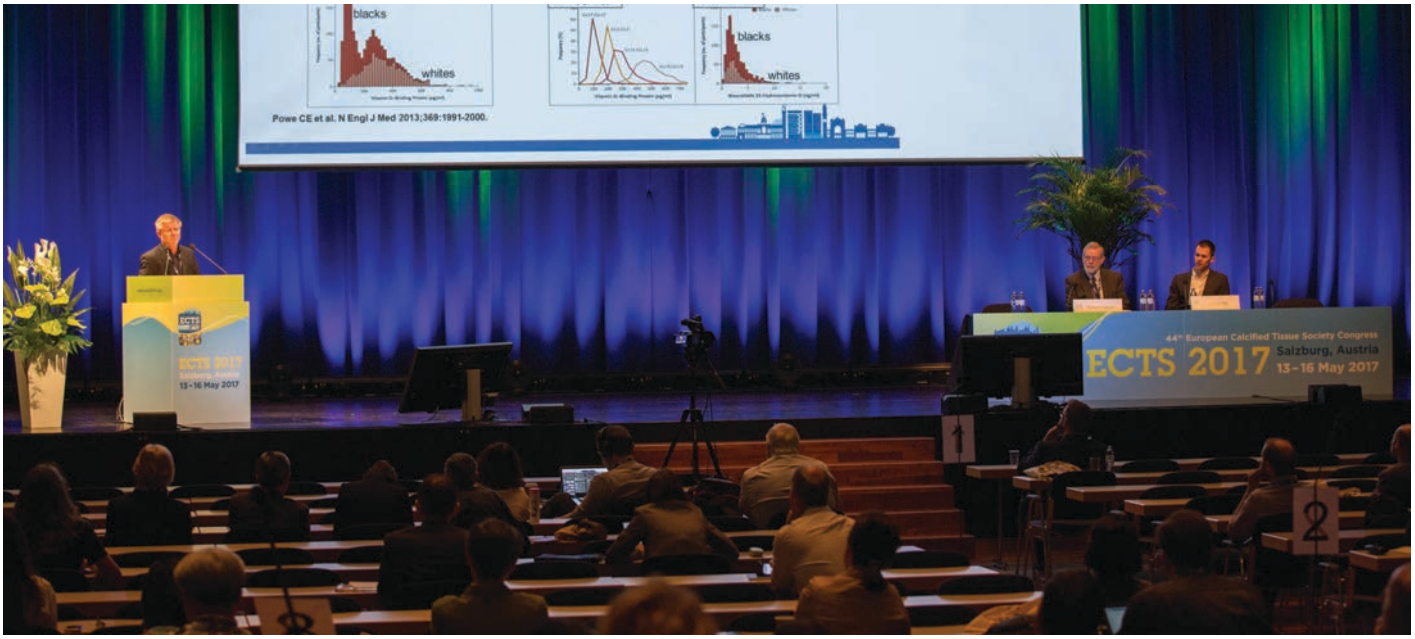
A DBP knock-out mouse has been studied by several groups. The first descriptions of these mice found them to be normal when receiving sufficient vitamin D, even with virtually no circulating vitamin D. But with vitamin D deficiency, the mice experienced secondary hyperparathyroidism and disrupted bone metabolism. The mice also showed a more sensitive gene expression response to 1,25(OH)D, and even though total 25(OH)D levels massively reduced, the homozygous mice actually have higher levels of free 25(OH)D compared to the wild type and heterozygous mice, which may have some protective effects.

Recent data obtained by Martin Hewison's group show that the levels of 25(OH)D in the kidney and spleen of the DBP-/- mice are not significantly affected by the lack of DBP, showing again that there are functions of vitamin D that are independent of DBP. They have also shown in wild type mice fed on diets containing either vitamin D2 or D3, that DBP has a higher binding affinity to vitamin D3 compared to vitamin D2. Differences in bone histomorphometry found by 8 weeks, and higher bone volume by 16 weeks, showed that the greater amount of free 25(OH)D in the vitamin D2 mice had a significant effect on their bone metabolism.

Clearly, 25(OH)D has effects on multiple organs that are both dependent and independent of DBP. This has to be considered depending on what type of study is being performed. Free 25(OH)D may eventually be shown a more sensitive marker for some specific questions, and it can now be measured directly by an ELISA kit. Also it could be interesting in future to investigate whether releasing more 25(OH)D from its bound to bioavailable form, feasible with certain free fatty acids or other nutritional factors, could have further effects on vitamin D functions.

Vitamin D and fall risk

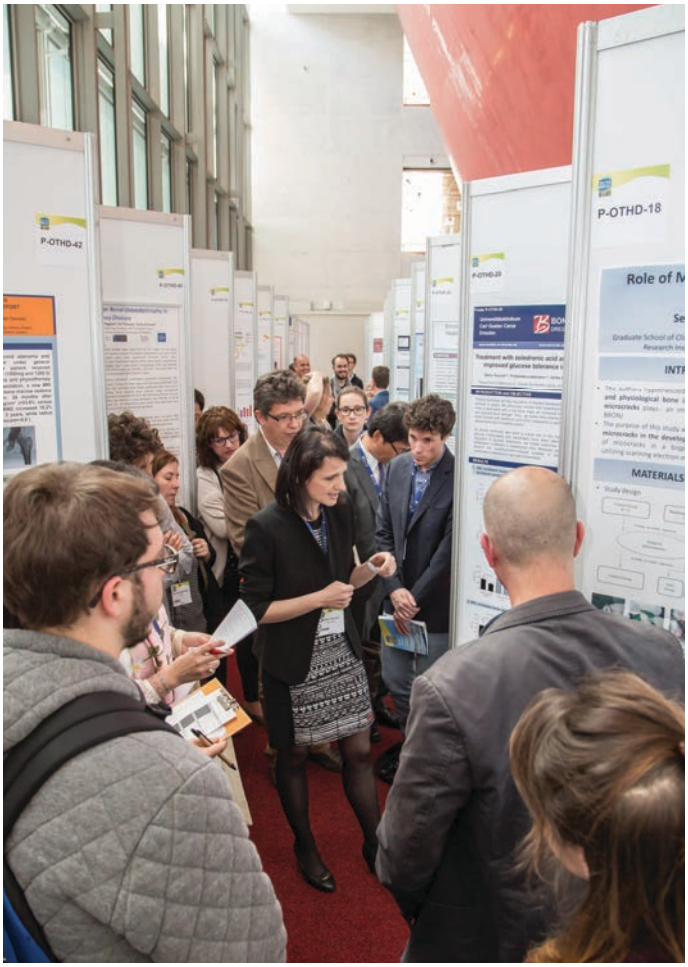
Terry Aspray (Newcastle, UK) started his talk by showing us a word cloud created by 30,000 people who had been affected by a fall - highlighting words such as pain, injury, embarrassment and fear. Up to a quarter of falls result in a fracture and in the UK, it is estimated that falls cost the country around £2 billion per year. The probability of experiencing a fall in any year rises from 21% in 46-65 year olds to 50% among the over 80s. Some individuals appear to be particularly at risk experiencing multiple falls, and the risk rises (to 66%) once a person has had a first fall.



The risk of falling is affected by intrinsic factors - such as chronic or acute disease - and extrinsic factors, in particular any hazards that could be present in a person's surroundings. Other important factors include the side effects of medication, and syncope (fainting) which affects half of the whole population at some point in our lives, and in older people is associated with orthostatic hypertension and cardiovascular disease. Clinical studies of falls have already investigated various interventions to prevent falls in the residential setting. A Cochrane Review published in 2012 concluded that active intervention can reduce falling rates by 36%, identifying exercise as a key factor in reducing risks, along with adequate levels of home staffing and home safety assessment to help reduce health and safety risks.

Vitamin D supplementation can improve musculoskeletal health and is associated with physical performance, while low vitamin D levels have been associated with falls. Many studies have therefore investigated whether vitamin D supplementation can reduce the risk of falls, but with complex and sometimes conflicting results. A meta-analysis published in 2009 found that regular vitamin D supplementation of 700-1000 IU each day reduced fall risk by 19% in the elderly, but another meta-analysis by Murad et al. in 2011 found the benefit of vitamin D monotherapy did not reach statistical significance.

It is plausible that higher doses of vitamin D can achieve a greater and more consistent reduction in risk of falls. Evidence so far from clinical trials suggests this is indeed the case, but that very high doses are associated with an increase in falls risk.



Concerning daily dosing, Smith and Gallagher recently reported a 12-month trial of seven different levels daily oral vitamin D supplementation, ranging from 400 to 4800 IU. A U-shaped curve relationship was found between vitamin D and incidence of falls, both by dose and serum 25OHD levels, with fall rates significantly decreased on the medium doses of 1600-3200 IU. However, fall rates increased as serum 25OHD exceed 40-45 ng/ml, and with the top two levels of daily dose (4000 and 4800 IU).

Adherence to daily supplementation can be poor, especially in the elderly, so investigation of monthly or even annual supplementation has been of interest. Sanders et al. found that annual oral 500,000 IU dose of vitamin D resulted in a 15% increase in incidence of falls, compared to placebo, with a higher risk in the 3 months following the dose. Terry Aspray is currently leading the VDOP study which is investigating vitamin D supplementation in 375 community-dwelling women and men at three monthly dose levels (12,000, 24,000 and 48,000 IU) for their effect on bone health, fractures and falls. Results are being prepared for publication later this year.

Several other studies of high monthly oral supplementation have been published. A RCT published in 2016 investigated very high monthly doses of vitamin D3 in 200 men and women over 70 who had experienced a prior fall. After 12 months the incidence of falls was significantly higher among those receiving 60,000 IU each month, and in those receiving 24,000 IU plus calcifediol, compared to those on 24,000 IU. The VIDA trial in New Zealand, just published, also investigated very high bolus supplementation versus placebo in over 5000 healthy volunteers aged 50-84 for a mean treatment duration of 3.4 years. Those receiving vitamin D were given a 200,000 IU initial dose followed by 100,000 IU each month. The overall rate of falls in the study was 52%, but there was no significant difference found between those receiving vitamin D and those on placebo.

Further studies are required to determine also any additional benefit of combining vitamin D supplementation with calcium. A beneficial effect on falls risk is suggested by Murad's 2011 meta-review and there is evidence from RCTs of a significant effect of combined therapy on hip fracture risk.

In summary, there is evidence that moderate monthly or daily doses appear to have a positive benefit for falls risk, but very high doses of vitamin D appear to increase the risk of falls. More robust evidence is required, including on the factors involved in falls. Terry Aspray emphasised that we need more studies that focus on falls as a primary outcome, and careful consideration of study design, if we are to conclusively decide this question.

In other news...

The contribution of inflammation to bone loss is likely to relate also to dietary factors. Two papers on dietary intervention from the CHANCES project, and the Women's Health Initiative found that higher adherence to a Mediterranean diet is associated with a lower risk of hip fracture. This may be due to antioxidant activities in the diet. Haring B et al., 2016, Benetou V et al., 2016

An update and meta-analysis of two previous systematic reviews on the effect of calcium intake on cardiovascular disease, drawing on trials published over the past 50 years, has concluded that Ca intake within tolerable upper intake levels (2000-2500 mg per day) is not associated with CVD risk in generally healthy adults. No statistically significant difference in CVD risk was found between groups receiving Ca supplementation, or calcium plus vitamin D supplementation, and those receiving placebo. Chung M et al., 2016

ECTS – a global forum for musculoskeletal research

ECTS was honoured to receive delegations from its partner societies in China (CSOBMR), Japan (JSBMR) and Korea (KSO and KSBMR), headed by presidents of these societies. Over 90 delegates attended from these countries. A special session involved the ECTS Academy hearing perspectives from these countries on What is New in Musculoskeletal Research.

Presidents from the East-Meet-West Programme

Also, ECTS congress is a regular meeting point for major EU- and national-funded research consortia. In the second half of this session, a series of pitch-talks allowed each consortium to present its latest progress in elevator-pitch style.

What is new in musculoskeletal research – the Eastern perspective

Mei Li from Peking Union Medical College Hospital, and a member of CSOBMR, presented an overview of musculoskeletal research in China, covering work on osteoporosis, metabolic bone diseases, genetic bone diseases, and bone biology. A major national study was highlighted, that is obtaining data to help define guidelines for diagnosis of bone diseases in China. Other groups are developing innovations in therapy involving regenerative medicine, pharmacogenomics, and are studying the molecular mechanisms of bone diseases. Concerning rare genetic bone diseases, several studies are examining the specific genetic mutations that are found in Chinese patients, for example in osteogenesis imperfecta more than 10 candidate genes have been found. Mei Li highlighted the benefit of training that Chinese scientists have received abroad and that China is looking forward to future cooperation with Europe to face future challenges together.

Takeshi Miyamoto from JSBMR presented some recent developments in Japanese research, focusing particularly on osteoporotic hip fractures. Japan has the world's oldest population with one in four aged over 65 and a high number of hip fracture patients. In the near future, 300,000 cases of hip fracture are expected per year. The two main contributors to hip fracture – bone fragility and falls risk – are a major area of study in Japan. Dr Miyamoto's group has identified HIF1α as a therapeutic target for post-menopausal osteoporosis, and an oral inhibitor protected against bone loss in mice. This may be a beneficial new therapy for the early stages of osteoporosis, avoiding some of the problems associated with bisphosphonates. Also, muscle weakness is considered the biggest risk factor for falls or slips causing hip fractures. Smad 2/3 proteins have been identified as therapeutic targets and inhibitors are being developed, with in vivo studies planned as the next step.

Taeyong Lee from Korea focused on advances in biomaterials, biomechanics, and bio-imaging. These are very exciting, multidisciplinary areas of research. Firstly, bioengineered materials for bone regeneration regenerative medicine were discussed. BMP2 can enhance bone formation, but is costly and can be easily denatured. A chemically immobilised BMP2 was presented that achieves the same bone regeneration but with a 5-10 times lower dose for the same effect. Nanofibres are also being developed to enhance bone regeneration by guiding cell migrations.

The effects of ageing on bone quality are just as important as the measurement of BMD. Taeyong Lee has developed a more rigorous analysis of bone biomechanics using finite element modelling, obtaining site-specific data on localised bone tissue distribution (buckling ratio), material properties, structure and geometry. Applying this analysis to samples from trials of antiresorptive drugs shows that overall BMD and structural properties won't always explain site-specific fractures. Data on trabecular bone scores obtained in Korea were discussed in comparison with scores obtained from Japan in the 1990s. Using the same statistical analysis, the rate of decline was significantly faster in Korea. A further study has indicated that changes in BMI could explain these differences. Finally, advances in clinical imaging using MRI and rapid CT scan were presented, with potential to provide detection of early cartilage changes in osteoarthritis.

EU and national research consortia at ECTS

DIMEOs is a German clinical research collaboration investigating early onset osteoporosis, in order to improve outcomes for this disease as well as develop improved early detection of common osteoporosis. Eight partners are involved with expertise in imaging, genotyping, exome sequencing, mouse models and clinical studies. Some results were presented at the Rare Bone Disease workshop at ECTS 2017.

RUBICON is an EU-funded Marie Skłodowska-Curie RISE network on connective tissue disorders involving the exchange of a planned 35 staff between 10 partners. Research achievements so far include identification of circadian clock-responding cartilage proteins, new insights into the molecular mechanisms of autosomal dominant osteopetrosis, the effect of unloading on osteoblasts, and proteomic analysis of extracellular matrix under hypoxic and normoxic conditions.

SYBIL is an EU-funded, 18 partner consortium, investigating the genetic changes leading to rare skeletal and cartilage disorders, which may also be relevant for osteoporosis and osteoarthritis. 'Omics' profiling is leading to new cell and animal models and systems biology is being used to study protein interactions. SYBIL has organised a satellite meeting at ECTS 2017 and partners also gave three oral presentations and several posters during the congress.

The Skeleton consortium is founded by the Italian Telethon foundation that funds research into rare monogenic diseases. It directly funds three institutes as well as extra mural research, with 25 scientists receiving funding overall. The network particularly connects the next generation of scientists with senior researchers and three meetings have been held since 2016. So far, €8m funding for research into rare genetic bone disease research has resulted in 238 publications.

TRR79 is a German funded consortium using materials science to help solve biomedical problems, targeting fractures and multiple myeloma. Biomaterials have been developed and tested in mouse models to help advanced healing. Certified two materials and seeking funding for clinical trials over next four years.

iBONE is a French and German collaboration with four partners, working to identify new therapeutic targets for osteoporosis. Analysis of mouse models and human tissues are being performed to select epigenetic regulators, that will be analysed further in vitro and in vivo for identification of therapeutic targets.

SKELMET is a German consortium of 5 groups working on the biology of bone metastasis. There are still no completely effective treatments and much research still has to be done. The consortium brings together expertise in imaging, cell and animal models, and clinical validation. They have identified new targets including DKK1 in breast cancer, established animal models and synergetic imaging tools, and also new tissue banks and tissue microarrays, which are available for external collaborators. The consortium has successfully applied for new funding and will continue their collaboration with up to 25 partners throughout Germany.

GEFOS is a large global consortium originating as an EU-funded FP5 project GENOMOS. The consortium is continuing to identify genetic determinants of osteoporosis using GWAS, followed by analysis of phenotypic pathways and functional assessment. They have developed a genome screen array optimised for clinical markers that can have optimised content. Current efforts are focused on collaboration with other projects to reach 10 million samples for GWAS. Research is also expanding into the interaction with the microbiome and use of new animal models including zebrafish.

Euroclast is an EU-funded Marie Curie Initial Training Network focused on osteoclast biology and the training of early stage researchers. The first researchers are now ending their three year training, though some will require further time to complete their PhD. Many of them have appreciated collaborating in a larger network. As well as PhD studies, Euroclast has provided training in complementary skills and given the researchers an understanding of alternative career pathways, for example in industry.

Osteogrow is an EU-funded project of 11 partners conducting clinical studies of a BMP6 compatible device for bone regeneration. This is unusual in being developed from academia rather than from industry. First time in man was achieved in Zagreb and phase 1 was passed with no safety issues. Currently the project is in phase II for spine regeneration. A new application to Horizon 2020 is seeking funding for the next clinical phase.

BMD as a surrogate marker of fracture risk

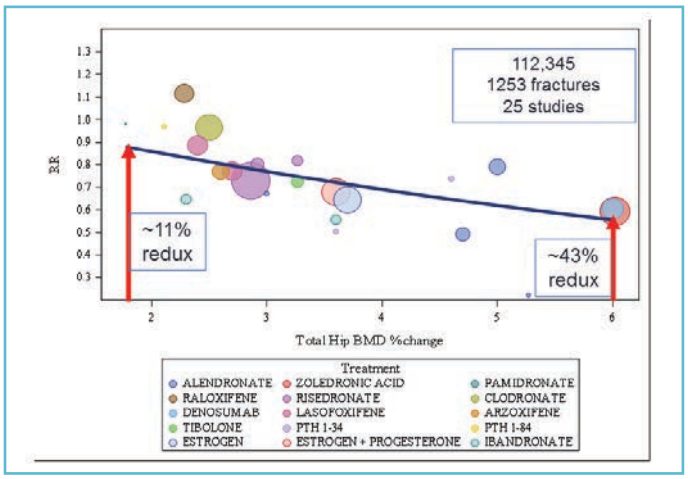
New osteoporosis treatments should provide a significant reduction in fracture risk, but using this as a clinical endpoint in RCTs requires very large, expensive trials and presents some ethical concerns related to treating low risk populations.

At ECTS 2017, **Dennis Black** reported on the FNIH Bone Quality Project, supported as a USA public-private partnership by multiple funding agencies and industry, which is aiming to develop BMD as a robust surrogate endpoint for fracture risk reduction. If regulators can approve this approach then there will be enormous benefits for future clinical trials, allowing them to be more efficient and bring new treatments more quickly to approval.

The Project is using a meta-analysis of image-derived biomarkers (DXA and QCT/FEA) and biochemical bone turnover markers, and so far has obtained data from 34 RCTs involving over >90,000 patients. Data collection has been challenging as the team have had to persuade pharmaceutical companies to release data that they normally do not share, including from trials of drugs that were not approved. Ultimately almost all the companies and other trials that were approached have agreed to collaborate.

DXA data collection has been the most successful so far with data for over 80,000 patients obtained. Preliminary results were presented. Hip BMD showed a strong relationship to fracture risk with the highest BMD gains associated with a 43% reduction. Also BMD is strongly related to risk of vertebral fractures risk, but less strongly to other fractures.

Published clinical studies have had varying durations, which are difficult to compare directly. The Project has investigated the shortest possible time in which DXA can reliably predict fracture risk in a RCT, investigating periods between 12 and 24 months. It appears that a strong correlation is established by 18 months. Intermediate level data analysis is continuing with application of innovative methods, for example linear mixed models, to address methodological problems such as missing DXA data. Measurement of biochemical markers may also be utilized.



Progress is being made towards registration of BMD as a surrogate marker for hip fracture risk in high-risk postmenopausal women. A letter of intent has already been received from the FDA, and an application will be submitted later this year.

At ECTS 2017, Dennis Black was awarded the ECTS Excellence in Research award for his contributions as a biostatistician to seminal studies of the epidemiology of osteoporosis, and many landmark randomized clinical trials of osteoporosis treatments. 10 of his many published papers have been cited more than 1000 times each, and he has an h-index=76

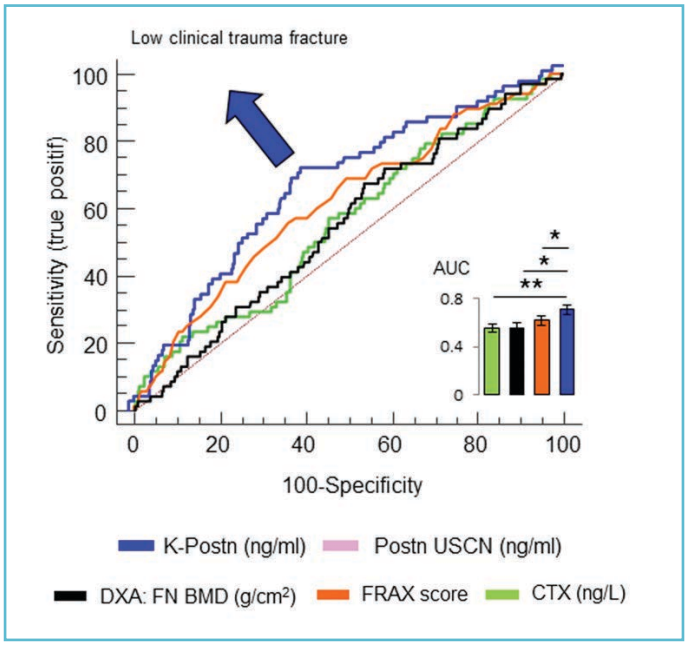
Periostin as a marker of cortical bone formation and fracture risk

Bone turnover markers are poorly associated to fracture risk and the IOF and IFCC currently do not recommend their inclusion in clinical assessments of fracture risk. Therefore, new clinical markers are required. Previous studies have already investigated periostin as a potential marker of fracture risk. Periostin is an extracellular matrix protein expressed in bone in the periosteum, which contributes to bone formation in response to mechanical loading and PTH. It is of particular interest as a marker of cortical bone structure.

Several oral presentations and posters at ECTS 2017 described work on periostin. Walsh and colleagues reported on a study of periostin levels in 166 men and women at three skeletal ages, finding an inverse correlation with tibia cortical thickness and density, and positive correlations with PINP, CTX and IGF-1. Higher levels of periostin were observed in the 16-18 age group, suggesting a role in IGF-1 driven cortical modelling, particularly in young adults.

Results from previous studies of periostin as a biomarker for fracture risk are conflicting, possibly as a result of the multiple isoforms of periostin, and differences in the design of periostin ELISAs developed by different companies. Most importantly, several other organs apart from bone secrete periostin.

Bonnet and colleagues have discovered a bone-specific periostin fragment K-Postn, resulting from cathepsin K digestion in the bone. A new ELISA has been developed for this biomarker, and a study in the GERICO cohort of 695 postmenopausal women has shown that this marker is associated with risk of incident low trauma fractures, independently of BMD, FRAX, bone microstructure and bone turnover.



In other news...

Results published from the Bone Mineral Density in Childhood Study have concluded that physical activity, in terms of more intensive exercise, has a clear benefit in children with a genetic predisposition to lower BMD in adulthood. Mitchell JA et al., 2016

Several new studies have examined the effects on the skeleton of bariatric surgery used to treat obesity. A nested case-control study of fracture risk in bariatric patients has found site-specific variations, with an increase in risk for upper limb, and clinical fractures (spine, hip, femur and pelvis), and a lower risk of distal lower limb fracture. In comparison, before surgery obese patients have a higher risk of distal limb fracture, low risk of upper limb fracture, and no difference in risk for clinical fractures, compared to normal individuals. Various changes in bone homeostasis may be responsible for these changes and patients receiving bariatric surgery should receive support from a bone specialist. Rousseau C et al., 2016

Bone and beyond

New results expanding our understanding of bone biology and its implications for bone diseases were presented at ECTS 2017. We highlight here some of the oral presentations given during the congress.

Role of platelet-derived growth factor receptor β clarified in osteoblasts

The PDGF (platelet-derived growth factor)/PDGFR system is expressed in numerous cells. Osteoblasts express both PDGFRα and PDGFRβ, and PDGF-BB is secreted by osteoclast precursors. Cyril Thouverey (Geneva) reported on an investigation of the role of PDGFRβ in osteoblast biology.

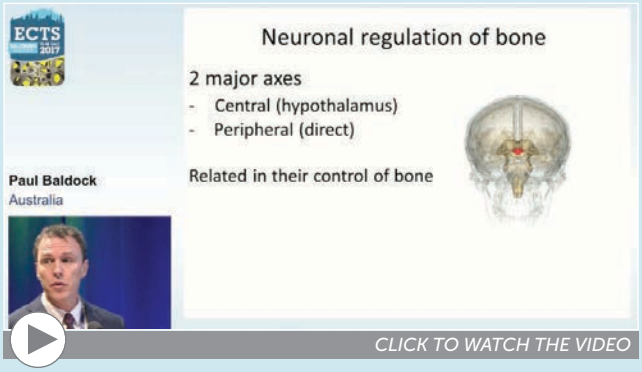
Mice expressing a floxed PDGFRβ-encoding gene were cross-bred with inducible Osx-Cre mice to generate mice lacking PDGFRβ in osteoblasts. Pdgfrb deletion was induced at 1.5 month of age. Bone phenotyping of mutant mice and controls at 4.5 months did not show any difference in cortical bone volume and thickness at femoral midshaft. However, an increased trabecular bone volume was found at the distal femur and the fifth lumbar vertebral body. Increases in expression of Osx, Alp and Ocn were measured in the tibia of mutant mice. In vitro experiments showed that PDGFRβ mediates PDGF-BB-induced inhibition of osteoblast differentiation through SRC signaling.

THY-1—a novel positive regulator of bone mass and strength

Thy-1 (CD90) is known to control fibroblast proliferation and differentiation. **Ann-Kristin Picke** (Dresden) and colleagues wanted to know if it also plays any role in the differentiation of MSC into osteoblasts or adipocytes. The disruption of this process to favour adipogenesis plays a key role in the development of osteoporosis and diabetes.

The group investigated bone phenotype, osteogenesis and adipogenesis in a Thy-1 knockout mouse, compared to the wild type. The knockout mouse gained weight with a higher amount of subcutaneous fat compared to the wild type, but with reduced trabecular and cortical bone thickness and elevated cortical porosity, reduced alkaline phosphatase expression, and reduced mechanical bone strength and elasticity. Ex vivo analysis of Thy-1–/– MSC obtained from the mouse showed a downregulation of markers for osteogenic differentiation and a corresponding rise in expression of adipogenic markers, including PPARγ and adiponectin. These results demonstrate clearly that Thy-1 is involved in osteogenesis from MSCs and is a novel positive regulator of bone mass. Further work will investigate the signalling pathways involved.

At the ECTS 2017 symposium "Nerves and Bone", Paul Baldock (Australia) spoke about the exciting new connections discovered between bone, energy metabolism and now also brain functions.



Induced global deletion of the glucocorticoid receptor impairs fracture healing

Endogenous glucocorticoids are important in many processes throughout the body. **Anna Rapp** reported on results obtained together with Yasmine Hachemi and others at the University of Ulm, Germany, shedding light on the role of endogenous glucocorticoids in fracture healing.

Fracture healing involves phases of inflammation, regeneration and bone remodelling. The role of glucocorticoids was investigated in each of these phases. Femur osteotomies were performed in a mouse model with inducible deletion of glucocorticoid receptor (GR), along with its wild type littermates. Molecular, cellular and bone phenotype changes were followed through the subsequent phases of bone healing. During the first six hours, IL-6 levels were elevated in serum in the GR deleted mouse, and levels of IL-1β were found almost three times higher in the hematoma, compared to the controls, but the levels of inflammation were otherwise unaffected. In the intermediate phase, no significant differences were found in the bone, cartilage or fibrous tissue.

However, after 28 days a significantly higher proportion of cartilage was found in the calli of the mouse model, a 21% reduction in BMD, and overall a much lower proportion of successfully healed bones – 29% compared to 86% among the wild type littermates.

The investigators concluded that the absence of GR has a major effect on fracture healing, through its impact on the final stage of endochondral ossification.

Claes Ohlsson received the Mike Horton Basic Science award at ECTS 2017.
He has developed an interdisciplinary team at the Centre for Bone and Arthritis Research at Gothenburg and has conducted pioneering studies of metabolic bone diseases in animal models and in longitudinal population-based clinical cohorts. He has published several seminal papers on the role of IGF-1, GH and sex steroids in bone.

Expression of SOST in mesenchymal stem cells from patients with osteoporotic fractures

Sclerostin, secreted by osteocytes, has been investigated for its role as a Wnt inhibitor in both rare bone diseases and osteoporosis. However, mesenchymal stem cells (MSCs) that differentiate to osteoblasts are also regulated by the Wnt pathway. **Jose A. Riancho** presented results from a study in Santander, Spain, which has examined the expression of SOST in MSCs obtained from the bone marrow of patients with hip fractures, and controls with osteoarthritis. RNAseq analysis, later confirmed by RT-qPCR, revealed an approximate ten-fold higher expression of SOST in the MSCs from hip fracture patients compared to the controls, accompanied by elevated RUNX2 and SP7 expression. Measurement of DNA methylation of 84 CpGs mapping to SOST, RUNX2 and SP7 regions showed 27 that were differentially methylated, consistent with the transcriptomic analysis and suggesting a likely epigenetic mechanism. The results suggest that epigenetic upregulation of SOST expression in MSCs may contribute negatively to the differentiation of MSCs into osteoblasts in patients with osteoporosis

ECTS/Amgen Bone Biology Fellowship 2017 to support prospective clinical studies on gut microbiota and bone health

Several important studies linking gut microbiota to bone health have been published, but only in animal models. Dr **Simone Bianciardi**, research fellow at the University of Siena, Italy and recipient of the ECTS Amgen Bone Biology Fellowship 2017 will undertake a prospective clinical study. This will be conducted in women experiencing menopausal transition due to ovariectomy over three years: 20 treated with probiotics and 20 on placebo. The study will investigate the effect of probiotics on gut permeability, inflammatory status, bone metabolism and BMD. A second study will identify the impact of gut microbiota on fracture risk, by comparing the gut microbiota of 15 postmenopausal women who have incident fragility fractures, to the gut microbiota of 15 healthy women.

Plenary oral presentation - Overexpression of human RANKL decreases skeletal muscle function and engenders insulin resistance

RANKL is part of the RANK/RANKL/OPG signalling pathway controlling osteoclast differentiation and activation. RANK is also expressed in skeletal muscle, fat and liver, and could therefore have a wider role especially in glucose metabolism. **Nicolas Bonnet** (Switzerland) has investigated this question in transgenic mice expressing human RANKL with low (Tg5516) and high (Tg5519) copy numbers, treated with OPG-Fc or vehicle for 4 weeks. A battery of tests for glucose metabolism, muscle function, treadmill performance and gene expression were performed.

At four months of age, the Tg5516 mice had only mild alterations in bone phenotype and no metabolic defects, while the Tg5519 mice had severe osteoporosis, a much lower muscle strength and treadmill speed, and insulin resistance. Tests using labelled 2-[14C] deoxyglucose indicated a lower glucose uptake in soleus, WATi and the brain of Tg5519, and after sacrifice they were found to have lower gastrocnemius and soleus mass, and decreased expression of para, LPL, Myh2 and Myh1 in the gastrocnemius. However, treatment of the Tg5519 mice with OPG-Fc was found to decrease or normalise these changes.

These results show that overexpression of RANKL may play an important role in the co-development of osteoporosis, type 2 diabetes and sarcopenia, which is an increasing concern for human health.

In other news...

Various sub-sets of stromal cells, osteoblasts, osteocytes, and hypertrophic chondrocytes secrete a C-type lectin domain protein, Clec11a, which has been found to promote osteogenesis and maintains the adult skeleton. The authors propose to rename Clec11a as osteolectin. Yue R et al., 2016

Many promoters of osteoclastogenesis have been studied, but very few negative regulators. A deficiency Guanine nucleotide-binding protein subunit α13 (Gα13) has been linked to a dramatic increase in osteoclast number and activity, through reducing RhoA activity and Akt/GSK3β/NFATc1 signalling pathway. Osteoclast-specific Gna13 conditional knockout mice have severe osteoporosis but can be protected from bone loss by Gα13 gain-of-function. Gα13 could therefore be manipulated as an endogenous "master switch" of osteoclastogenesis for the treatment of bone diseases. Wu M et al., 2017

Korean researchers have found that WHI-131, a compound already shown to have anti-inflammatory, anti-allergic, and anti-leukemic potential in animal models, is also a potent inhibitor of osteoclast function and promotes osteoblast differentiation. These effects have been found through investigations using in vitro cell cultures and in vivo mouse models of bone loss and bone formation. Through this dual action, WHI-131 is a potential candidate for treatment of osteoporosis. Cheon YH et al., 2016

Increased levels of leptin have been found to activate Jak2/Stat3 signalling in bone marrow stromal cells in mice. This resulted in increased adipogenesis and reduced osteogenesis in the limb bones of wild type mice. However, mice with conditional deletion of leptin receptor from their limb bone marrow stromal cells did not experience this effect. Moreover, on a normal diet their limb bone adiposity was decreased and osteogenesis increased in comparison with the wild type. Yue R et al., 2016

A case-cohort study of pro-inflammatory cytokines in men has identified several as markers of fracture risk. Men with elevated levels of 3 or more markers experienced the highest hazard ratios for fracture, especially in the hip. Cauley JA, 2016

Treatment with parathyroid hormone (PTH) can help reduce fractures in osteoporosis, but the mechanism is not yet known. A recent study has shown that bone marrow adipocytes are uniquely responsive to PTH and can influence osteogenesis and adipogenesis. Deletion of the PTH1R receptor in mouse mesenchymal stem cells resulted in lower bone formation and higher bone marrow adipose tissue. Bone marrow adipocytes originating in these stem cells were found to have higher expression of RANKL, and levels of RANKL were elevated in bone marrow and serum. Administration of PTH to control mice was found to reduce bone marrow adipose tissue, and similar findings were reported in male osteoporotic patients. Fan Y et al., 2017

Researchers at Columbia University have pioneered investigations into the endocrine activities of bone. A new study has suggested a role for osteoblast-secreted protein Lcn2 in the regulation of appetite in the brain. Experiments have shown binding of Lcn2 to the MC4R receptor in the hypothalamus and activating a MC4R-dependent appetite suppression pathway. In addition Lcn2 contributes to glucose homeostasis through inducing insulin secretion. Mosialou I et al., 2017

The Karsenty lab at Columbia has now shown that levels of circulating osteocalcin can double during aerobic exercise, and that osteocalcin signalling in the muscle myofibers improves efficiency of their nutrient uptake and catabolism. Also, osteocalcin is responsible for much of the exercise-related release of IL-6 and administration of exogenous osteocalcin can both boost the exercise capacity of 3 month old mice and restore a youthful exercise capacity in 15-month old mice. Mera et al., 2016

Fulzele et al. have investigated the action of osteocyte secreted sclerostin on whole body metabolism. Three mouse models expressing high sclerostin levels were found to have normal glucose and insulin metabolism, but progressive loss of white adipose tissue. Beige adipocytes were found increased in gonadal and inguinal white adipose tissue, with a reduced canonical β-catenin signalling, suggesting that sclerostin and perhaps other secreted factors from bone have a role in the regulation of beige adipogenesis. Fulzele et al., 2017

Dead chrondrocytes are found in damaged joint cartilage, but has not been clear if the loss of chondrocytes is actually involved in cartilage damage. A study has selectively killed articular surface chondrocytes in mice knee joints, and observed the effects with rapid 3D confocal microscopy and histology. Surprisingly, no significant deterioration in the cartilage was observed as much as 8 months later. This shows that chondrocyte death does not contribute to cartilage damage. Instead, the study determined that chondrocytes adopt a catabolic phenotype as a result of initial damage following injury. Minjie Zhang et al., 2016

Neutrophins including nerve growth factor (NGF) are known to be secreted in peripheral tissues and direct innervation. A paper from Thomas Clemens' group shows how NGF-TrkA signalling between osteoblast progenitors and nerve cells is involved in the innervation of developing long bones, promoting also vascularization and ossification. Both inhibition of TrKA signalling, and deletion of NGF in perichondral precursor cells in mouse models were found to disrupt ossification and reduce postnatal bone mass and length. Tomlinson RE et al., 2016

The connections between the microbiome and osteoporosis have not previously been addressed, though it is likely the microbiome affects nutritional intake, inflammation, and other factors related to bone health. A study in sex steroid deficient mice, which normally experience gut permeability, upregulation of osteoclastogenic cytokines, and bone loss, has found that these effects are not observed in germ-free mice. Also, twice-weekly treatment of the mice with probiotics was found to protect against bone loss. These results suggest a key role for gut microbiota and gut permeability in the development of osteoporosis. Li JY et al., 2016

Reducing fracture risk in patients with neurological disease

Patients with neurological diseases, especially Parkinson's disease and stroke, are at a higher risk of fracture, and also experience worse outcomes after a fracture. In the ECTS 2017 symposium on Nerves and Bone, Ken Poole (Cambridge) discussed the clinical evidence and current efforts to address this challenge.

Stroke patients are likely to lose BMD after their stroke, and are at a higher risk of recurrent falls. A 2009 study of Dutch prescription records found stroke associated with a two-fold increase in risk of hip or femur fracture, with the risk highest for those experiencing a stroke less than three months prior to fracture. The risk diminishes with time but does not return to baseline. A later study in Germany suggested that stroke patients without a functional impairment were more at risk of osteoporotic fracture at most sites, while those with functional impairment had no elevated risk compared to the reference group that had experience no stroke. The same group studied fracture risk in patients with idiopathic Parkinson's disease, finding again that those without functional impairment were at higher risk.

This evidence suggests that retaining mobility increases the risk of falls for those with neurological disease. Also, changes in the care environment appear to be the biggest predictor of fall risk. Pathogenic factors in fracture risk among patients with neurological disease include visual problems, malnourishment, and side effects of medication that can all be potentially addressed through improved management of care.

Ken Poole then discussed several clinical intervention trials. Patients with neurological disease face particular challenges in receiving some medications, for example oral bisphosphonates can be difficult to swallow. Ken Poole's group previously conducted a small phase II RCT of zoledronate in hemiplegic stroke patients, finding that treatment protected BMD in the hemiplegic hip, compared to controls that lost an average of 5.5% of their hip BMD. In 2005, Sato et al. published very promising results from a trial of risedronate in 2005, but a critical review of this group's work revealed major methodological flaws, resulting in a retraction of this paper and others by the same group.

So far no clinical study has been conducted on osteoporosis therapy in stroke patients with fracture as an end point. It is challenging to assemble the necessary size of study among stroke patients. However, so far there is no suggestion that patients receiving zoledronate do worse than those who are not treated, and even a single dose during the high risk period may be beneficial.

Other interventions that should be investigated further are modification of vitamin D supplementation, and changes to a patient's environment to reduce risks of falls, as part of an individualised approach. FRAX does not specifically address neurological disease as a risk factor. However, the QFracture algorithm, calibrated for use in the UK to calculate risk of fracture over one to ten years, does provide inputs for several neurological conditions, including dementia, epilepsy and Parkinson's disease.

Eastell and Cummings highlighted in their 2016 review that "there are nearly as many patients with medical conditions that substantially increase the risk of hip fracture as there are people with osteoporosis by femoral neck bone mineral density". Patients with neurological conditions form a significant part of this group. Greater cooperation between bone researchers and clinicians, and neurologists caring for this group of patients, will certainly help to clarify how active interventions can reduce their risk of fracture in future.

In other news...

Data from the 25 year prospective Dubbo Osteoporosis Study has been analysed to investigate how muscle weakness relates to post-fracture mortality. Groups of study participants were selected that had been measured at least twice for muscle strength before or after fracture. Analysis of mortality during the follow up period showed that muscle weakness was associated with 15% of premature deaths after fracture in women and 23% in men. This is the first prospective study to address muscle mass as a factor in fracture risk. Pham et al., 2017

A study of US Medicare prescription databases has shown that drugs that are known to be associated with increased fracture risk are still being prescribed after a hip, shoulder or wrist fracture. While its important to treat the conditions affecting the patient, accelerated bone loss or increased risk of falls will be a likely result with these treatments. The authors suggest that other options for addressing secondary fracture risk may be considered. Munson JC et al., 2016

Insights from Outside – expanding your horizons

The ECTS 2017 "Insights from Outside" plenary lecture, and several other sessions, introduced a range of emerging concepts and hot topics with speakers from outside the bone filed.

Plenary lecture - Using biobanks to study the genetic basis for common diseases

Using the example of his research into chronic obstructive pulmonary disease (COPD), **Ian Hall** (Nottingham, UK) explained in his plenary lecture how Big Data and biobanks are important resources for investigating the genetic basis of many diseases.

COPD is associated with smoking, but only some smokers develop the disease, suggesting a strong genetic component. COPD is in part defined by reduced lung function. A genome-wide association study of COPD, the UK BiLEVE study, has been performed using genetic material obtained from the UK Biobank. The UK Biobank has recruited 500,000 patients who have undergone phenotyping, completed a questionnaire, donated blood and urine, and have given informed consent. The whole Biobank is in the process of being genotyped.

The BiLEVE study selected 50,000 samples from the Biobank, including only those with complete data and European ancestry. This sample was further stratified into groups of heavy smokers and those that never smoked, and for each of these, three further sub-groups with low, medium and high lung function (FEV1). The samples were analysed using a custom array, identifying six novel genome-wide significant signals of association with extremes of lung function and COPD risk. The study also discovered five new genome-wide signals associated to smoking behaviour. These results were published in 2015, and have potential implications for targeting of smoking cessation efforts. Additional studies, recently published looking at lung function as a quantitate trait, have increased the number of genetic factors predicting FEV1 (and therefore potentially COPD risk) to 97. Given that COPD places an extra burden on healthcare this raises the possibility of targeting interventions at those smokers who have a higher risk of COPD.

For musculoskeletal disorders, the use of Big Data can help predict treatment response, define predictive factors for severity and disease subtypes, define new therapeutic targets and improve our knowledge of pathophysiology. The UK Biobank collects several relevant data fields including hand grip strength, physical activity, BMD/DXA, types of treatment and fractures.

Ian Hall emphasised that the UK Biobank will still be open to international collaboration after the UK leaves the European Union. The data can be accessed for research by any user, private or public, from anywhere in the world, as long as the study is ethically sound, has been approved by UK biobank and all resulting data is archived with the biobank.

Tendinopathy

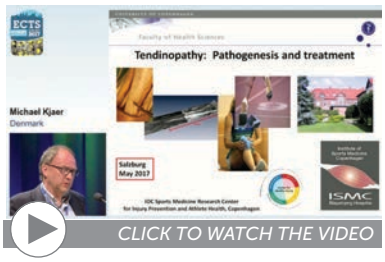
Tendinopathy is common form of tendon overuse injury, experienced as a result of sport or occupational activities. The ECTS 2017 symposium on tendinopathy discussed latest research addressing the causes and treatment of the condition.

Malcolm Collins (Cape Town, South Africa) spoke about the progress towards identifying genetic factors involved in a predisposition to tendon injury. The biological mechanisms of tendinopathy are poorly understood, and over recent decades inflammatory and degenerative mechanisms have been proposed. The current model views tendinopathy as a multi-factorial condition with genetic, intrinsic and extrinsic components. Some individuals do appear to be more susceptible to tendon injury and also vary in the time of healing and recovery.

Malcolm Collins' group have focused on identifying genetic markers of Achilles and elbow tendinopathy. Rare diseases including collagen fibril and collagen disorders, e.g. Ehlers-Danlos Syndrome can suggest candidate gene polymorphisms to investigate for an association to tendinopathy. In particular, variants in genes for type V and type XI collagen have been identified, that are thought to influence the size, arrangement and biomechanical properties of tendon fibres. Work is also progressing in the use of next generation and whole exome sequencing to identify more markers of tendinopathy and define its biological mechanisms.

However, there are huge ethical issues to address, especially with the easy availability of direct-to-consumer genetic testing and the high demands placed on sports professionals.

Michael Kjear (Copenhagen, Denmark) discussed the pathogenesis and treatment of tendinopathy. Tendon tissue has very little turnover in adults, with 95% of the collagen being stable upon reaching adulthood. However, a current hypothesis is that there are still some dynamic changes in the remaining collagen, especially in response to mechanical loading. Tendinopathy, arising from repeated overload and insufficient recovery, has been observed by electron microscopy to involve accumulation of disorganized tissue between the fascicles as well as distorted cells, swelling, and angiogenesis, and an increase in collagen III in the affected area.



Studies of tendon therapy have long established that muscle strength training, rather than rest, speeds recovery. In fact, inactivity has also been found detrimental to tendon health. Several recent studies have investigated the optimal type of training to improve outcomes. In general, controlled, slower strength training is better for tendinopathy in around 70-75% of cases, and there is no real difference in outcomes between eccentric and concentric training.

The benefit of using NSAIDs is still being clarified. Inflammation has been observed at least in the early phase of tendinopathy. However, it's also known that NSAIDs inhibit the normal physiological response to exercise. Other therapies include shockwave therapy, which is at least shown to help calcific tendinopathy, and platelet-rich plasma injection, which is still popular among sports clinicians but there is still little evidence of any benefit.

In conclusion, proper clinical investigation of tendon therapies has progressed a great deal over the past 10 years, and right now there are major advances in our understanding of the pathogenesis. For treatment, it appears that no one treatment will be 100% effective and most will benefit from a combination of approaches.

Malcolm Collins and Michael Kjaer are participants in the EU-funded RUBICON network, developing a programme of global staff exchanges for research into connective tissue disorders, including tendinopathy.

Autophagy – an essential biological process

Autophagy is a highly conserved process of degradation and recycling of cell components that are no longer required, or that have become damaged or malfunctional in some way. Hence autophagy plays an essential role in maintaining cell and organ homeostasis, and dysregulation of autophagy has a causative role in many diseases, including skeletal pathologies. But, the mechanisms of autophagy are still not completely understood.

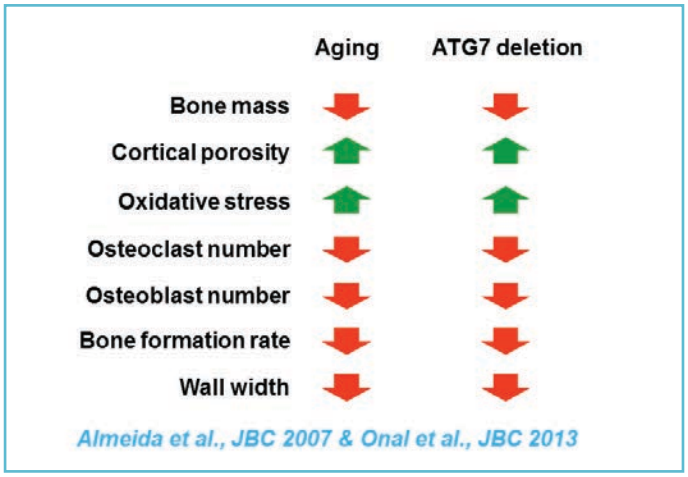
In our ECTS 2017 Preclinical Workshop on Autophagy, two leading researchers working in this field, **Sharon Tooze** and **Charles O'Brien**, explained some of the latest developments.

Several kinds of autophagy have been identified, including macroautophagy, microautophagy, chaperon-mediated autophagy and xenophagy. Control of autophagy involves a large family of ATG genes. Macroautophagy is the main pathway employed by cells and involves the formation of a double membrane – an autophagosome - to surround and transport the targeted organelle to the lysosome for degradation. One of the earliest events triggering formation of autophagosomes is the translocation of ATG9 vesicles, and the activation of the ULK and Beclin1 complexes at the site of membrane formation.

Sharon Tooze (Francis Crick Institute, UK) was responsible in 2006 for confirming that autophagosome formation was dependent on ATG9. She presented some of her group's recent work in mammalian cell lines to define more precisely the protein-protein interactions involved this step of autophagosome formation. Collaboration with the Francis Crick electron microscopy facility has allowed the group to utilise cryo-soft x-ray and correlative light microscopy to image intracellular structures under near-native conditions.

The current picture obtained through these studies is that ATG9 trafficking occurs by vesicles originating from both the Golgi complex and endocytic compartments. This process is mediated by small GTPases, which confer specificity to the transport step, and TRAPIII, a large tethering complex which allows the selective transport vesicle to the correct membrane.

Charles O'Brien (USA) discussed the link between glucocorticoid- and aging-induced bone loss and autophagy. He has a long-standing interest in how bone is lost during ageing and in response to glucocorticoids. Previous findings by his group show that autophagy is promoted in osteoblasts and osteocytes during glucocorticoid-induced bone loss, and reduced in ageing. This provided a rationale for the generation of a conditional Atg7-ko (Atg7-cko) animal model to specifically ablate this protein from the osteoblast lineage (from pre-osteoblasts to osteocytes depending on the Cre model he used), and to analyse its bone phenotype with or without glucocorticoid challenge.



The results show that the Atg7-cko model with ablation in mature osteoblasts and osteocytes, has features resembling those of aged mice, even at a young age (see figure below). In particular, this model has low bone turnover and cortical porosity. Intriguingly, challenging these mice with an excess of glucocorticoids did not worsen the bone phenotype, but rather prevented some osteocyte cell death via induction of the autophagic flux.

Disrupting Atg7 earlier in the osteoblast lineage caused a more severe bone phenotype, with spontaneous fractures observed in half the mice by one month of age. This occurs because of a delay in the formation of the osteocyte canalicular network, adding to the previously described abnormalities to give a mechanically unfit bone.

The results indicate that autophagy is necessary for the transition from osteoblast to osteocyte, since the pre-osteoblast phase, and open a new avenue in the field of bone autophagy.

More "Insights from Outside"....

Further presentations recorded from our preclinical workshops can be viewed online.

- A preclinical workshop on Liquid Biopsies included Aija Line (Latvia), speaking on extracellular vesicle-borne small RNAs as potential markers of disease progression, and Catherine Alix-Panabie (Montpellier) on circulating tumour cells.
- The preclinical workshop on Ribosomal Proteins in Health and Disease included talks by Rebekka Schneider-Kramann (Germany) on the role of ribosomal proteins in myelodysplastic syndrome, and Alyson MacInnes (Amsterdam) speaking on a novel ribosomopathy linking translational fidelity to intellectual disability, autism, and dysmorphism.

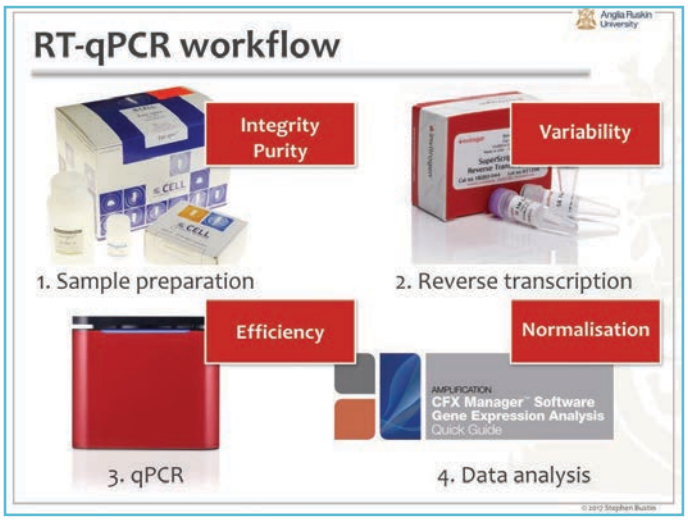
The ECTS Iain T Boyle Award recognises the contribution and progress of younger scientists working in the field of bone and calcified tissue. In 2017 it was awarded to Dr **Carmine Settembre**, a group leader at Telethon Institute of Genetics and Medicine (TIGEM), Naples, and Assistant Professor of Medical Genetics at the Federico II University in Naples. He is investigating the FGF-autophagy axis in bone physiology with support from an ERC Starting Grant.

qPCR studies lag far behind in quality control and reporting

Quantitative real-time PCR (qPCR) is an essential tool used regularly by most researchers participating in ECTS 2017. So, there was huge interest in the Basic Science Update on Technology, when speaker Stephen Bustin questioned the validity of many published qPCR results.

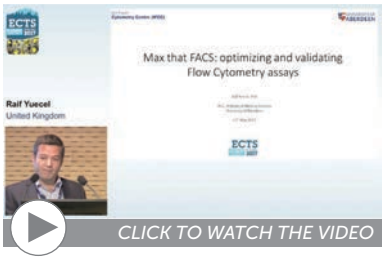
Stephen Bustin is well-known for his expertise on qPCR methodology and reliability, having published many papers on the subject and acted as an expert witness. He was also involved in the development of the MIQE Guidelines in 2009, which set out the minimum information that should be provided in publications of qPCR experiments. His presentation at the ECTS 2017 Basic Science Update on Technology showed how variability can be introduced at all stages of qPCR experiments, including primer design and validation, RNA extraction, the critical reverse transcription stage, the qPCR step and data analysis. It is not unknown for published studies to use an incorrect primer, or a primer that also amplifies a pseudogene or a closely related target. The choice of PCR reagents, including the reverse transcriptase and PCR master mix, is a major source of variability. Possible confounding effects involving reference genes are not often considered, neither the importance of sufficient replicates of the reverse transcription step. Some studies have described a positive result based on differences in mRNA abundance as little as 1.5-fold, but without rigorous control of variability, such a result is likely to be meaningless. Often, a lack of detailed description of qPCR materials and methods in published papers makes it impossible to reproduce the results, and to verify their validity.

Stephen Bustin illustrated these problems by discussing examples of randomly selected published studies in the bone field and found that many lack reporting of essential quality control steps and have fundamental methodological flaws. He also conducted a random survey of qPCR users among ECTS 2017 delegates that he has described further in this short communication. This found that a majority of these considered qPCR to be simple and reliable, but only a small minority carried out checks on RNA integrity and purity. PCR specificity and efficiency was checked only by those who considered themselves expert users. Only three individuals among the ECTS delegates questioned by Stephen Bustin were aware of the MIQE guidelines.



In the same session, a presentation given by **Sharon Bahia** from the European Collection of Authenticated Cell Cultures (ECACC) showed how problems with human cell line cross-contamination and misidentification are being effectively addressed. Its been known since the 1960s that cross contamination can occur, resulting in misidentified cell lines being used in experiments and erroneous results being published. The ECACC and similar repositories around the world now employ rigorous authentication of their cell lines, with STR profiling or DNA barcoding of cell lines established. Researchers are recommended to implement their own quality control steps during cell culture procedures and to build this critical step into their research methodology and grant proposals. A free handbook is available from the ECACC website – Fundamental Techniques in Cell Culture, Laboratory Handbook -Third Edition – we are all encouraged to make use of this when designing our cell culture protocols.

In his talk, Stephen Bustin said that "qPCR is around 30 years behind cell culture". Several decades after the first discoveries of cross-contaminated cell lines, major journals and funders now require that cell lines are authenticated before results are sent for publication. The hope is that it will not take as long for the problems in qPCR to be addressed.



The Basic Science Update on Technology also included a talk by **Raif Yuecel** on good practice in multicolour flow cytometry. With equipment ever evolving and more and more labels being detectable simultaneously, the correct use of controls is paramount. He echoed the concerns of the other speakers in this session that the community is not always sufficiently careful in experimental design and interpretation of results. He described a flow chart with the key points to pay attention to. He concluded by giving some examples of of new methods in flow cytometry, especially his group's work on adapting the technique to address the challenge of analysing and sorting microvesicles from blood plasma.

Meeting the Experts at ECTS

Our popular ECTS Meet the Expert sessions allowed delegates to hear leading experts discuss their specialist knowledge, and to ask questions. Half were on clinical and half on preclinical topics, and the programme also featured a talk from a Nature editor.

Optimal replacement with vitamin D
Terry Aspray, United Kingdom

How to manage the young person with osteoporosis
Jennifer Walsh, United Kingdom

What is the mechanism for glucocorticoid-induced osteoporosis?
Lorenz Hofbauer, Germany

Thought you knew everything about PCR? Variability of results depend on reserve transcription of PCR
Stephen Bustin, United Kingdom

Bone vasculature
Anjali Kusumbe, United Kingdom

Pain and cancer in bone
Toshiyuki Yoneda, Japan

Patients at high fracture risk: beyond BMD and FRAX
Steven R. Cummings, USA

Long-rerm treatment of osteoporosis
Dennis Black, USA

Rare bone diseases
Maria Luisa Bianchi, Italy

Difficult cases in glucocorticoid-induced osteoporosis
Kenneth Saag, USA

Avoiding pitfalls in epidemiological studies of osteoporosis
Bo Abrahamsen, Denmark

Bone phenotyping in mice
Duncan Bassett, United Kingdom

Impact of inflammation on osteoblasts in inflammatory arthritis
Ellen Gravallese, USA

Navigating the publishing process at scientific journals
Randy Levinson, USA

In other news...

Irving Weissman and collaborators have published a human stem cell differentiation atlas, derived from comprehensive analysis of purified mesodermal cell lineages, using whole population and single cell analysis with RNA-seq, ATAC-seq and surface markers. They developed efficient induction of all lineages to 80-99% purity. The atlas is available as a resource for future development of regenerative medicine. Koh WK et al., 2016

Advances in regenerative medicine for cartilage defects have been limited by the difficulties in in vitro expansion of chondrocytes. Chinese researchers have isolated human cartilage-derived progenitor stem cells from fully differentiated chondrocytes, under low glucose 2D culture conditions. These cells have been found to expressed MSC marker CD146, and a similar phenotype to bone marrow MSCs but with a greater chondrogenic potential. Cartilage formation using these cells has been demonstrated in pilot in vitro and in vivo studies. Yangzi Jiang et al., 2016

Deep tissue 3D imaging is usually limited by the scattering caused by biological tissues. A new protocol has been developed for whole bone optical clearing, without affecting endogenous fluorescence. Imaging with light sheet fluorescence microscopy was then combined with a computational method to detect and count labelled osteoprogenitor cells, with no sampling required. This is a very exciting development, providing new possibilities to study cells within transparent bone, especially in reporter mice. Greenbaum A et al., 2017

New insights into bone disease in cancer

Plenary oral presentation - Regulation of breast cancer tumorigenesis, metastasis and osteolysis by IKKε

IκB kinase subunit epsilon (IKKε), a key component of the NFκB signalling pathway important for bone remodelling and inflammation, was identified as a breast cancer oncogene in 2007, and is highly expressed in human and mouse breast cancer cell lines. However, its role in breast cancer metastasis has not yet been defined.

In his plenary oral presentation, **Ryan Bishop** (Sheffield, UK) presented results obtained in breast cancer cell lines and mouse models of breast cancer through stable knockdown of IKKε, or by pharmacological inhibition using IKKε/TBK-1 inhibitor, Amlexanox.

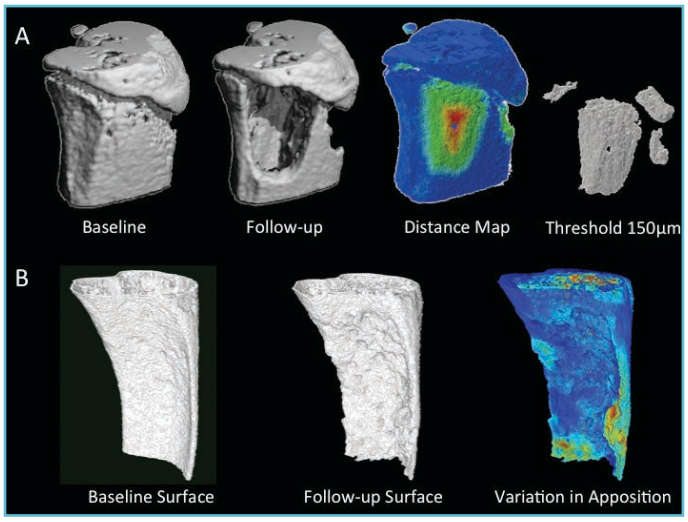
In MDA-MB-231 human cancer cells, inhibition of IKKε reduced cell migration, invasion and viability, while its overexpression stimulated migration and viability. In bone marrow cell culture, the normal stimulation of osteoclast formation by conditioned medium obtained from MDA-MB-231 cells was found to be dependent on IKKε. Treatment of an immunocompetent breast cancer mouse model with Amlexanox showed a reduction in mammary and skeletal tumour burden, and reduced osteolysis. In both cell and animal models, the therapeutic effect of Docetaxel was potentiated when used in combination with Amlexanox. In mice, a reduced incidence of metastases to bone, brain and spleen was found with this combined treatment, in comparison to individual treatments or vehicle.

Overall, these results show that IKKε inhibition, either alone or in combination with chemotherapy, is a promising approach for the treatment of metastasis in breast cancer.

Tracking the progression of osteolytic and osteosclerotic lesions using time-lapse micro-CT

Standard bone structural evaluations are often inadequate to determine localised changes in bone, found in bone metastases in osteotropic cancers. Graeme Campbell reported on work at Kiel, as part of the German SKELMET project, to develop new methods for obtaining detailed information on the progression of osteolytic and osteosclerotic lesions from analysis of time-lapse micro-CT images.

Two orthotopic mouse models of breast cancer were used in the study. The first group were injected with human osteolytic MDA cells, and treated with alendronate or placebo. The second group were injected with osteosclerotic human MCF-7 cells. Weekly micro-CT imaging was performed over 12 weeks. Images were processed by registration to the baseline, with mapping of osteolytic bone loss or osteosclerotic apposition calculated through computational analysis of the baseline and follow-up images (see figure below).



In this way, detailed microstructural data were obtained in a time-series for both experiments, along with standard BMD, BV/TV and separation measurements. As expected, the time-lapse microCT analysis showed the development of osteolytic lesions, with the mice treated with alendronate developing lesions with reduced volume (18.6% less by 4 weeks) than the untreated mice. However, BV/TV and separation were only slightly deviated from baseline. Only two of the MCF-7 mice developed tumours so the data is only qualitative, but increased SDD was observed by 5 weeks, indicating osteosclerotic growth.

This approach has therefore been shown to detect metastatic progression in bone, and effects of bisphosphonates on osteolytic lesions, that may not have been measured by standard methods. Further studies will use this approach to investigate novel treatment strategies.

Bidirectional regulation of osteosarcoma associated bone formation by exogenous and tumour-derived Sema3a

Daniëlle De Ridder (Sheffield, UK) presented an interesting study that deals with Semaphorin 3a and its role in osteosarcoma. Her results show that overexpressing Sema3a in KHOS cells or treatment with recombinant Sema3a significantly suppresses migration and invasion, while concurrently decreasing osteoclast formation in different in vitro models. This was due to Gsk3β phosphorylation, β-catenin overexpression and reduced NF-κB activation.

However, Sema3a overexpression in KHOS cells did not affect in vivo growth in xenograft experiments, but rather reduced ectopic bone formation, while treatment of KHOS inoculated mice with Sema3a increased bone volume in non-inoculated limbs.

In conclusion, Sema3a seems to be an interesting therapeutic agent to be used in association with classic chemotherapy, but further studies are needed to assess its efficacy.

Control of osteosarcoma by RSK2 inhibition-induced polyploidy

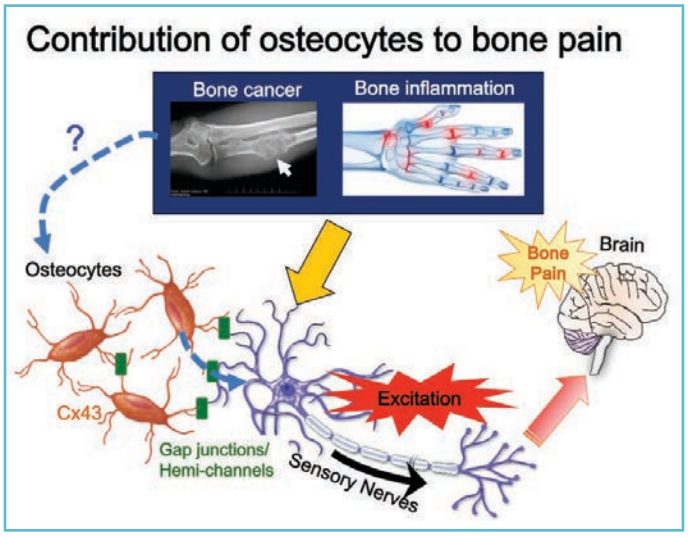
Jean-Pierre David (Hamburg, Germany) described the role of Rsk2 (ribosomal S6 kinase 2) in c-fos-induced osteosarcoma. In particular, the group used the c-fos transgenic (cFosTg) mouse model that spontaneously develops osteosarcoma, and used different approaches to inhibit Rsk2 either pharmacologically or by genetic manipulation. P53-mesenchymal stromal cells-deficient mouse was also used as osteosarcoma model. Rsk2 inhibition significantly reduced growth of osteosarcoma cells deriving from the cFosTg mouse, and intriguingly, the cells also became at least tetraploid for the most part, as assessed by cytofluorimetry. Similar results were observed in cFosTg mice with genetically inactivated Rsk2. This was also true for the P53 mouse model-derived-osteosarcomas, and for U2OS and SaOS in vitro. Large scale gene expression analyses revealed that Rsk2 ablation blocks the cells in the mitotic phase of the cell cycle, most likely because of mitotic catastrophe during chromosome segregation.

This study suggests that RSK inhibitors might be a potential therapeutic approach for osteosarcoma, although more studies are needed to assess the safety of the treatment.

Bone pain-modifying actions of osteocytes via connexin43-mediated communications with sensory nerves

Bone pain is one of the more significant and distressing aspects of many bone diseases as experienced by patients. Bone is heavily innervated by sensory nerves in close cell-cell contact with osteocytes. **Masahiro Hiasa**, one of our East meets West award winners, gave an oral presentation of his work investigating whether osteocytes play a role in modulating axogenesis and bone pain.

Close contact between osteocytes and sensory nerves was confirmed in observations of bone obtained from a DMP-1/GFP mouse that showed direct physical contact between GFP+ osteocytes and calcitonin gene related peptide-positive sensory nerves. Further in vitro experiments showed that sensory nerve cells transferred calcein to osteocytes, while co-culture with osteocytes enhanced acid-induced sensory nerve excitation.



It was then investigated whether connexin 43 (Cx43), which forms gap junction channels that mediate cell-cell coupling between bone cells, could also be involved in an interaction with nerve cells. Blocking this protein with a selective inhibitor GAP27, or knockdown of Cx43 in the sensory nerve cells did indeed block the previously observed excitation of sensory nerves by osteocytes. GAP27 was then found to decrease sensory nerve excitation and quantified bone pain (hyperalgesia and allodynia) in mice injected with mouse breast cancer cells. In addition, bone pain was significantly alleviated in osteocyte-specific connexin 43 knock-out mice that were injected with breast cancer cells.

The study therefore reveals new details of the interactions between sensory nerves and bone cells, showing that osteocytes play an important role in modulating bone pain and are a potential and alternative therapeutic target.

A role for vitamin D receptor in breast cancer metastasis and epithelial to mesenchymal transition

Lack of vitamin D promotes cancer growth in bone, partly due to indirect effects of vitamin D deficiency on bone causing alterations within the bone microenvironment. However, there is evidence that there are also direct effects (via the vitamin D receptor (VDR)) of vitamin D on cancer cells that have so far been scarcely investigated. In his studies on the effects of the VDR in breast cancers that were conducted at the ANZAC Research Institute, University of Sydney, Australia, Konstantin Horas (Würzburg, Germany) described how a stable VDR-knockdown (VDR-KD) was generated in a breast cancer cell line (MDA-MB-231) and tumour cells were then injected intracardially in nude mice to check for metastatic spreading pattern compared to control cells that express the VDR. Results showed that VDR-KD cells have a more invasive phenotype promoting breast cancer metastasis to bone. Moreover, epithelial-to-mesenchymal transition (EMT) markers were significantly affected by VDR-KD. These results appear be confirmed in human cancer patients, where VDR expression of tumours seems to be inversely correlated with metastasis and patient prognosis.

ECTS Awards

ECTS/Amgen Bone Biology Fellowship

Supported by AMGEN

Dr Simone Bianciardi

Steven Boonen Clinical Research Award

Supported by AMGEN

Professor Nick Bishop

Excellence in Research Award

Professor Dennis Black

Mike Horton Basic Science Award

Professor Claes Ohlsson

Iain T Boyle Award

Dr Carmine Settembre

Philippe Bordier Clinical Award

Professor Bente Langdahl

East-meets-West Presidential Research Award

Supported by AgNovos Healthcare

The three highest-ranking abstracts from China, Japan and Korea received this award.

Japan

- Bone pain-modifying actions of osteocytes via connexin43-mediated communications with sensory nerves (**Masahiro Hiasa**)
- Neuronal HGF regulates breast cancer progression and bone pain induction (**Tatsuo Okui**)
- Effect of fibroblast growth factor 1 (FGF-1) on chondrocytes through CCN2 regulation and its possible role in osteoarthritis (**Abdellatif Elseoudi**)

China

- Gene mutation spectrum and genotype-phenotype correlation in Chinese osteogenesis imperfecta patients revealed by targeted next generation sequencing (**Fang Lv**)
- Differences in femoral neck structure between elderly Caucasian and Chinese populations: a Perth-Beijing cohort study (**Ling Wan**)
- GPATCH1 and AKAP11 identified by GWAS and targeted resequencing regulate osteogenic mineralization (**Ka Fai Cheng**)

Korea

- Biomarkers for bisphosphonate-related osteonecrosis of the jaw (**Jin-Woo Kim**)
- The transformation of mature osteoblasts into bone lining cells during bone loss by mechanical unloading (**Sang Wan Kim**)
- Olecranon fractures have features of osteoporotic fracture (**Hyun Sik Gong**)

New Investigator and Travel Awards

Supported by AgNovos HEALTHCARE *and* ELSEVIER

The ECTS New Investigator awards are available to ECTS members who submit an abstract to the congress,.

- Regulation of breast cancer tumorigenesis, metastasis and osteolysis by IKK? **(Ryan T. Bishop)**
- Peculiar osteopetrotic patients: new insights on bone biology from exome sequencing **(Eleonora Palagano)**
- The relation between radio-graphic vertebral fractures and trabecular bone score: a population based study **(Fjorda Koromani)**
- Micropetrosis of osteocyte lacunae originates from accumulation of calcified nano-spherites and varies between healthy, osteoporotic and bisphosphonate-treated bone (**Peter Milovanovic**)
- New insights into the CLCN7-dependent Autosomal Dominant Osteopetrosis type 2 (ADO2): a systemic disease (**Antonio Maurizi**)
- Genome-Wide Association Study Identifies SREBF1, IRX5, PAX9 AND IGF2BP3 as Genetic Determinants of Dental Maturation (**Olja Grgic**)
- HMGB1 accelerates regeneration of multiple tissues by transitioning stem cells to G(Alert) (**G. Lee**)
- Establishing race- and gender-specific reference intervals for pyridoxal 5-phosphate to better identify adult hypophosphatasia using data from the NHANES programme (**Philip Nicklin**)
- Apolipoprotein A-I Prevents Osteoporosis and Promotes Osteogenesis of Mesenchymal Stem Cells via STAT3, CXCL6, and CXCL8 (**Yu-Chuan Liu**)
- Pleiotropic effects of genetic variants associated with different phenotypes on osteoporosis risk (**Maria Christou**)
- High bone turnover in mice carrying a pathogenic Notch2-mutation causing Hajdu-Cheney syndrome (**Timur Yorgan**)
- Change in bone mineral density (BMD) or bone turnover markers (BTM) did not predict risk of vertebral fracture after discontinuation of alendronate in the FLEX study (**Stephen H. Chang**)
- Quantitative 3D-morphometry of vertebra reveals severe pathological changes in an osteogenesis imperfecta zebrafish model carrying a collagen type I glycine substitution (**Imke Fiedler**)
- Leveraging genetic associations to evaluate clinical risk factors for osteoporotic fractures (**Katerina Trajanoska**)
- Bidirectional regulation of osteosarcoma associated bone formation by exogenous and tumour-derived Sema3a (**Daniëlle de Ridder**)
- Thy-1 - a novel positive regulator of bone mass and strength (**Ann-Kristin Picke**)
- Osteosarcoma development by non-canonical Wnt signalling (**Kazuhiko Matsuoka**)
- Dissecting the Mechanisms of Progressive Osteolysis In Gorham-Stout Disease (**Michela Rossi**)
- Loss of p53 compensates osteopenia in murine Mysm1-deficiency (**Anna Kovtun**)
- Identification of a novel secreted factor (KIAA1199) that enhances skeletal stem cell motility and migration and is up-regulated during fracture healing: role of Wnt signalling (**Kaikai Shi**)
- Phenotypic study of a novel mouse model for Crouzon syndrome with acanthosis nigricans (**Maxence Cornille**)

Feedback on ECTS 2017

From twitter

@Hermann-Agis
It was a wonderful congress here in Salzburg! Thank you all who made it possible #ECTS2017 #ECTS

@JajaSalam
Having a wonderful time at #ECTS2017 in Salzburg. Excellent talks and more to come!

@BrittleBoneUK
Great so many of our medics @AcademyEcts #ECTS2017 learn lots chaps

@Chuesa
Amazing lecture by Nick Bishop at #ECTS2017

From congress delegates

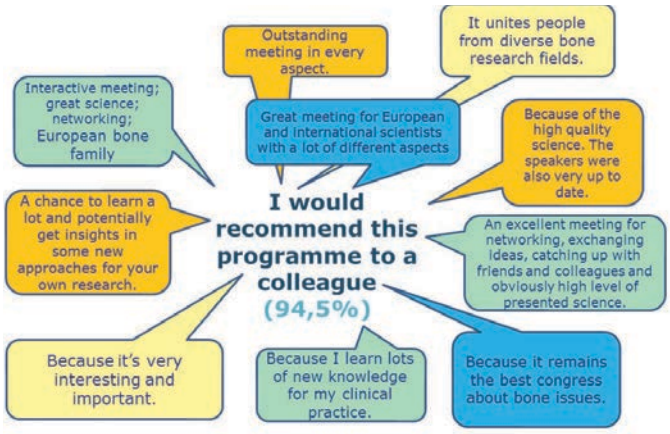
"For me it's the first time at ECTS, but it will be worth coming back next year. The programme is great, so many things. I like to read the posters and see the connections to our field. The presentations are highly interesting, and the short oral poster presentations were really useful, 3 minutes each, to get a broad view of what is going on- that was a good idea."
(PhD student)

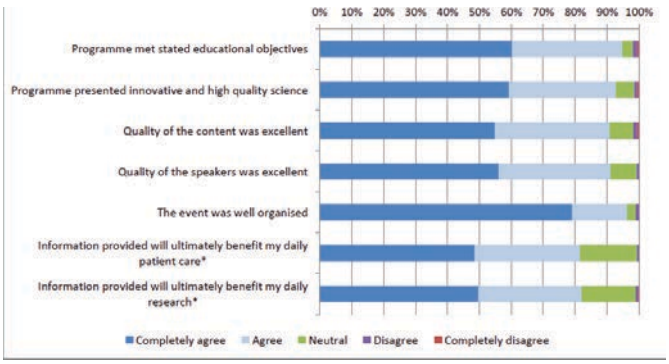
"As a basic researcher I love to be in the clinical sessions to know what the clinical people need and what I could provide for them. Mostly basic science and clinical conferences run separately and you don't get the two so close together as in ECTS."
(PhD student)

"The arrangements are excellent. I have learned the latest developments in the bone field. From now on I'm going to come every year."
(Assistant Professor)

From the delegate survey

400 delegates completed the delegate survey through the ECTS 2017 congress app, with the results now analysed showing high levels of satisfaction with the congress quality and organization (see below).
Overall, 94.5% of responding delegates would recommend the programme to a colleague, and 92.5% would be definitely or probably interested to attend ECTS congress again.





ECTS through the year

ECTS organises networking and educational activities throughout the year. We recently created a new website at ectsoc.org, which is regularly updated with information about ECTS activities and has an archive of educational material. You can also follow our twitter account @ECTS_soc.

ECTS also publishes a monthly newsletter, which is open for contributions and will keep you updated on all ECTS activities and news from the bone field.

Our educational activities are also now supported by an ongoing webinar series "Bone, Muscle and Beyond", which we will launch in September 2017. ECTS also organizes Clinical Training Courses. This year two courses on Bone Biomarkers were held as part of the pre-congress programme. The ECTS PhD training courses held every year are very popular (this year in Paris, July 8th-11th 2017).

ECTS Academy

ECTS Academy (@AcademyEcts) is our daughter society, created to support new investigators in developing their careers. Its activities include networking, mentoring, and organizing the New Investigator program at the ECTS annual meeting. The Academy also runs a webinar series with presentations from researchers who have obtained an ERC grant, gaining from their expertise both in science and career development.

An application process is required to join and membership lasts 5 years. At ECTS 2017, a further 10 new members were welcomed into ECTS Academy - Nerea Alonso (UK), Ulrike Baschant (Germany), Björn Busse (Germany), Roy Heusschen (Belgium), Abbas Jafari (Denmark), Savarana Ramasamy (UK), Ilaria Roato (Italy), Katherine Staines (UK), Elena Tsourdi (Germany), Annegreet Veldhuis-Vlug (Netherlands) – congratulations!