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SBMS
The Swiss Bone and Mineral Society

SVGO
Schweizerische Vereinigung gegen die Osteoporose

ASCO
Association Suisse contre l'Ostéoporose
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New cartilage quality evaluation using cyclic nano indentation: case study with a tool DMOAD compound active in the rat MNX model

C. Lavet¹, D. Amantini², D. Merciris³, S. Meurisse³, L. Lepescheux⁴, P. Pastoureau⁵, P. Deprez⁵, P. Clement-Lacroix⁵, P. Ammann¹

¹Division of Bone Diseases, Department of Internal Medicine Specialties, Geneva University Hospital, Geneva, Switzerland; ²Galapagos SASU, Romainville, France; ³Institut de Recherches Servier, Suresnes, France

Measurement of cartilage material level properties are lacking in small osteoarthritis (OA) animal model. We evaluated an indentation technique allowing cartilage depth dependant quality in femurs from SHAM and meniscectomized (MNX) rats treated with vehicle and a disease modifying osteoarthritis drug (DMOAD) selected on its in vivo efficacy.

Surgical joint destabilization was performed in 10-week-old female Lewis rats. DMOAD was delivered by oral dosage (20 mg/kg/d) until sacrifice (21 days post-surgery). Femur was used for phase-contrast micro-computed tomography allowing determination of hyaline cartilage thickness of each condyle as well as trabecular and cortical subchondral bone (SB) morphometric parameters. The quality (indentation depth and Young's modulus) of each condyle of the femoral cartilage was evaluated through bioindentation. Cartilage of proximal tibia was evaluated and scored using the OARSI method.

MNX animals showed higher tibial OARSI score and a deterioration of the trabecular SB and to more mineralized SB bone plate. In MNX, depth of indentation increased and Young's modulus decreased at each cartilage depth investigated in the medial condyle (respective average +74 % and −35 %), indicating softening of the cartilage, while μCT analysis showed increased hyaline cartilage thickness. At the lateral condyle indentation depth and Young's modulus also respectively increased and decreased without morphologic alteration. DMOAD prevented OA progression in the femur and tibia and normalized femoral biomechanical and trabecular SB alterations without SB bone plate mineralization.

This technique generated data on cartilage quality which were correlated to well-validated readouts and allows to differentiate OA and DMOAD effects.

Osteocyte-specific ablation of Pparγ improves energy metabolism and prevents fat accumulation but not bone loss in response to a high fat diet

J. Brun, S. L. Ferrari, N. Bonnet

Service des Maladies Osseuses, Hôpitaux Universitaires, Genève, Switzerland

Pparγ is a master transcriptional regulator of energy metabolism. We demonstrated that Dmp1-Cre/Lox-mice (KO) have increased bone mass and improved energy metabolism. Here we investigated if Pparγ-deficiency in Dmp1 cells can prevent high fat diet effects on these parameters. For this purpose, WT and KO male mice aged of 16 weeks received a high fat or chow diet (HF 60 % vs CD 10 % of fat) for 12 weeks. Lean and fat, bone structure, metabolic rate and tissue temperature were evaluated respectively by echoMRI, microCT, labmaster and infrared camera.

As expected vertebral BV/TV was higher in KO (+39 % vs WT, p < 0.01) and lower in HF (+12 % vs CD) mainly due to an effect on thickness (+17 % vs CD, p < 0.01) but there was no interaction between diet and genotype. Cortical structure was not affected by diet. Under HF, movement, VO2 and heat were higher in KO (+41 %, +13 %, +13 % vs WT, p < 0.05). Body temperature was also higher, particularly in the BAT-neck region (+1.5 % vs WT, p < 0.01). UCP1&3 and PPARβ&γ expression in BAT was higher in KO (84 %, 139 %, 125 % and 167 % vs WT, p < 0.01). Histology and UCP1 expression indicate a browning of the WAT. As a result, glucose and insulin tolerance test were improved in the KO (AUC −22 % and −9 % vs WT, p < 0.05). Finally, HF induced fat mass increase was prevented in KO (+17 % vs CD and +44 % vs CD in WT, p < 0.05) whereas increased in lean mass was greater (+14.2 % vs CD and +9.6 % vs CD in WT).

In conclusion, ablation of Pparγ by Dmp1-Cre improves bone mass but does not prevent the deleterious effects of HF on bone. In contrast, it improves BAT activity and insulin sensitivity, preventing fat accumulation and improving glucose homeostasis. How bone regulate energy metabolism under the control of Pparγ remains to be determined.

Fracture repair in bisphosphonate-treated osteoporotic bone

Michel Hauser, Mark Siegrist, Silvia Dolder, Willy Hofstetter
Bone Biology & Orthopaedic Research, Department Clinical Research, University of Bern, Switzerland

Post-menopausal osteoporosis, which is characterized by an increase in bone resorption and high bone turnover, is treated with bisphosphonates to block osteoclast activity. Since bone remodelling is a critical step in fracture healing, it is hypothesized that bisphosphonate therapy may impair the bone’s ability to repair fractures.

Ovariectomy(OVX)-induced oestrogen deficiency is used as a model for post-menopausal osteoporosis. Twelve week old mice were OVX and 8 weeks later, the bisphosphonate Alend-
Bone-implant contact and peri-implant bone fraction, mostly monitored using in vivo microCT. Post mortem, samples under-osseointegration (0, 3, 6, 9, 14, 20 and 28 days post-op) were examined. No significant difference in bone mass or mechanical properties was observed between groups.

The efficacy of local bisphosphonate and BMP-2 delivery in improving bone mass and mechanical implant stability

Linda Freitag¹*, Christian Günther¹*, Laura Kyllönen¹*, Ursula Eberli¹, Stephan Zeiter¹, David Eglin¹, Vincent A. Stadelmann¹

¹AO Research Institute Davos, Davos, Switzerland; *equivalent contributions

Because of low bone mass and reduced mechanical properties, fixation in osteoporotic patients is still challenging. In the present study, our aim was to improve implant stability in osteoporotic bone using a hyaluronan hydrogel for local delivery of bisphosphonates (BP) and bone morphogenetic protein 2 (BMP-2). The hypothesis was that BP would prevent early resorption in response to interventional trauma and BMP-2 would support bone formation, which is impaired in osteoporotic bone.

41 adult female wistar rats were divided into 7 groups: groups 1 and 2 were the healthy controls, groups 3 to 7 were ovariectomized at 13 weeks. All animals received a BaSO4-PEEK miniscrew in the proximal tibia at 25 weeks. In groups 2 and 4, pure hydrogel was pipetted into the drill hole before screw insertion. ZOL-BMP2 loaded hydrogel was given in group 5. Group 6 received zoledronate systemically, group 7 zoledronate systemically and BMP-2 peri-locally (sub-cutaneous).

Bone mineral density (at 12, 24 and 29 weeks) and implant osseointegration (0, 3, 6, 9, 14, 20 and 28 days post-op) were monitored using in vivo microCT. Post mortem, samples underwent histological examinations.

Our data showed that pure gel is bioactive in terms of implant fixation. ZOL-BMP2-gel induces significant increase of bone-implant contact and peri-implant bone fraction, mostly through reduced resorption. In our model, systemic administration did not induce better fixation.

In conclusion, local ZOL-BMP2 delivery with a hyaluronan hydrogel to improve implant stability in osteoporotic bone is a potent alternative to systemic drug administration at significantly lower doses.

2. Short communications clinical

Risk and predictors of subsequent fractures after an atypical femoral fracture

Emmanuel Biver¹, Marie Claude Audet¹, Robin Peter², Raphaël Meier³, Brigitte Uebelhart¹, Thierry Chevalley¹, Kuntheavy Ing Lorenzini², René Rizzoli³, Serge Ferrari³

¹Division of Bone Diseases, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland; ²Division of Orthopedic Surgery, Department of Surgery, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland; ³Division of Visceral and Transplant Surgery, Department of Surgery, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland; ⁴Division of Clinical Pharmacology and Toxicology, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland

Objective: Fracture risk after an atypical femoral fracture (AFF) in patients discontinuing or pursuing anti-resorptive drugs (AR) is unknown. The objective of this study was to investigate the incidence and predictors of new fragility fractures and second AFF after an AFF.

Methods: In a longitudinal case-control study, incidence and predictors of subsequent fragility fractures and of second AFF were assessed in 50 subjects with AFF compared to 50 subjects with typical femoral fracture (TFF) matched by age (±1 year) and gender, and 50 previous or current bisphosphonate users (BP) matched for gender from the Geneva Retired Cohort.

Results: Twenty one patients (42 %) with previous AFF sustained a new fragility fracture, compared with 30 (60 %) in the TFF group and 7 (14 %) in the BP. The risk of a new fracture in the AFF group was similar to the TFF (p = 0.553), but higher than in BP users (p = 0.003). AFF predicted subsequent fragility fracture independently of age, BMI, prior fractures history, osteoporosis status, duration of AR drugs and continuation of AR drugs during follow-up (HR 2.88, CI 95 % 1.20–6.94, p = 0.018). Second AFF occurred in 23 subjects with AFF (46 %), more frequently in subjects with low BMI and longer duration of AR treatment prior to the initial AFF. Second AFF were more frequent in subjects who continued AR drugs after first AFF (83 % vs 35 %, p = 0.030).

Conclusions: AFF is an independent predictor of subsequent fragility fractures. A bone anabolic agent may be considered after an AFF as AR treatments prior to the AFF or continued thereafter is a risk for a second AFF.
Rebound-associated vertebral fractures after denosumab discontinuation: A series of 8 women with 42 spontaneous vertebral fractures

Olivier Lamy, Delphine Stoll, Elena Gonzalez Rodriguez, Didier Hans, Bérengère Aubry-Rozier

Center of Bone Diseases, Bone and Joint Department, Lausanne University Hospital, Lausanne, Switzerland

Osteoporosis (OP) treatments are given for a limited period of time because of a risk/benefice balance. Reversibility of OP treatment is observed by the measurements of bone markers turnover (BMTs) and bone mineral density (BMD). The effect on vertebral fracture (VFx) is difficult to evaluate. The OP treatment discontinuation is associated with an increase of BMTs and a more or less rapid decrease of BMD. Denosumab (Dmab) discontinuation is associated with a severe rebound effect on BMTs and BMD for near 24 months. A recent publication suggests an increase of VFx (Osteoporosis Int 2015 Oct 28).

We report the cases of 8 postmenopausal women. They received Dmab 60 mg every 6 months for 2 to 8 doses. The 8 women were on calcium and vitamin D. A wide biological assessment excluded a secondary cause of OP. VFx were documented by MRI.

Five OP women without any prior fragility fracture were treated every 6 months with 4 to 6 Dmab doses. Dmab was stopped because there was no more OP on BMD (3 women 55, 56 and 59 y old), the aromatase inhibitors were stopped (77 y old) and according to the wish of the patient (77 y old). 9 to 16 months after Dmab discontinuation, they presented respectively 5 (D11, D12, L2-L4), 9 (D7-D9, D12-L5), 2 (D11 and D12), 5 (D12-L2) and 9 (D5-D9 and D11-L2) symptomatic spontaneous (SS) VFx.

A 65 y old woman with osteoporosis and 1 prevalent VFx was treated every 6 months with 8 Dmab doses. Ten months after Dmab discontinuation she presented 6 SSVFx (D5, D8, D12, L2-L4).

These 62 y old woman (osteopenia, treated with aromatase inhibitors) received 2 Dmab doses every 6 months. The subsequent Dmab dose was forgotten. Twelve months after the last Dmab dose she presented a D10 SSFxF.

A 71 y old woman (one prevalent VFx and one hip fracture) received 2 Dmab doses with a delay of 11 months because of a lack of compliance. Eleven months after the last Dmab dose she presented 5 SSFxF (D12, L2-L5).

These 8 cases show a severe increased risk of vertebral fractures in the 9 to 16 months after the last injection of Dmab. The occurrence of these fractures can be explained by the severe rebound effect observed after denosumab discontinuation. It is urgent to: 1) inform the health authorities and patients of this risk; 2) determine treatment regimens before or at the time of denosumab discontinuation.

Prior exposure to bisphosphonates prevents the rebound of bone turnover markers after denosumab therapy

Brigitte Uebelhart, René Rizzoli, Serge L. Ferrari

Service des Maladies osseuses; Département des spécialités de médecine, Hopitaux Universitaires de Genève, Genève, Switzerland

Cessation of denosumab (Dmab) treatment is followed by a transient rebound of bone turnover markers (BTM), with an accelerated bone loss and the possibility of a transient increase in bone fragility and fracture risk. We investigated whether bisphosphonate (BPs) therapy prior to Dmab could attenuate this rebound.

In a retrospective longitudinal study, we assessed changes in serum CTX levels in 35 patients (31 women, 4 men, mean age 68.6 years [range 50–84]) up to 17 months after Dmab cessation. Of them, 18 patients had received BP prior to Dmab (mean exposure 6.7 years, range 1–13; mean gap time between BPs exposure and Dmab initiation 3.2 years, range 0–14).

Dmab treatment lasted from 6 months to 4.5 years. In subjects who received only one Dmab injection (n = 8), CTX did not rebound after cessation. In 7 out of 9 patients treated with Dmab (mean 5 injections, range 3–8) and without prior exposure to BPs, CTX increased +117% (range 36–233) above the upper limit of premenopausal range by 12 months (range 6–18) after Dmab. In contrast, in 15 out of 18 patients treated with Dmab (mean 3.7 injections, range 3–7) and previously exposed to BPs, CTX remained in the normal range.

This study indicates that the rebound in bone turnover markers occurring after cessation of denosumab in patients who have received multiple injections may be prevented by prior exposure to BPs, likely related to the persistence of BPs in bone. Thus, in the patients with prior long acting BPs exposure, denosumab cessation may not be a concern.

Is there an optimal Trabecular Bone Score (TBS) lumbar spine vertebrae combination to predict Major Osteoporotic Fracture (MOF)? The OsteoLaus Cohort Study


Center of Bone diseases, Rheumatology service, Bone and Joint Department, Lausanne, University Hospital, Lausanne, Switzerland

Guidelines recommend to use the average bone mineral density (BMD) over L1 to L4 for osteoporosis diagnosis and prediction of fracture. Exclusion of vertebrae is recommended according to specific rules (ISCD position). Optimal combination is unknown for TBS, a surrogate of bone micro-architecture. The aim of this study is to test several TBS vertebrae combinations in regard to MOF prediction.

The OsteoLaus cohort included 1500 women 50 to 80 years old. All women had a detailed questionnaire, BMD and TBS.
measurements and vertebral fracture assessment. The primary outcome was the prevalence of MOF according to TBS per-vertebral combination. L1-L4 TBS was used as reference value.

Out of 1466 women included in the study (mean age 64.5 ± 7.6 years), 12.7% suffered from MOF. The odd ratios per standard deviation decreased (OR) were 1.53 (1.29–1.80) and 1.80 (1.50–2.15) for the spine and total femur BMD respectively. Adjusted (age and glucocorticoids status) OR and area under the curve of different combination of vertebrae can be found in Table 1 for TBS.

It seems that excluding L4 improves the fracture risk prediction. Further prospective studies are needed to confirm these results.

### POSTER SESSION

**Associations between TBS and BMD, trabecular microstructure and fat mass in the Geneva Retirees Cohort**

Emmanuel Biver, Claire Durosier, Thierry Chevalley, René Rizzoli, Serge Ferrari

Division of Bone Diseases, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland

**Background:** A high proportion of fracture occurs in osteopenic subjects. Trabecular Bone Score (TBS) is a texture parameter obtained from the spine DXA image which predicts fractures. The objective of this study was to investigate the associations between TBS, trabecular (Tb) bone microstructure and fat mass in postmenopausal women.

**Methods:** Seven hundred forty five women (age 65.0 ± 1.4 years) enrolled in the Geneva Retirees Cohort with areal BMD and TBS data were included in the study. Trabecular (Tb) volumetric bone density (vBMD) and microstructure at the distal radius and tibia were assessed by HR-pQCT (Xtreme CT, Scanco Medical, Bassersdorf, Switzerland). Body composition was assessed by DXA.

**Results:** TBS was positively correlated with lumbar spine aBMD (r = 0.49), radius and tibia Tb vBMD (r = 0.35 and 0.29, respectively) and Tb number (r = 0.29 and 0.23, respectively), p < 0.001 for all. TBS was negatively correlated with trunk fat mass (r = −0.19, p < 0.001). In 431 women (58%) with osteopenia (at least one T-score at the spine, hip or femoral neck between −2.5 and −1, with none ≤−2.5), 91 (21%) had low TBS (≤1.2). Osteopenic women with low TBS (≤1.2) had lower lumbar spine aBMD (−3.9%, p < 0.001); a trend for lower distal radius Tb vBMD and number (−3.8% for both, p = 0.060 and 0.144 respectively); and higher trunk fat mass (+37%, p < 0.001) compared to osteopenic women with TBS > 1.2.

**Conclusion:** These data suggest that lower TBS values compared to BMD observed in some osteopenic patients are mainly related to fat mass rather than to trabecular microstructure.

**Monitoring live human mesenchymal stem cell differentiation and subsequent selection using fluorescent RNA-based probes**

Bojun Li1, René Rothweiler2, Claudia Loebel1, Mauro Alini1, Martin J. Stoddart1,2

1 AO Research Institute Davos, Davos, Switzerland; 2 Albert Ludwig University of Freiburg, Freiburg, Germany

Investigating mesenchymal stem cell differentiation requires time and multiple samples due to the destructive endpoint assays performed. Osteogenesis of human bone marrow derived mesenchymal stem cells (hBMSCs) has been widely studied for bone tissue engineering. Recent studies show that the osteogenic differentiation of hBMSCs can be assessed by quantifying the ratio of two important transcription factors (Runx2/Sox9). In previous studies, these transcription factors can only be detected via destructive methods, either intra-cellular immunostaining or PCR. Here we demonstrate a new technique to observe mRNA expression of two genes in individual live cells using two fluorescent probes specific for Runx2 or Sox9 mRNA. The changes of mRNA expression in cells with or without osteogenic induction can be observed in a non-destructive manner. In addition, the osteogenic hBMSCs can be separated based on the relative intracellular fluorescence of Sox9 in relation to Runx2 using fluorescence activated cell sorting (FACS). The isolated cells show different proliferate rates and osteogenic differentiation potential. Relatively homogeneous cell populations with high osteogenic potential can be isolated from the original heterogeneous osteogenically induced hBMSCs within the first week of induction. This offers a more detailed analysis of the ef-
fectiveness of new therapeutics both at the individual cell level, e.g. number of responding cells, and the response of the population as a whole. By identifying and isolating differentiating cells at early time points, prospective analysis of differentiation is also possible, which will lead to a greater understanding of MSC differentiation.

Tissue mechanics of piled critical size biomimetic and biominerizable nanocomposites: formation of bioreactor-induced stem cell gradients under perfusion and compression

Walter Baumgartner1, Manfred Welti2, Nora Hild3, Samuel C. Hess3, Wendelin J. Stark2, Gabriella Meier Bürgisser1, Pietro Giovanoli1, Johanna Buschmann1

1Division of Plastic and Hand Surgery, University Hospital Zurich, Zurich, Switzerland; 2Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Zurich, Switzerland

Perfusion bioreactors are used to solve problems in bone tissue engineering with respect to sufficient nutrient and oxygen supply especially in critical size bone grafts. Biominerizable and biocompatible nanocomposite materials are attractive and suitable scaffold materials for bone tissue engineering because they offer mineral components in organic carriers. Human adipose derived stem cells (ASCs) can potentially be used to increase bone healing, especially when seeded onto a porous electrospun scaffold.

Electrospun nanocomposite disks of poly-lactic-co-glycolic acid and amorphous calcium phosphate nanoparticles (PLGA/a-CaP) were seeded with ASCs and eight disks were stacked in a bioreactor running with normal culture medium (no differentiation supplements). Under continuous perfusion and uniaxial cyclic compression, load-displacement curves as a function of time were assessed during 9 days. Stiffness and energy dissipation were recorded. Moreover, stem cell densities in the layers of the piled scaffold were determined as well as their morphologies and differentiation status (endothelial cell differentiation, chondrogenesis and osteogenesis).

While the stiffness of the cell free constructs increased over time based on the transformation of the a-CaP nanoparticles into flake-like apatite, ASC-seeded constructs showed a constant stiffness. Stem cell density gradients were histologically determined with a linear increase from the bottom to the top of the pile (r²>0.95) [1]. Cell morphology was influenced by the flow rate, with stem cells getting more roundish at higher flow rates. Some osteogenesis was found upon osteopontin immunostaining, while no endothelial cell differentiation and no chondrogenesis was triggered.

The fabrication of a critical size bone graft is presented based on a biominerizable bone-biomimetic nanocomposite with preserved stiffness when seeded with ASCs. The cell densities of ASCs inside the piled construct varied with a linear gradient. Beginning osteogenesis was triggered by the dynamic culture conditions including perfusion and compression.


BMP antagonists modulate RANKL dependent osteoclast formation

Eliza S. Hartmann1,2, John Choy1,3, Silvia Dolder1, Klaus A. Siebenrock2, Willy Hofstetter1, Frank M. Klenke1,2

1Bone Biology & Orthopaedic Research, Department Clinical Research, University of Bern, Bern, Switzerland; 2Department of Orthopaedic Surgery, Inselspital, Bern University Hospital, Bern, Switzerland; 3Graduate School of Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland

Osteoinductive growth factors, including BMP2 are used in bone regenerative medicine. Induction of antagonists may be the cause for the need of supraphysiological amounts of BMP2 and for BMP2-associated osteolysis. Herein, we hypothesize that antagonists not only limit BMP efficacy but also mediate effects like osteoclastogenesis.

Primary murine OB (2-days old C57BL/6J mice) were cultured in media containing 1,25(OH)2D3 (0/10–8/10–9 M) ± BMP2 (33/166 nM). Transcript levels of BMP3, Noggin and Gremlin-1 were analyzed by qRT-PCR. M-CSF dependent Osteoclast Precursor Cells (C57Bl/6) were grown in media containing M-CSF (30 ng/ml) and RANKL (2.5/20 ng/ml)+Noggin, Gremlin-1 and BMP3 (33 nM). Effects of antagonists on OC development were assessed by adding the proteins at days 0–6, 0–3 (proliferation), 4–6 (differentiation). OCs were visualized by TRAP staining, multinucleated (n≥3) cells were counted.

BMP2 dose-dependently increased mRNA levels of Noggin and Gremlin-1 and decreased BMP3 mRNA in OBs. Noggin and Gremlin-1 with 2.5 ng/ml RANKL enhanced OC formation 10-fold as compared to RANKL alone. Addition of antagonists increased OC formation 3-fold when added during days 0–3, as compared to days 4–6.

OBs exposed to BMP2 express increased levels of mRNA encoding BMP antagonists Noggin and Gremlin-1. The increase in the synthesis of antagonists may account for the low bioefficacy of exogenously added BMP2. Furthermore, the antagonists synergize with RANKL in increasing OC formation when added early in OC development. Consequently, increased expression of antagonists in response to BMP2 may account for the risk of BMP2-associated osteolysis.
An organ-on-chip model of the endothelial barrier to study the role of perivascular cells in bone regeneration

M. Herrmann¹, Z. Wang², M. Alini³, L. Barbe⁴, S. Verrier¹
¹AO Research Institute, Davos, Switzerland; ²CSEM, Landquart, Switzerland

The identification of mesenchymal stem cells at perivascular sites of the endothelial barrier, suggests that pericytes have a role as multipotent progenitors. Microfluidic technologies have shown the potential to closely mimic the vascular microenvironment and represent an alternative to animal models. The aim of this project was to develop a microfluidic system comprised of a 3D microvascular network embedded in a hydrogel enabling the investigation of perivascular cells in a physiologically relevant context.

A microfluidic mold was fabricated out of polycarbonate and comprises 3 different layers creating an empty chamber upon assembly. A removable microcapillary placed within the chamber enables creation of a microchannel within the gel. Collagen type I was injected into the chamber and polymerized at 37 °C for 60 min. Microchannels were created by careful retraction of the capillary. The chip was connected to a reservoir of endothelial growth medium and perfused using a micro pump. Microchannels were seeded with human umbilical vein endothelial cells (HUVECs) and observed by time-lapse microscopy.

Regular channels (diameter 150 μm) could be created. Time-lapse microscopy revealed efficient cell attachment and complete coverage of the surface of the microchannel. Good viability of HUVECs was observed and vessel sprouting occurred 28 h after initiation of perfusion.

We have successfully developed a perfused microvascular model. The embedding of microchannels within a hydrogel matrix will enable to study cellular cross-talk and cell migration.

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The positive effect of hormone replacement therapy on bone mineral density and trabecular bone score persists after its withdrawal: the OsteoLaus Cohort

CHUV, Lausanne University Hospital, Lausanne, Switzerland

Hormone replacement therapy (HRT) increases BMD but controversy exists regarding residual effect after withdrawal. We aimed to explore the effect of HRT on BMD and bone microarchitecture, assessed by TBS.

The Osteolaus study included 1,500 women (50–80 years). BMD at lumbar spine (LS), femoral neck (FN) and total hip (TH) as well as TBS were measured. After exclusion of women with current or past anti-osteoporotic treatments, the remaining women were classified according to HRT status as: Never (NU, n=617), Current (CU, n=282) and Past (PU, n=380) Users.

The 3 groups differed in age: 67.4±6.8, 64.0±6.8 and 62.1±8.0 years for PU, CU and NU respectively (p<0.001). After adjustment for age and BMI, BMD and TBS values decreased significantly according to HRT status (CU > PU > NU, p<0.01) with significant between-groups difference for all BMD LS and TH values. BMD FN and TBS differed only when CU were compared to PU or NU (PU vs NU for TBS: p=0.066). TBS was negatively associated with age: BMI-adjusted slopes for 10-year increment were −0.051 (−0.060; −0.041), −0.032 (−0.048; −0.017) and −0.022 (−0.038; −0.005) in NU, PU and CU respectively (p<0.05). Similar pattern was seen for adjusted slopes of BMD with age except for the comparison between PU and NU at FN.

We show for the first time that current HRT use is associated with a significantly better preservation of TBS. The benefits of HRT use for the TBS and the BMD at LS and TH, seem to persist in PU.

An innovative formulation of alendronic acid 70 mg weekly: a buffered effervescent tablet

A. Tireford¹, A. Dowling²
¹Labatec Pharma SA; ²EffRx Pharmaceuticals SA

Alendronate is still considered as a gold-standard therapy for the treatment of osteoporosis.

The aim is to present an innovative formulation, a buffered effervescent tablet containing an equivalent of 70 mg alendronate.

A bioequivalence study vs alendronate 70 mg weekly tablet as well as a scintigraphic study investigating the effect of the new formulation on gastric emptying and gastric pH are presented. Data from post-marketing experience are given.

A bioequivalence study was conducted in 70 female and 45 male subjects aged 45 to 73 years. Bioequivalence was assumed if the 90% CI of both the treatment ratio T/R of \( \text{A}_{\text{eff}} \) (cumulative amount excreted into urine during the entire period of sample collection) and of \( E_{\text{max}} \) (maximum excretion rate) were within 80%–125%. For \( A_{\text{eff}} \), T/R was 88.14% (82.92–93.69) and for \( E_{\text{max}} \), T/R was 90.44% (84.85–96.41), allowing to conclude that both formulations are bioequivalent and thus therapeutically equivalent.

The scintigraphic study showed that gastric pH after ingestion of the buffered solution was immediately buffered to levels above pH 3 meaning that the risk of exposing the stomach and oesophageal lining to acidified alendronate is negligible.

Post-marketing experience since the launch of the buffered alendronate 70 mg effervescent tablet in the US and in EU supports a very promising tolerability profile.

The buffered alendronate 70 mg effervescent tablet is therapeutically equivalent to alendronate 70 mg weekly tablet and it...
presents advantages which could result in increased compliance and persistence in osteoporosis treatment.

**M-CSF- and GM-CSF-dependent haematopoietic progenitor cells give rise to osteoclasts**

*Nina Ruef, Silvia Dolder, Willy Hofstetter*

Bone Biology & Orthopaedic Research, Department Clinical Research, University of Bern, Bern, Switzerland

Levels of circulating cytokines are elevated in inflammatory diseases. In synergism with 1,25(OH)²D₃, TNFα and IL-17A induce the release of GM-CSF by murine osteoblasts *in vitro*, resulting in a change in the haematopoietic microenvironment of osteoclasts (OC) and osteoclast progenitor cells (OPC). Herein, the effects of GM-CSF on OC development were further studied.

Non-adherent M-CSF dependent OPC obtained from bone marrow were grown for 3 days in media supplemented with M-CSF, with GM-CSF or M-CSF/GM-CSF. The potential of the three OPC pools to develop into OC was assessed by subsequent culture with M-CSF/RANKL.

OPC precultured with M-CSF, GM-CSF and M-CSF/GM-CSF all gave rise to OC. In GM-CSF and M-CSF/GM-CSF treated OPC, levels of transcripts encoding dendritic cell marker CD11c, DC-STAMP and OC-STAMP were 100x higher than those in M-CSF treated OPC. After starting M-CSF/RANKL-treatment, transcripts for CD11c, DC-STAMP and OC-STAMP decreased gradually. In progressing cultures, DC-STAMP/OC-STAMP transcripts increased again along with OC development, as also observed in M-CSF treated OPC. Levels of transcripts encoding OC markers NHA2, CTR and Acp5 increased with OC formation in all OPC pools and correlated with number of OC.

GM-CSF treated OPC express dendritic markers but upon removal of GM-CSF and stimulation with M-CSF/RANKL they reverse their phenotype and give rise to OC with similar resorption activity as OC generated from M-CSF treated OPC. Progenitors generated in presence of GM-CSF have a high potential to form OC and may lead to an increased number of OC upon homing to bone, causing accelerated bone resorption.

The **BMP2 variant L51P rescues bone formation of human mesenchymal stem cells in the presence of intervertebral disc cells**

*Adel Tekari¹, Hans-Jörg Sebald², Lorin M. Benneker³, Benjamin Gantenbein¹*

¹Tissue and Organ Mechanobiology, Institute for Surgical Technology & Biomechanics, University of Bern, Bern, Switzerland; ²The Spine Center, Thun, Switzerland; ³Department of Orthopedic Surgery & Traumatology, Inselspital Bern, Bern, Switzerland

The intervertebral disc (IVD) is an avascular tissue with a nearly absent self-healing potential. While the clinical gold standard treatment of disc-related degeneration is spinal fusion, minimally invasive surgical options such as laparoscopic anterior spinal fusion exits and results in an incomplete disectomy of the disc. We previously showed that IVD cells hinder the osteogenic differentiation of mesenchymal stem cells (MSC) *in vitro* (1). Within the present study, we aimed to investigate the contribution of bone morphogenetic proteins (BMP) in bone formation of MSC.

Human bone marrow-derived mesenchymal stromal cells were co-cultured with IVD cells in the presence of L51P, a BMP2 variant with osteoinductive potential via inhibition of noggin (2). The osteogenic differentiation was evaluated by gene expression, alkaline phosphatase (ALP) activity and histology.

IVD cells expressed BMP antagonists, namely noggin, gremlin and chordin as measured by transcript and protein levels. The osteogenic differentiation of MSC was hindered by IVD cells as detected by Alizarin red staining and ALP activity. L51P added to the cultures induced bone formation by interfering with the IVD cells’ secreted BMP antagonists.

**Conclusions:** The IVD cells secrete BMP antagonists, which are responsible for bone non-fusion. The concept of antagonizing endogenous BMP inhibitors with L51P may represent a promising clinical option to augment bone regeneration during spinal fusion.


**Zehnjährige Denosumab-Behandlung bei postmenopausalen Frauen mit Osteoporose: Ergebnisse der FREEDOM-Erweiterungsstudie**


¹Bern University Hospital; ²Michigan Bone & Mineral Clinic; ³University of Florence; ⁴Laval University & CHU de Québec Research Centre; ⁵Hôpital Edouard Herriot; ⁶San Francisco Coordinating Center, CPMC Research Institute, & UCSF; ⁷Krakow Medical Centre; ⁸Medical University Graz; ⁹University of British Columbia; ¹⁰University of Liège; ¹¹Paris Descartes University; ¹²UoC; ¹³Amgen Inc.; ¹⁴Leiden University Medical Center

Osteoporose ist eine schwere chronische Erkrankung, die eine langfristige Behandlung erfordert. Daher sind langfristige Wirksamkeits- und Sicherheitsdaten von großer Bedeutung. Denosumab (DMAb) wird weltweit in über 80 Ländern zur Behandlung von postmenopausalen Frauen mit Osteoporose angewandt. Die Wirkung der DMAb-Behandlung über bis zu 10 Jahre wurde in der 3 Jahre dauernden FREEDOM-Studie
and in the 7 year duration of the extension of the study. In the preceding article, we present the results of the last year of extension, which are based on a 10-year-long, continuous DMAb treatment.

During the extension, the proband women received 60 mg DMAb every 6 months along with calcium and vitamin D. For this analysis, the long-term group received a 10-year-long DMAb therapy (3 years within the FREEDOM study and 7 years as part of the extension); the crossover group received a 7-year-long DMAb treatment (3 years placebo within the FREEDOM study and 7 years DMAb within the extension).

Of the 4550 proband women who entered the extension phase, 2784 (61%) continued to participate in the study at the beginning of year 10. Of these proband women, 2212 (80%) completed their final visit after 10 years, 120 (4%) dropped out, and 452 (16%) were still enrolled in the study at the time of submission of this article. In the long-term group, further significant increases in bone density were observed; the average 10-year increases compared to the FREEDOM baseline values were 21.6% for the lumbar spine and 9.1% for the proximal femur. The crossover group showed cumulative 7-year increases of 16.3% (lumbar spine) and 7.3% (proximal femur) compared to the baseline values of the extension study (▶ Fig. 1). All p < 0.0001 compared to the baseline values of FREEDOM, to the baseline values of the extension, and to the previous measurement. In both groups, similar sustained decreases in bone turnover markers were observed. The effect of treatment weakened in a characteristic way. The incidence rates of new vertebral and non-vertebral fractures remained low. Overall, the incidence of adverse events and serious adverse events remained unchanged compared to the previous extension study.

The DMAb treatment for up to 10 years was associated with a sustained decrease in bone turnover and a sustained increase in bone density. The benefit-risk profile of DMAb remained unaltered in this aging population of postmenopausal women.

Correlation between the T-score obtained for the DMO of the total hip (HT) and the incidence of non-vertebral fractures (NVFX) under treatment with denosumab (DMAb) for up to 10 years maximum

1Hôpital universitaire de Genève, Genève, Switzerland; 2University of Vé- rone, Verona, Italy; 3University of Laval and CHU de Québec Research Centre, Quebec, QC, Canada; 4Helen Hayes Hospital, West Haverstraw, NY, United States; 5Centre Médical de Cracovie, Cracovie, Poland; 6CCBR, Rio de Janeiro, Brazil; 7University Autònoma de Barcelona, Barcelona, Spain; 8Université de Liège, Liège, Belgium; 9Amgen Inc., Thousand Oaks, CA, United States; 10New Mexico Clinical Research and Osteopa- risis Center, Albuquerque, NM, United States

Objective: To study the relationship between the T-score obtained for the DMO of the total hip (HT) and the incidence of non-vertebral fractures (NVFX) during a 10-year treatment with DMAb.

Materials and methods: Women who received DMAb during 3 years as part of the FREEDOM study (N = 3902); a large part of these (N = 2343) were recruited for the extension phase and continued to take DMAb for an additional 7 years, totaling 10 years of continuous treatment. A repeated-measures model was used to estimate the T-scores during DMO for each subject during the entire follow-up, especially at the time of NVFX in all subjects at risk at the time of the fracture. The Cox proportional hazards model was adjusted for the time of NVFX, indicating the response to treatment, and the evolution over time of the T-score of HT during the DMO as a covarying variable during the time.

Results: The incidence of NVFX was lower with a higher T-score for the HT during the DMO (Fig. 1). For example, des

Abb. 1 Die durchschnittlichen 10-Jahres-Steigerungen gegenüber den FREEDOM-Ausgangswerten
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The role of iron in the development of osteoclasts

Romina Cabra1,2,3, Wenjie Xie1,2,3, Silvia Dolder1, Willy Hoffs-tetter1,2

1Bone Biology & Orthopaedic Research, Department of clinical research, University of Bern, Bern, Switzerland; 2Swiss National Centre of Competence in Research, NCCR TransCure, University of Bern, Bern, Switzerland; 3Graduate School, Cell Biology, University of Bern, Bern, Switzerland

Introduction: Iron is the most abundant trace metal in humans. To avoid iron metabolism disorders such as anemia, iron homeostasis must be tightly regulated. Membrane iron transporters, such as ferroportin and DMT1, are critical proteins for the regulation of systemic and cellular iron homeostasis. Recently we found an increase in transcript levels of DMT1, a membrane transporter for iron uptake, during the in vitro development of osteoclasts from progenitor cells. We therefore wish to investigate the direct effects of iron on the development of osteoclast lineage cells focusing on one target, DMT1.

Methods: M-CSF-dependent, non-adherent osteoclast progenitor cells derived from C57BL/6J mice were grown in culture medium supplemented with two DMT1 inhibitors (AE147 and MPO11A1) and a reference compound (CISMBI) at concentrations of 0, 1, 3, 10, 30, and 60 µM. AE147 and MPO11A1 were previously synthesized and tested by the groups of Rey-mond and Hediger, which are part of the NCCR TransCure network. After cultures of 3 and 5 days, osteoclastogenesis was assessed by the activity of the osteoclast marker enzyme TRAP in cell lysates. Cell viability was determined using an XTT-Assay.

Results: Cell viability and osteoclastogenesis decreased dose-dependently in the presence of both competitive inhibitors, with MPO11A1 exerting stronger inhibition than AE147. In contrast, CISMBI did not affect cells viability yet fully blocked osteoclastogenesis at 60 µM.

Conclusion: Specific DMT1 inhibitors appear to negatively affect osteoclastogenesis. However, whether this is a consequence of a specific effect of perturbed iron transport or of unspecific toxic effects requires further studies.
### Programm (Stand bei Drucklegung)

**Annual Meeting SVGO/ASCO und SBMS 2016**

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| 09.05 Uhr | „State of the Art“-Lecture Preclinical
Evolution of bone tissue engineering strategies
Prof. Ivan Martin, Basel
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| 09.40 Uhr | Short communications preclinical
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| 10.30 Uhr | Kaffeepause                                                                  |
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| 11.40 Uhr | „State of the Art“-Lecture Translational
Hypophosphatasia: From Bench to Bedside
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| 12.15 Uhr | Lunch                                                                        |
| 13.00 Uhr | SVGO/ASCO (Auditorium Ettore Rossi)
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| 13.30 Uhr | „State of the art“-Lecture Preclinical
Mineral disorders in teeth and potential strategies for their repair: the „genetics/stem cells/tissue engineering“ amalgam
Prof. Thimios Mitsiadis, Zürich |
| 13.40 Uhr | Nouveautés dans les traitements de l’ostéoporose
Prof. Serge Ferrari, Geneva |
| 14.00 Uhr | Prévention de la perte osseuse lors du traitement du cancer du sein par anti-aromatases
Dr. med. Emmanuel Biver, Geneva |
| 14.30 Uhr | Update Guidelines/Research
HRT: Neue Empfehlungen SMG, NICE und Endocrine Society
Priv.-Doz. Dr. Petra Stute, Bern |
| 15.00 Uhr | Einfluss der Sarkopenie auf das Frakturrisiko
Prof. Heike Bischoff-Ferrari, Zürich |
| 16.20 Uhr | „State of the Art“-Lecture Clinical
Identification of patients at high fracture risk
Fem Dr. med. Karine Briot, Paris
Chair: S. Ferrari |
| 16.45 Uhr | Assemblée Générale SVGO/ASCO                                                |

**SBMS (Kursraum 1)**

**Assemblée Générale SVGO/ASCO**