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Preclinical Abstracts

Circulation Periostin: A Novel Serum Marker of Cortical Bone Structure in Human

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Introduction Periostin is a matricellular protein, which is mainly expressed by periosteal cells and osteocytes in bone. Periostin down-regulates Sost gene expression and is necessary for the cortical bone response to loading and to PTH treatment. We previously reported that circulating periostin levels (cPostn) correlate with periosteal bone formation and cortical thickness (CtTh) independently of bone turnover in mice treated with PTH. In the present study, we investigated the relationship between cPostn and cortical structure in human healthy subjects.

Methods We measured cPostn, bone turnover markers, aBMD by DXA, bone trabecular (Tb), and cortical (Ct) parameters at the distal radius and tibia by HR-pQCT (XtremeCT) in 242 healthy women and 59 men, aged 64.9 ± 1.4 (SD) years. To test the association between cPostn and bone parameters, we divided the subjects into 3 tertiles of cPostn (low: 1277 ± 148, medium: 1636 ± 94, high: 2140 ± 311 ng/ml) and applied an ANOVA and ANCOVA with adjustment for gender. Multiple regressions were used to assess the relation between cPostn, bone mineral mass, microstructure, and turnover markers.

Results Mean cPostn was 1633 ± 410 ng/ml (min 814, max 3490 ng/ml). cPostn was higher in men than in women (+8.6 %, $p < 0.01$), whereas P1NP and CTX were lower in men than in women (–20 % and –21 %, $p < 0.01$). Distal radius total bone area, cortical area (Ct.area), and perimeter were higher in the highest vs lowest cPostn tertile (+6.4 %, +7.7 %, and +3.8 %, $p < 0.05$; $p = 0.12$–0.34 after adjustment for gender). BV/TV was higher in the highest tertile (+12.4 % vs low tertile, $p < 0.01$) and disappeared after sex-adjustment. cPostn positively correlated with Ct.area and Ct.perimeter of distal radius ($r = 0.12$, $p < 0.05$, both) and tibia ($r = 0.15$, $p < 0.05$, both). These correlations remained significant after adjusting for P1NP, CTX, or whole body bone mineral mass, but not for gender. There was no difference in whole-body bone mineral mass, spine and proximal femur aBMD, PTH, P1NP, and CTX between cPostn tertiles. Separate analyses per tertile in men and women indicated similar, but non-significant trends. cPostn was associated with PTH levels, but only in the subgroup of subjects with higher PTH levels (> 6.3 pM, $r^2 = 0.09$, $p < 0.01$).

Conclusions These results recorded in a homogeneous population of healthy 65-year-old subjects indicate a positive correlation of cPostn with cortical bone structure, independently of bone turnover markers as previously observed in mice, but likely through a sex-dependent effect.

In-Vivo Monitoring of Osteosarcoma Primary Tumour Growth with Fluorescence and Luciferase Imaging

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Introduction In osteosarcoma (OS), monitoring of metastases is very important, as this is the most important prognostic indicator for patient survival. In our laboratory, we study OS in several mouse models. Using lacZ-transduced tumour cells [1] we can monitor metastatic dissemination down to the single cell level by X-gal tissue staining, but only ex vivo. Moreover, we are faced with the problem that routine methods tend to overestimate tumour volume and the true amount of living tumour cells, due to necrosis inside the primary tumour. We therefore looked into additional techniques to monitor primary tumour growth and metastasis in vivo. Here we present results obtained with an intratibial, metastasizing OS model in SCID mice making use of OS cells that stably express the light-producing enzyme Luciferase (Luc), or the fluorescent proteins mCherry (mCh) or dsRed (dsR), in addition to the lacZ gene.

Methods Female SCID mice were intratibially injected with either Luc/lacZ-, dsR/lacZ-, or mCh/lacZ-tagged osteolytic, highly metastatic 143B OS cells. Mice were scanned every week in the IVIS Lumina XR to monitor Luc, dsR, and mCh expression, and primary tumour sizes were measured using caliper and micro-CT. To monitor Luc activity, mice were injected with the Luc substrate Luciferin immediately before Luc monitoring. For dsR and mCh monitoring, excitation wavelengths were 557 nm for dsR and 587 nm for mCh, with the dsRed (575–650 nm) and Cy5.5 emission filters (695–770 nm), respectively. Scans took between 10 and 15 min, with 2 mice scanned simultaneously. Twenty-eight days after tumour cell injection the animals were sacrificed, the lungs excised and stained with X-gal. Both macro- (> 0.1 mm) and micro-metastases (< 0.1 mm) were counted.

Results Already 24 hrs after injection of Luc-expressing tumour cells, a clear Luc signal was detected in the injected hind limb, which increased further over time and peaked on day 28 after tumour cell injection. The dsR signal could only be detected on day 28 due to a high nonspecific tissue autofluorescence. mCh-expressing tumours were detected with intermediate sensitivity. In all groups of tumour-bearing mice, lung metastases were readily detectable with in-vivo micro-CT, but when using optical imaging we were only able to confirm this finding in mice with Luc-expressing tumours. Unexpectedly, post-mortem X-gal staining showed that dsR-expressing tumours produced more macrometastases than mCh- and Luc-expressing tumours. Finally, we found a strong correlation between caliper-measured and micro-CT-measured tumour volumes in all groups of mice.

Conclusions Luciferase tagging of tumour cells is a sensitive approach to monitor osteosarcoma tumour growth in vivo. Tumour cell-derived mCherry fluorescence yielded the most consistent signal during primary tumour growth, whereas dsRed fluorescence was found to be unsuitable. Micro-CT is the method of choice for monitoring lung metastasis. These techniques allow us to perform live imaging of primary tumour growth and metastasis in OS mouse models, and enable us to monitor treatment effects over time.

References:

1. Arlt MJ, Born W, Fuchs B. Improved visualization of lung metastases at single cell resolution in mice by combined in-situ perfusion of lung tissue and X-Gal staining of lacZ-tagged tumor cells. J Vis Exp 2012; 66: e4162.

Decreased Cement Stiffness Does Not Improve the Anchorage of Augmented Implants in Osteoporotic Bone

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Introduction Implant migration, or cut-out, is becoming an increasing problem in surgical fracture treatment in osteoporotic bone.

Polymethylmethacrylate- (PMMA-) based cements are promising in the field of implant augmentation to countervail this problem. However, their routine clinical use is still controversial due to associated risks, such as leakage, chemical necrosis, or fat embolism. These risks are strongly correlated with the amount of cement used. It is hypothesized here that the usage of less stiff cement would allow the application of smaller cement volumes in order to reduce those risks without sacrificing the mechanical benefits of augmentation.

Methods The influence of cement stiffness on the loading of osteoporotic bone was investigated by mechanical testing and finite element (FE) analysis with an emphasis on the bone-cement interface. Furthermore, we investigated what pattern of cement distribution would lead to the most favourable load-bearing situation at the bone-cement interface via FE methods.

Results/Conclusions Cements with a decreased Young's modulus led to significantly inferior biomechanical performance. FE analysis revealed that these cements only slightly reduce the maximal Von Mises stresses in bone tissue compared to commercially available cements. Hence the stiffness modifications of commercial PMMA-based cements with a decreased Young's modulus seem not to improve the load distribution at the bone-cement interface, do not reduce peak stresses, and thus do not allow the usage of smaller amounts of cement without impairing the implant anchorage. For this reason, the main benefits of cement application could be an increased load-bearing area between bone and implant and an increased number of simultaneously highly loaded bone elements which can only be achieved when providing a certain stiffness. The most beneficial cement distribution is still under investigation.

Spirulina Alga Prevents Impairment of Peak Bone Mass Acquisition Induced by an Isocaloric Low-Protein Diet

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Introduction New food strategies should be developed to fight against child malnutrition and growth retardation in developing countries. Spirulina alga, one of the richest sources of vegetable protein, contains all essential amino acids. It easily grows in tropical regions. We hypothesized that impaired peak bone mass acquisition (PBMA) caused by dietary protein deficiency could be prevented by Spirulina supplementation in growing rats.

Methods One-month-old female rats were fed an isocaloric diet containing 10 % casein (Con10), 5 % casein (Con5), or 5 % casein + 5 % Spirulina (Con5+Spi5) during 8 weeks. Cortical and trabecular bone microstructure were analyzed by micro-CT and areal BMD by DXA. Bone strength was evaluated by tibia midshaft 3-point binding test and proximal tibia compression test. Serum IGF-I was measured.

Results As compared with the Con10 group, isocaloric low-protein diet decreased proximal tibia areal BMD (–10 %, $p < 0.0001$), bone trabecular volume (BV/TV; –41 % $p < 0.01$), and trabecular thickness (Tb.Th; –10 %, $p < 0.05$), resulting in a lower ultimate strength (US; –18 %, $p < 0.01$). All these parameters were significantly higher in the Con5+Spi5 group which showed similar values as the Con10 group. In tibia cortical middiaphysis, there was a trend towards lower values (areal BMD: –4 %, $p < 0.056$, cortical bone volume [Ct.BV]: –7 %, $p = 0.063$, and US: –7 %, NS), while Con5+Spi5 group showed significantly higher cortical bone parameters than Con5 group (areal BMD: +6 %, $p < 0.05$; Ct.BV: +12 %, $p < 0.01$; US: +10 %, NS). Serum IGF-I was also lower in the Con5 group compared to the Con5+Spi5 and Con10 groups (380.5 ± 10.1, 437.0 ± 12.4, and 437.9 ± 18.5 ng/ml, respectively; $p < 0.05$).

Conclusion We demonstrate that Spirulina supplementation effectively prevents cortical and trabecular bone alterations, as well as bone strength decrease induced by isocaloric dietary protein deficiency during growth, in association with the maintenance of optimal IGF-I levels. Spirulina is an effective nutrient to prevent impaired PBMA in protein-deficient growing rats.

Clinical Abstracts

Strontium Ranelate Treatment Improves Bone Mineral Level Properties in Human Transiliac Bone Biopsy Specimens

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Introduction Bone strength, hence fracture risk, is dependent on bone geometry, microstructure, and bone material level properties. We have reported that microstructure and material level properties contribute independently to the increase in bone strength in rats treated with strontium ranelate for 2 years, as evaluated by μCT-based finite element analysis.

Methods We investigated the effects of strontium ranelate (SrRan) treatment on bone material level properties of transiliac bone biopsy from postmenopausal osteoporotic patients in 3 studies. In a cross-sectional study, 12 specimens (6 in 2 g/day SrRan-treated patients for 3 years and 6 in the placebo group) were analyzed (in the treated group 3 samples were paired). In a longitudinal study, 80 paired biopsies were obtained at baseline and after 6 or 12 months of treatment with 2 g/day SrRan. Elastic modulus, hardness, and working energy were blindly analyzed by nanoindentation in the middle of the cortex and of trabecular nodes under humid conditions. Significance of differences was evaluated by student t-test.

Results In the cross-sectional study, SrRan treatment was associated with higher cortical elastic modulus, hardness, and working energy by +11.0 % ($p = 0.05$), +28.1 % ($p = 0.0001$), and +11.2 % ($p = 0.01$), respectively. In 3 paired biopsies, SrRan for 3 years increased these variables by +21.8 ± 20.8 %, +48.7 ± 22.8 %, and +13.8 ± 16.7 %, respectively, as compared to baseline (cortex). In the longitudinal study, cortical elastic modulus, hardness, and working energy increased by +20.3 ± 7.4 % ($p = 0.001$), +18.3 ± 6.6 % ($p = 0.001$), and +13.0 ± 5.7 % ($p = 0.001$) after 12 months, respectively. Similar differences were observed in trabecular bone, however, no significant effects were observed after 6 months of treatment.

Conclusion Overall, these results detected in 92 human biopsies suggest that changes of bone material level properties in bone specimens collected in patients treated with strontium ranelate could contribute to increased bone strength and to fracture risk reduction.

Can We Improve the Reproducibility of Vertebral Fracture Assessment (VFA) Readings? An Exploratory Study Based on the OsteoLaus Cohort

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Introduction Osteoporotic vertebral fractures are highly prevalent and a major cause of morbidity and mortality in industrialized countries. The vertebral fracture assessment (VFA), obtained with bone mineral density testing via dual-energy X-ray absorptiometry (DXA), has been validated for vertebral fracture diagnosis in clinical settings. The International Osteoporosis Foundation (IOF) and the International Society of Clinical Densitometry (ISCD) both proposed specific guidelines for VFA interpretation. But the reproducibility of VFA reading is not known in population-based cohort screening. The aim of this study was to estimate the benefits of using IOF/ISCD guidelines for VFA reading and to estimate the level of reproducibility within and between raters for VFA in a population-based cohort. To assess these estimations, 2 statistical tools were used: Cohen's and uniform kappa coefficients.

Methods 360 women were randomly selected from the larger cohort OsteoLaus. We calculated inter- and intra-reading reproducibility of the 2 kappa statistics both before and after applying an IOF/ISCD-adapted guideline (IIR). All readings were performed independently

by 2 osteoporosis experts with extensive experience reading VFA but no prior training in the IOF/ISCD courses.

Results Overall, 12 % of vertebrae were unreadable, with this percentage as high as 48 % for levels T4 and T5. Depending on the reader, the prevalence of VF varied from 3 % to 4 %, with 3–4 % grade 1, 0.6–1.3 % grade 2, and 0.03–0.2 % grade 3 VF. Before IIR application, inter-reader reproducibility with Cohen's kappa was 0.35–0.71 and 0.74–0.98 with the uniform kappa coefficient for all criteria. After applying the IRR recommendations, inter-reader reproducibility was 0.28–0.68 and 0.70–0.99, respectively. All the results were better for lumbar spine than for dorsal spine.

Conclusions We concluded that kappa uniform is better adapted for the screening in a population-based cohort (events rare and repartition not uniform) than kappa of Cohen. The uniform kappa statistic is more accurate as a measure of reproducibility than Cohen's kappa for VFA reading. Moreover the IOF/ISCD guidelines did not increase reproducibility in VFA reading when readers are already experts in osteoporosis.

Long-Term Exercise Intervention in Older Adults: 4-Year Follow-Up of a Randomized Controlled Trial of Music-Based Multitask Training (Jaques-Dalcroze Eurhythmics)

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Introduction Long-term prospective controlled data supporting the efficacy of exercise to prevent physical decline in old age are scarce. In a 1-year randomized controlled trial [1], we showed that a 6-month music-based multitask exercise programme (ie, Jaques-Dalcroze eurhythmics) improved gait and balance and reduced falls in older adults. We conducted a 4-year extension of this trial aimed at assessing the effects of long-term practice of the multitask exercise regimen.

Methods We planned a long-term follow-up extension of a 1-year randomized controlled trial in which 134 community-dwellers aged ≥ 65 years at increased risk of falls received an immediate or delayed 6-month music-based multitask exercise programme, with outcomes assessed at baseline, 6 months, and 1 year. Four years following original trial enrolment, 52 subjects (baseline mean ± SD age: 75 ± 8 years) who (i) have maintained exercise programme participation through the 4-year follow-up visit (long-term "intervention group", n = 23) or (ii) have discontinued participation following original trial completion ("control group", n = 29) were studied. They were reassessed in a blind fashion, using the same procedures.

Results At 4 years, linear mixed-effects models showed significant gait (gait speed, p = 0.006) and balance (unipodal stance time, p = 0.015) improvements in the intervention group, compared with the control group. Also, intervention subjects did better on Timed Up & Go, Five-Times-Sit-to-Stand, and hand grip strength tests than controls (p < 0.05, for all comparisons). Furthermore, the exercise programme had a protective effect on falls (relative risk, 0.69; 95-% confidence interval: 0.5–0.9; p = 0.008).

Conclusions This 4-year extension of a randomized controlled trial suggests that long-term maintenance of a music-based multitask exercise programme later in life is a promising strategy to prevent age-related physical decline. It highlights that sustained adherence to exercise programmes is required to reduce fall risk.

References:

1. Trombetti A, Hars M, Herrmann FR, et al. Effect of music-based multitask training on gait, balance, and fall risk in elderly people: a randomized controlled trial. Arch Intern Med 2011; 171: 525–33.

Le dénosumab améliore significativement le « Trabecular Bone Score » (TBS), un indice de la microarchitecture trabéculaire, dans l'ostéoporose postménopausique

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Objectif Le « Trabecular Bone Score » (TBS) est un nouvel indice structurel dérivé des mesures par DXA au niveau de la colonne lombaire. Il est corrélé aux paramètres tridimensionnels microarchitecturaux de l'os trabéculaire, considérés comme prédicteurs de fractures. Le TBS pourrait améliorer l'identification de patients à haut risque de fractures vertébrales indépendamment de la densité minérale osseuse (DMO) [Hans JBMR 2011; Boutroy JBMR 2010]. Le dénosumab (Dmab) réduit le remodelage osseux, augmente la DMO et diminue les fractures vertébrales chez la femme souffrant d'ostéoporose postménopausique. Nous avons analysé l'effet du Dmab sur le TBS sur une période de 3 ans, ainsi que la relation entre le TBS et la DMO lombaire chez les femmes dont les mesures par DXA de l'étude de FREEDOM étaient transposables en TBS.

Méthodologie L'étude FREEDOM était une étude randomisée, menée en double aveugle pendant 3 ans auprès de femmes ménopausées dont le T-score, mesuré par DXA, au niveau de la colonne lombaire ou de la hanche totale était compris entre –2,5 et –4,0. Les femmes ont reçu 60 mg de Dmab tous les 6 mois. Une partie de ces femmes ont été enrôlées dans la sous-étude DXA, où les mesures de DXA lombaires ont été effectuées après le 1er, 6e, 12e, 24e et 36e mois d'étude. Les radiographies disponibles ont été évaluées rétrospectivement en aveugle à l'aide d'un nouveau logiciel (TBS iNsight® v1.9, Med-Imaps, Pessac, France) et le TBS a été déterminé pour le début de l'étude ainsi que pour les mois 12, 24 et 36. En vertu d'études précédentes, les valeurs de TBS suivantes ont été convenues : TBS > 1.35, microarchitecture normale ; TBS entre 1.35 et > 1.20, microarchitecture détériorée ; et TBS ≤ 1.20, microarchitecture détruite de manière étendue.

Résultats Il y avait une valeur TBS d'entrée et ≥ 1 valeur de TBS lors d'une visite suivante pour 285 femmes (128 placébo, 157 Dmab). Chez ces femmes, la moyenne d'âge s'élevait à 73 ans, la DMO lombaire moyenne correspondait à un T-score de –2.79, et le TBS lombaire moyen était de 1.20. En plus d'une augmentation stable de la DMO lombaire sous Dmab (9.8 % après 36 mois), les valeurs TBS ont augmenté de façon consistante, continue et significative par rapport au placébo et aux valeurs d'entrée. Seule une petite partie de la variation du TBS au début de l'étude était due à la DMO ($r^2 < 0,07$). Par ailleurs, les variations dans le changement de TBS au cours de l'étude n'étaient en grande partie pas liées au changement de DMO, indépendamment du fait que les valeurs étaient exprimées en valeurs absolues ou en pourcentage du changement (tous les $r^2 < 0,06$). Ceci indique que des informations indépendantes de la DMO ont été récoltées en utilisant le TBS.

Conclusion Chez la femme souffrant d'ostéoporose postménopausique, le dénosumab améliore le TBS, un indice de la microarchitecture trabéculaire au niveau de la colonne lombaire, de manière significative indépendamment de la DMO.

Deutsche Übersetzung

What Is the Performance in Vertebral Fracture Discrimination by Bone Mineral Density (BMD), Microarchitecture Estimation (TBS), and FRAX in Stand-Alone, Combined, or Adjusted Approaches: The OsteoLaus Study

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Introduction TBS, a novel grey-level measurement derived from lumbar spine DXA image texture, is related to microarchitecture and fracture risk independently of BMD. FRAX estimates the 10-year probability of major osteoporotic fracture (MOF) using risk factors that contribute to fracture risk independently of femoral neck BMD. The aim of the study is to compare the performance of FRAX versus TBS-adjusted FRAX using the Leslie B et al [1] method to better identify women at high fracture risk.

Methods The OsteoLaus cohort (1400 women 50–80 years living in Lausanne, Switzerland) started in 2010. CRF for OP, FRAX, lumbar spine and hip BMD, VFA by DXA, and TBS are recorded in OsteoLaus. Sensitivity and specificity in regard to vertebral fracture grade 2 and 3 have been calculated for all bone modalities as stand-alone or combined approaches. The adjustment of FRAX by TBS has also been calculated. Integrated discrimination index and net reclassification improvement have also been investigated.

Results We included 911 women: mean age 65.2 ± 7.9 y, BMI 25.7 ± 4.4, mean lumbar spine BMD 0.931 ± 0.163 (T-score –1.04 SD), TBS 1.289 ± 0.100. As expected, correlation between BMD and site-matched TBS is low (r^2 = 0.16). Prevalence of VFx grade 2/3 and MOF are 7.5 % and 15.0 %, respectively.

When used to reclassify fracture risk, this gave a significant increase in integrated discrimination index for VFx (+2.5 %, P < 0.001), with net reclassification improvement +7.6 % for VFx (p < 0.001).

Conclusion An incremental improvement in fracture identification was seen by using lumbar spine TBS in combination with FRAX. If validated in prospective cohorts, lumbar spine TBS may become clinically useful for enhancing fracture prediction from FRAX.

References:

1. Leslie WD, et al. Lumbar spine TBS is a FRAX independent risk factor for fracture: the Manitoba BMD Cohort. ISCD Annual Meeting, Tampa, Florida, 2013.

Die Langzeitbehandlung mit Denosumab führt zu einer anhaltend niedrigen Frakturinzidenz bei postmenopausalen Frauen mit Osteoporose ≥ 75 Jahre

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Ziel In der 3-jährigen Zulassungsstudie FREEDOM führte Denosumab (Dmab) bei postmenopausalen Frauen mit Osteoporose zu einer Erhöhung der Knochenmineraldichte (BMD) und einer Verminderung der Inzidenz neuer vertebraler, nichtvertebraler und Hüftfrakturen [Cummings 2009]. Bei Frauen, die aufgrund ihres Alters von ≥ 75 Jahren ein erhöhtes Frakturrisiko hatten, bewirkte Dmab eine Verringerung des Risikos für Hüftfrakturen um 62 % [Boonen 2011]. Die Wirkung einer Dmab-Langzeittherapie über bis zu 10 Jahre wird momentan in der offenen Verlängerung der FREEDOM-Studie untersucht. Mit zunehmendem Alter steigt die Frakturinzidenz, insbesondere die von Hüftfrakturen bei Frauen ≥ 75 Jahre. Daher haben wir die Frakturhäufigkeit und die BMD-Zunahme bei Frauen ≥ 75 Jahre, die über insgesamt 6 Jahre mit Dmab behandelt wurden, genauer charakterisiert.

Französische Übersetzung ⊙

Table 1: Lamy O, et al.

	Sensitivity	Specificity
Spine BMD (–2.5 T-score threshold)	29.4 %	82.7 %
Lowest BMD (–2.5 T-score threshold)	35.3 %	80.9 %
Spine TBS (–1.200 threshold)	51.5 %	77.1 %
FRAX MOF (20-% threshold)	38.2 %	94.8 %
Lowest BMD or TBS thresholds	64.7 %	65.4 %
Spine TBS or FRAX MOF (20-% threshold)	63.2 %	74.4 %
TBS adjusted FRAX All fracture (20-% threshold)	50.0 %	89.9 %

Methodik In der Verlängerungsstudie erhielt jede Frau 60 mg Dmab alle 6 Monate sowie eine tägliche Supplementierung mit Kalzium und Vitamin D. Wir untersuchten die Frakturinzidenz und BMD-Zunahme bei allen Frauen, die über 6 Jahre mit Dmab behandelt worden waren (Langzeitgruppe [LZ]) sowie bei den Frauen der Subgruppe, die zu Beginn der FREEDOM-Studie ≥ 75 Jahre alt waren (Hochrisikogruppe [HR]).

Ergebnisse Die FREEDOM-Ausgangswerte der LZ-Gruppe (n = 2343) und der HR-Gruppe (n = 662) waren vergleichbar, mit der Ausnahme, dass die Patientinnen der HR-Gruppe im Mittel älter waren (LZ-Gruppe: 72 Jahre; HR-Gruppe: 78 Jahre) und einen niedrigeren mittleren BMD-T-Wert an der Gesamthüfte hatten (LZ-Gruppe: –1,9; HR-Gruppe: –2,1). Trotz des zunehmenden Alters der Patientinnen war die Behandlung mit Dmab in den Jahren 4–6 mit einer anhaltend niedrigen Inzidenz neuer vertebraler, nichtvertebraler und Hüftfrakturen assoziiert. Zudem war die Frakturinzidenz in der HR-Gruppe in den Jahren 4–6 vergleichbar mit den beobachteten Häufigkeiten in den Jahren 1–3 bei Frauen ≥ 75 Jahre. Die BMD an der LWS und der Gesamthüfte nahm über 6 Jahre kontinuierlich zu und war bei Frauen ≥ 75 Jahre vergleichbar mit denen der gesamten LZ-Gruppe. Trotz fortgeschrittenerem Alter waren unerwünschte Ereignisse (UE) und schwerwiegende UE in der HR-Gruppe der Verlängerung vergleichbar mit denen der HR-Gruppe in der FREEDOM-Studie.

Fazit Diese Ergebnisse unterstreichen die starke und konsistente frakturensenkende Wirkung und das Sicherheitsprofil einer fortgesetzten Behandlung mit Dmab über 6 Jahre. Denosumab stellt eine Therapieoption für Frauen mit hohem Frakturrisiko dar. Das gilt vor allem für Patientinnen ≥ 75 Jahre, bei denen Frakturen, wie z. B. der Hüfte, durch den Abbau des kortikalen Knochens exponenziell zunehmen.

Behandlung mit Denosumab in postmenopausalen Frauen über 7 Jahre: Klinische Frakturen in den ersten 4 Jahren der FREEDOM-Verlängerungsstudie

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Ziel Denosumab (DMAb) demonstrierte in der 3-jährigen FREEDOM-Studie eine Verminderung des Risikos für vertebrale, nichtvertebrale und Hüftfrakturen. Die unverblindete Verlängerungsstu-

Französische Übersetzung ⊙

die untersucht die Langzeiteffekte von DMAb für bis zu 10 Jahre. Wir zeigen hier die Inzidenz von klinischen Frakturen (nichtvertebral und klinisch vertebral) von den ersten 4 Jahren der Verlängerung, was einer DMAb-Exposition von bis zu 7 Jahren entspricht.

Methodik In der FREEDOM-Erweiterungsstudie erhielten alle Teilnehmerinnen 60 mg DMAb alle 6 Monate und täglich eine Kalzium/Vitamin-D-Ergänzung. Für die vorliegende Analyse erhielten die Frauen aus der FREEDOM-DMAb-Gruppe für weitere 4 Jahre DMAb, was einer Behandlungsdauer von 7 Jahren entspricht (Langzeitgruppe); Frauen aus der FREEDOM-Placebogruppe erhielten DMAb für insgesamt 4 Jahre (Überkreuzgruppe). Für das 7. Studienjahr wurden nichtvertebrale Frakturen, klinische vertebrale Frakturen, Knochenumbaumarker und unerwünschte Ereignisse gesammelt.

Ergebnisse Von 5928 für die Verlängerung qualifizierten Frauen wurden 4550 (77 %) eingeschlossen (n = 2343 Langzeit; n = 2207 Überkreuz). Die jährliche Inzidenz für nichtvertebrale Frakturen blieb niedrig; in der Langzeitgruppe: 1,4 %, 1,2 %, 1,6 % und 1,5 % entsprechend für die Jahre 4–7 unter DMAb-Behandlung, was auf einen anhaltenden Effekt von DMAb hinweist; in der Überkreuzgruppe: 2,5 %, 1,9 %, 2,2 % und 1,0 % entsprechend für die Jahre 1–4 unter DMAb-Behandlung, was mit der DMAb-Gruppe aus der FREEDOM-Studie konsistent ist. Auch die Rate von klinischen vertebralen Frakturen war in beiden Gruppen niedrig. Auf die Expositionszeit normierte unerwünschte und schwere unerwünschte Ereignisraten waren zwischen der Langzeit- und Überkreuzgruppe ähnlich. Ereignisse, die einer ONJ entsprachen, wurden in 5 bzw. 3 Individuen entsprechend der Langzeit- und Überkreuzgruppe beobachtet. Zwei Femurfrakturen, welche als atypisch entsprechend eingestuft wurden, wurden beobachtet (1 Langzeit, 1 Überkreuz).

Fazit Die Behandlung mit Denosumab für bis zu 7 Jahre zeigt nach wie vor ein günstiges Risiko-Nutzen-Profil und war verbunden mit einer niedrigen Inzidenz von klinischen (nichtvertebralen und klinisch vertebralen) Frakturen.

Kompatibilität kortikaler Festigkeitsparameter der distalen Tibia mittels DXA und HR-pQCT bei prämenopausalen Frauen

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Einführung In Ergänzung zur Knochenmineraldichte (BMD) können strukturelle Parameter die Beschreibung der Knochenfestigkeit verbessern. Mittels eines neuen Index, welcher sich aus der BMD und dem polaren Trägheitsmoment (pMOI) direkt aus einer Doppel-Energie-Röntgenabsorptionsmetrie-Messung (DXA) berechnet, konnte *ex vivo* im Bereich der distalen Tibia-Diaphyse (T-DIA) die Festigkeit des Knochens ebenso gut vorausgesagt werden wie durch die hochauflösende, periphere quantitative Computertomographie (HR-pQCT). Der Korrelationskoeffizient zwischen beiden Methoden betrug $R^2 = 0{,}88$ [1]. Bislang ist nicht bekannt, ob sich dies auch *in vivo* bestätigen lässt.

Methoden Eine Kohorte gesunder gehfähiger, nichtschwangerer Frauen zwischen 20 und 40 Jahren, welche im Grossraum Davos leben, wurde aus einem kommerziellen Adressregister nach dem Zufallsprinzip rekrutiert. 485 Frauen wurden per Post angeschrieben. Diejenigen, die sich schriftlich bereit erklärten teilzunehmen und die Einschlusskriterien erfüllten, wurden eingeschlossen. Die Frauen wurden mittels HR-pQCT (XtremeCT™, Scanco Medical AG, Brüttisellen, Schweiz) an der Tibia sowie mittels DXA (Hologic Discovery C™, Hologic, Bedford, MA, USA) am Schenkelhals und an der Tibia untersucht. Zwecks Berechnung des pMOI wurde die Dicke der tibialen Kortikalis (CTh) mit beiden Methoden ermittelt. Bei den DXA-Scans geschah dies aufgrund von DICOM-Abbildungen. Dabei kam ein eigens entwickeltes Analysetool zum Einsatz, welches aus MATLAB™ (MathWorks™, Natick, MA, USA) generiert worden war. Entsprechend der vormals beschriebenen Methodologie ist damit auch die Kalkulation des pMOI für den DXA-Scan möglich [1]. Die beiden Festigkeitsindizes, bestehend aus dem Produkt von volumetrischer (vBMD, HR-pQCT) bzw. flächenbezogener (aBMD, DXA) Knochendichte multipliziert mit dem pMOI der jeweiligen Methode, wurden miteinander korreliert.

Resultate 72 gesunde prämenopausale Frauen konnten eingeschlossen werden. Das Durchschnittsalter lag bei 33,8 (± 5,2) Jahren, bei einer durchschnittlichen Körpergrösse von 167,6 (± 6,2) cm und einem BMI von 23,2 (± 4,1) kg/m^2. Die durchschnittliche BMD des Schenkelhalses lag bei 0,853 (± 0,119) g/cm^2, was einem durchschnittlichen Z-Score von 0,0 SD (± 1,1 SD) entspricht. An der distalen Tibia-Diaphyse zeigte die Kombination von Knochendichte und pMOI, welche mittels beider Messmethoden separat ermittelt wurde, eine hohe Korrelation von $R^2 = 0{,}74$.

Schlussfolgerung In einer Kohorte zufällig ausgewählter prämenopausaler Frauen besteht eine hohe Korrelation der kortikalen Kombinations-Indizes aus Struktur und Knochendichte, welche durch DXA bzw. HR-pQCT generiert wurden.

Literatur:

Popp AW, Windolf M, Senn C, et al. Prediction of bone strength at the distal tibia by HR-pQCT and DXA. Bone 2012; 50: 296–300.

Basic Research Abstracts

Ppar Beta Deficiency Induces Muscle and Bone Loss with Aging but Does Not Impair the Bone Biomechanical Response to Loading: A Sarco-Osteopenic Mouse Model

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Introduction Pparβ is a crucial transcription factor for muscle fatty acid oxidation. Pparβ$^{-/-}$ mice have reduced muscle strength, exercise performance, and also a decreased skeletal response to exercise. Here we investigate the influence of Pparβ on muscle and bone loss with aging, and its role on the bone biomechanical response to loading.

Methods Pparβ$^{-/-}$ and Pparβ$^{+/+}$ mice were monitored at 1, 3, and 18 months of age. Muscle function was evaluated by handgrip and locomotor activity. Six-month-old Pparβ$^{-/-}$ and Pparβ$^{+/+}$ males were subjected to in-vivo axial compression of the tibia, using a peak load of 12 N, at 0.1 Hz, during 7 min, 3 days/week for a period of 2 weeks, while the right unloaded tibia served as paired control. Bone and TB (total body) lean mass was analyzed by DXA, trabecular and cortical microarchitecture by ex-vivo micro-computed tomography, biomechanical properties by 3-point bending, and bone formation indices by histomorphometry of the tibia. Relative expression of Ppar and myostatin mRNA (normalized for GAPDH) was evaluated in gastrocnemius and femurs by qRT-PCR.

Results At 1 month of age, lean mass, skeletal muscle function, and bone parameters did not differ between Pparβ$^{-/-}$ and Pparβ$^{+/+}$. From 1 to 3 months of age, Pparβ$^{-/-}$ had lesser gain in lean mass (+64 % vs +88 % in Pparβ$^{+/+}$, $p < 0.01$) and TB BMD (+66 % vs +73 % in Pparβ$^{+/+}$, $p < 0.05$). Mean force and running distance were significantly lower in Pparβ$^{-/-}$. CtTV, CtBV, and strength were also reduced in Pparβ$^{-/-}$ (0.56 ± 0.02 mm^3, 0.34 ± 0.01 mm^3, 19.7 ± 1.3 N vs 0.62 ± 0.02 mm^3, 0.38 ± 0.02 mm^3, 25.1 ± 1.4 N, respectively in Pparβ$^{+/+}$, $p < 0.05$). From 3 to 18 months, differences between genotypes in TB lean and bone mass, mean force, and running distance further increased. At 18 months of age, Pparβ$^{-/-}$ had lower BV/TV, CtTV, CtBV, Ec-PsBFR and Ec-PsMP/BPm, and strength compared to Pparβ$^{+/+}$. Circulating myostatin increased more with age in Pparβ$^{-/-}$ than Pparβ$^{+/+}$ (4.4- vs 2.9-fold, $p < 0.05$). Myostatin gene expression is also higher in Pparβ$^{-/-}$ vs Pparβ$^{+/+}$ both in muscle (+25 %, $p < 0.05$) and bone (+33 %, $p < 0.05$). However, following axial compression,

the increase in BMD, CtTV, PsBFR, and PsMP/BP was similar in both genotypes.

Conclusions These results indicate that Pparβ plays an important role in the acquisition and maintenance of muscle and bone mass. Hence, in absence of Pparβ, sarcopenia and osteoporosis develop with aging, paralleling an increase in myostatin. However, the skeletal response to direct loading is maintained, suggesting that bone alterations are due to the loss of muscle functions and partly reversible.

RANKL Immobilized on β-TCP Induces and Maintains Osteoclast Formation

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Introduction β-tricalcium phosphate (β-TCP) biomaterials have been approved for the repair of osseous defects. However, in large defects the substitution of the biomaterial by authentic bone is inadequate to provide sufficient long-term mechanical stability. We aimed to develop composites of β-TCP ceramics and receptor activator of nuclear factor κ-B ligand (RANKL) to enhance the formation of osteoclasts and thereby stimulating material resorption.

Methods RANKL was immobilized on β-TCP ceramics by (i) superficial adsorption, leading to passive short-term release, and (ii) co-precipitation together with calcium phosphate, resulting in an incorporation of the protein into a crystalline layer of calcium phosphate and a cell-mediated long-term release. Murine osteoclast precursors were seeded onto the ceramics. After 15 days, the formation of osteoclasts was evaluated with tartrate-resistant acidic phosphatase (TRAP) staining and quantified with TRAP activity. Additionally, the expression of the osteoclast markers calcitonin receptor and cathepsin K was quantified by real-time PCR.

Results Superficially adsorbed RANKL did not induce the formation of osteoclasts on β-TCP ceramics. When co-precipitated onto β-TCP ceramics, RANKL induced the formation of osteoclasts as demonstrated by the development of TRAP-positive cells and a 2-fold increase in TRAP activity, which was similar to that observed in positive controls. Development of osteoclast lineage cells was further confirmed by an increase in the levels of transcripts encoding cathepsin K and calcitonin receptors.

Conclusions Our study shows for the first time that RANKL immobilized on β-TCP ceramics induces the formation of osteoclasts. However, to support osteoclast formation, a long-term release system for the growth factor is required. RANKL co-precipitation may induce osteoclast differentiation in 2 steps. Firstly, a residual passive release of the protein may induce osteoclastogenesis. Subsequently, matured osteoclasts mediate the release of RANKL by resorbing the protein-containing calcium phosphate layer, thereby perpetuing their differentiation and activation. It remains to be demonstrated whether the formation of osteoclasts leads to a stimulation of biomaterial resorption. Experiments focusing on the resorptive activity of osteoclasts formed on β-TCP ceramics are ongoing.

Development of a S100 Cell-Based ELISA for Screening of Stimuli Inducing Redifferentiation of Human Articular Chondrocytes

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Introduction Redifferentiation of human articular chondrocytes (HAC) prior implantation has been intensively investigated due to their dedifferentiation during monolayer expansion. Deposition of proteoglycans and collagen type II in 3D cultures is a common readout for the efficacy of different chondrogenic stimuli. These models limit the possibility of large, comprehensive, and well-controlled comparative studies of HAC redifferentiation induction. We have previously identified S100 protein as a marker of HAC redifferentiation potential [1]. Here we investigated re-expression of S100 in HAC as a redifferentiation readout in a 2D microplate cell-based assay (CELISA) amenable to high throughput screening of redifferentiation-inducing factors.

Methods An S100 cell-based ELISA (S100 CELISA) was developed in 96-well microplates using S100-positive melanoma cell line A2058, and validated using monolayer expanded HAC at different stages of dedifferentiation: HAC cultured for 48 h (P0; differentiated/S100+), 3 passages (P3; partially dedifferentiated/mixture of S100+ and S100–), and 3 passages in the presence of TGFβ1 and FGF2 (P3+TF; dedifferentiated/S100–). S100 induction was determined with the CELISA assay in P3 and P3+TF HAC incubated with 100 ng/ml of TGFβ1 and BMP4, and in P3 HAC from 3 different donors using different concentrations of BMP4, during one week. S100 expression was normalized to the amount of DNA using SYBR green.

Results CELISA results showed high S100 expression in P0 HAC, lower yet detectable expression in P3 HAC, and no S100 detection in P3+TF HAC. S100 expression was induced in P3 but not in P3+TF HAC incubated with BMP4, in accordance with published data assessing HAC redifferentiation in monolayer [2]. In contrast, incubation with TGFβ1 did not induce S100 in P3 or P3+TF HAC. An increase in S100 expression was confirmed with P3 HAC from 3 different donors stimulated with increasing concentrations of BMP4.

Conclusion The S100 CELISA could offer an advantageous alternative, in terms of time and cell amount requirements, to 3D models for testing factors/conditions to induce HAC redifferentiation. We are currently validating the assay by comparing BMP4 induction of S100 during monolayer expansion of HAC with their redifferentiation potential in 3D pellets.

References:

1. Giovannini S, Diaz-Romero J, Aigner T, et al. Population doublings and percentage of S100-positive cells as predictors of in vitro chondrogenicity of expanded human articular chondrocytes. J Cell Physiol 2010; 222: 411–20.
2. Benz K, Breit S, Lukoschek M, et al. Molecular analysis of expansion, differentiation, and growth factor treatment of human chondrocytes identifies differentiation markers and growth-related genes. Biochem Biophys Res Commun 2002; 293: 284–92.

Effects of Circulating Sclerostin on Bone and Lean Mass in Male Mice

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Introduction Sclerostin, a product of osteocytes, is known to inhibit Wnt signalling by binding the LRP5/6 receptor [1]. Its production is reduced by mechanical stimulation [2]. Although serum sclerostin levels correlated positively with age and BMD, data from cohorts on the association between serum sclerostin levels and bone fragility are discordant. Recent data showed that high serum sclerostin levels were associated with a lower fracture risk [3].

Methods We investigate the effects of injections of a mouse sclerostin protein (MuScl) on bone and lean tissue in male mice. In vitro, MuScl fully binds LRP6 resulting in a quick inhibition of Wnt activity. Eight-week-old male mice (n = 6 per group) were injected with MuScl (1 mg/kg, iv, 3 days/week) or vehicle for 3 weeks. The 2 last weeks, the left tibia was simultaneously subjected to axial compression (12 N, at 0.1 Hz, during 7 min, 3 days/week), whereas the right tibia served as non-loaded control. BMD, BMC, and body composition were measured longitudinally by DXA. Trabecular and cortical microarchitecture of the 2 tibias, the left femur, and L5 vertebrae were performed ex vivo by μCT. Sclerostin and OCN levels were measured. Muscle mass was estimated by weighting gastrocnemius and tibialis muscles.

Results As expected, serum Sclerostin levels increased more in the MuScl group than in the vehicle group (+162 % vs +44 %; p = 0.063),

whereas OCN level was not affected by MuScl (p = ns). Compared to vehicle, MuScl tended to increase total body BMD and tibia BMD (+8 % vs +6 %, p = 0.060; +13 % vs +10 %, p = 0.174, respectively). Trabecular BV/TV was increased in MuScl group (13.93 ± 0.93 % vs 11.03 ± 0.96 %; p = 0.056) and the connectivity density was significantly higher in the MuScl vs vehicle group (122.34 ± 11.53 1/mm^3 vs 82.48 ± 11.06 1/mm^3; p < 0.05). Other bone parameters measured were not affected by MuScl. MuScl tended to inhibit lean mass gain (+2.7 % vs +4.35 %; p = 0.113) and tibialis weight was significantly lower in MuScl vs vehicle group (0.053 ± 0.004 g vs 0.037 ± 0.003 g; p < 0.05). The mechanical stimulation had improved tibia BMD, trabecular and cortical microarchitecture, but no synergistic effect was measured between mechanical stimulation and sclerostin.

Conclusions High-dose sclerostin administration improved whole body bone mass and tibia trabecular microarchitecture without affecting osteocalcin levels and other bone parameters measured. Interestingly, MuScl impaired lean mass and muscle mass of tibialis, which suggests a new role of sclerostin in bone-muscle interactions.

References:
1. ten Dijke P, Krause C, de Gorter DJ, et al. Osteocyte-derived sclerostin inhibits bone formation: its role in bone morphogenetic protein and Wnt signaling. J Bone Joint Surg Am 2008; 90 (Suppl 1): 31–5.
2. Robling AG, Niziolek PJ, Baldridge LA, et al. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. J Biol Chem 2008; 283: 5866–75.
3. Szulc P, Bertholon C, Borel O, et al. Lower fracture risk in older men with higher sclerostin concentration: A prospective analysis from the MINOS study. J Bone Miner Res 2013; 28: 855–64.

Promotion of Neovascularization in Tissue-Engineered Bone Implants

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Introduction The sufficient supply with nutrients and growth factors is of critical value for tissue-engineered implants. In large-size implants the ingrowth of vessels often fails. Thus, neovascularization has to be promoted by the implant. The use of co-cultures of mesenchymal stem cells (MSC) and endothelial progenitor cells (EPC) is a promising therapeutic approach for tissue-engineered implants with the ability to integrate in the surrounding tissue. The aim of the current study was to promote neovascularization (in vitro, in vivo) of hydroxyapatite-containing polyurethane (PU) scaffolds.

Methods MSC and EPC were isolated from human bone marrow using Ficoll and MACS®, respectively. PU scaffolds were seeded with MSC and EPC in different proportions in the presence of autologous platelet lysate. Constructs for in-vitro observation were incubated in a medium suitable for both osteogenic and endothelial differentiation. For in-vivo analysis constructs were implanted subcutaneously in nude mice and excised after 8 weeks. Neovascularization was examined on cryosections stained with toluidine blue or in immunofluorescence staining of endothelial marker.

Results We observed neovascularization in the scaffolds in vitro and in vivo. Most importantly, in vivo a perfusion of scaffolds was observed, which was never detected in cell-free scaffolds, confirming anastomosis of newly formed vessels in the scaffold with the host vascular system.

Conclusion In conclusion, we could demonstrate that the presence of EPC is highly effective in inducing neovascularization in tissue-engineered constructs.

Osteoanabolic Effect of Alendronate and Zoledronate on Bone Marrow Stromal Cells (BMSCs) Isolated from Osteoporotic Patients and Its Implications for Their Mode of Action in the Treatment of Age-Related Bone Loss

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Introduction Bisphosphonates are a well-characterized class of synthetic compounds structurally related to pyrophosphate and are thought to mediate their antiresorptive actions primarily through inhibition of osteoclast activity. However, there is now an increasing number of reports alluding to the idea that the preventative effects of bisphosphonates on bone loss may be additionally mediated through their anabolic effects on cells of the osteoblastic lineage. In the present study, we evaluated the potential for aminobisphosphonates to enhance the development of bone-forming osteoblasts from BMSCs isolated from aged osteoporotic patients.

Methods BMSCs were isolated from a total of 10 aged female osteoporotic donors undergoing routine surgical procedures for proximal femur fracture. Osteoporosis was confirmed in these patients using dual-energy X-ray absorptiometry, where a bone mineral density T-score of –2.5 standard deviations or less at the contralateral hip and/or lumbar spine was considered osteoporotic. In 3 of the cases, patients had been receiving alendronate treatment for more than 3 years. The influence of aminobisphosphonate treatment on BMSC osteogenesis was assessed by the quantitative measurement of alkaline phosphatase (ALP) activity and qRT-PCR analysis of known osteogenic markers. Mineralized matrix formation by BMSC-derived osteoblasts was visualized and quantified using Alizarin red staining.

Results BMSC cultures treated with osteogenic medium supplemented with zoledronate demonstrated a significant increase in Alizarin red staining after 3 weeks as compared to cells cultured in osteogenic medium alone. Similarly, cultures of differentiating BMSCs isolated from patients receiving alendronate treatment also demonstrated an increased propensity for osteoblastogenesis, even in the absence of further in-vitro stimulation by zoledronate. The stimulatory effect of aminobisphosphonate treatment on mineralized matrix formation by BMSC-derived osteoblasts was independent of any alterations in ALP activity, although significant decreases in osteopontin mRNA expression levels were observed.

Conclusions The results presented here demonstrate for the first time that aminobisphosphonate treatment of osteoporotic BMSCs enhances their capacity for osteoblast formation and subsequent mineral deposition, thus supporting the concept of aminobisphosphonates as having an osteoanabolic effect in osteoporotic patients. Furthermore, our findings suggest that inhibition of osteopontin may represent a novel approach for enhancing osteoblastogenesis in osteoporotic BMSCs.

Effect of IL-1 Beta During Osteogenic Differentiation of Human MSCs

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Introduction Bone fracture repair involves skeletal tissue regeneration, with fracture healing being analogous to embryonic bone development. Inflammation plays an important role in fracture healing through the release of local and systemic regulatory factors that aid in efficient tissue repair. One of these factors, interleukin 1β (IL-1β), has a bimodal expression pattern with peaks during the initial inflammation stage and remodelling phase of endochondral fracture repair.

RunX2 is a key transcription factor associated with osteogenic differentiation and is required for bone formation. In contrast, the Sry-

related transcription factor Sox9 is a key regulator of cartilage differentiation and especially expressed during chondrogenesis. The first aim of the study was to determine the effects of IL-1β on differentiation and mineralization in human mesenchymal stem cells (MSCs). Furthermore, we hypothesized that osteogenic differentiation of human MSCs is controlled through a balance in the expression of the 2 transcription factors RunX2 and Sox9 which drives the direction of MSC differentiation towards bone development.

Methods Bone marrow of different donors (n = 5) was obtained with ethical approval and written consent of the patients. MSCs were isolated through Ficoll density gradient centrifugation and cultured in monolayers over a period of 28 days. During this period, MSCs were cultured in osteogenic medium (100 nM dexamethasone, 5 mM β-glycerol phosphate, and 50 μg/ml ascorbic acid) with or without IL-1β (10 ng/ml) supplementation. DNA content, alkaline phosphatase (ALP) activity, alizarin-red S quantification, ^{45}Calcium (^{45}Ca) incorporation, and expression of osteogenic specific genes were assessed (n = 3). To elucidate the roles of molecular determinants in bone forming cells, we analysed Sox9 and RunX2 expression patterns in comparison to the ^{45}Ca incorporation.

Results Stimulation with IL-1β had a significant increase in cellular proliferation compared to control medium following 14, 21, and 28 days of culture. Furthermore, IL-1β supplementation significantly enhanced incorporation of ^{45}Ca on days 21 and 28 compared to osteogenic induced cultures without IL-1β. The typical peak of ALP-activity on day 14 in osteogenic induced MSCs was shifted from day 14 to day 7 if cells were stimulated with IL-1β. Furthermore, the ratio between RunX2 and Sox9 on day 7 showed a positive correlation to the incorporated ^{45}Ca on days 21 and 28.

Discussion The present study has shown that IL-1β enhances proliferation and direct mineralization of hMSCs in vitro. IL-1β-stimulated cells enter maturation prior to non-stimulated osteogenic induced hMSCs as monitored by ALP activity. Our data further imply that the ratio between RunX2 and Sox9 mRNA expression is a decisive factor towards differentiation of hMSCs. IL-1β increased the RunX2-Sox9 ratio already on day 7, which confirms an enhanced mineralization potential in the IL-1β-stimulated group.

Conclusion We have shown that IL-1β stimulates the cells to inflammation, increasing proliferation, and differentiation. Furthermore, we propose that the Runx2-Sox9 ratio is a promising early screening method for osteogenicity of human MSCs.

Biodegradable Fiber-Reinforced Nanocomposites for Bone Void Filler Applications

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Introduction Conventional calcium phosphate (CaP) scaffolds used as bone void fillers do not withstand high static and dynamic loads as they exhibit low fracture toughness. Of particular importance are load-bearing properties for scaffolds used in subchondral applications, where shocks induced by articulation and movements are translated to. Fracture toughness and compressive strength of CaP scaffolds can be increased by engineering fiber-reinforced composite material with fiber volume fractions higher than 1 %, provided the components are homogeneously dispersed. Due to the reduced size, nanoscale materials allow a higher degree of component dispersion compared to the use of microscale components. The aim of the present study was to engineer a biodegradable fiber-reinforced cement using homogeneously dispersed nanoscale poly-L-lactic acid (PLLA) fibers and CaP nanoparticles.

Methods We manufactured non-wovens based on PLLA by electrospinning to obtain mean fiber diameters in the range of 250 nm. In order to ensure a homogenous distribution of individual fibers within the CaP matrix, 1 part electrospun nanofiber meshes were sonicated at –80 °C together with 7 parts (w/w) β-tricalcium phosphate (TCP) nanoparticles. One part of the resulting dispersion was mixed with 2 parts (w/w) 2.6 M aqueous solution of monocalcium-phosphate monohydrate and allowed to react in a cement reaction.

Results Sonication resulted in separation of the fibers and a homogeneous dispersion within the TCP particle matrix. The resulting set bone void filler composed of Brushite and 5 % (v/v) PLLA fibers was characterized with regard to morphology and mechanical properties. It was found that the reinforced material exhibited an approximately 90-% increase in compressive strength when compared to non-reinforced control cement. SEM analysis revealed a homogeneous distribution of the fibers within the Brushite matrix.

Conclusions Combined particle-fiber mesh sonication is a suitable method to homogeneously disperse electrospun fibers and particles and presents a method to engineer composites with fiber volume fractions > 5 % suitable for bone void filler applications.

ΔNp63α and GLI2 Association in Osteosarcoma Progression

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Introduction Osteosarcoma (OS) is the most common type of primary bone cancer. It arises in bone during periods of rapid growth and primarily affects adolescents and young adults. ΔNp63α and GLI2, a hedgehog signalling component, are transcription factors capable of inducing motility, invasion, and metastasis in many types of cancer but their role in OS is poorly understood. We are investigating the influence of ΔNp63α and GLI2 in OS progression. We could show that expression of ΔNp63α and GLI2 is upregulated in invasive OS cell lines. In ΔNp63α overexpressed SaOS-2 cell lines (SaOS-2-ΔNp63α), GLI2 expression was evident when compared with the empty vector (SaOS-2-EV) where little or no expression could be detected. Treatment with GLI2 inhibitor (GANT61) in SaOS-2-ΔNp63α and 143B cells drastically reduced the expression of GLI2 when compared with SaOS-2-EV. Cell cycle analysis revealed that GANT61 treatment induced G0/G1 arrest in SaOS-2-ΔNp63α and 143B cells.

Methods ΔNp63α stable SaOS-2 cell lines were generated by retroviral infection. Expression levels of ΔNp63α and GLI2 were analysed by western blot (WB) and real-time PCR (qRT PCR) in OS cell lines. Co-localization analysis of ΔNp63α and GLI2 were performed by immunocytochemistry. FACS was utilised for determination of cell cycle stage after treatment with GANT61.

Results ΔNp63α and GLI2 were upregulated in invasive OS cell lines as revealed by WB and qRT PCR. Immunocytochemistry revealed that ΔNp63α and GLI2 show colocalisation in the nucleus in SaOS-2-ΔNp63α and 143B cells. Treatment with GANT61 for 24 hours induced G0/G1 arrest in SaOS-2-ΔNp63α and 143B cells.

Conclusions Our results indicate that there might be a mutual interaction between ΔNp63α and GLI2 in OS progression. To further elucidate the functional role, SaOS-2-ΔNp63α cells will be injected in an established intratibial mouse model and treatment with GANT61 will be carried out.

Biomechanical Concepts Translated into a Bone Tissue Engineering Application

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Introduction Tissue engineering scaffolds can be used as bone substitutes in revision knee arthroplasty (RKA). In RKA, bone defects are often located in load-bearing regions. We have previously shown that mechanical stimulation can be used as an osteogenic signal [1]. The goal of this study was to determine if a physiologic load transmitted by the tibial tray of the RKA could be used as an in-situ osteogenic signal to the scaffolds filling the bone defects.

Methods In order to answer this question, we proposed a novel translation procedure having 4 steps: (1) determining the mechanical stimulus in RKA scaffold using finite element modelling, (2) designing an animal study to measure bone formation spatially and temporally using micro-CT imaging with and without applying the estimated mechanical stimulus, (3) identifying bone formation parameters for the loaded and non-loaded cases appearing in a recently developed mathematical model for bone formation in scaffold [2], and (4) using the identified bone formation parameters and the mathematical model to estimate over time the stiffness and the bone formation in the bone-scaffold construct in RKA.

Results The geometry of scaffolds used in the FEM of RKA was imported in COMSOL (Comsol AB, Stockholm, Sweden) and meshed with tetrahedral elements. Two different sets of identified parameters for the bone formation model were used, one for non-loaded and one for loaded scaffolds. Diffusion equation was solved inside the scaffold, assuming that the boundaries adjacent to the scaffold are osteogenic. The models were solved for a time period of 36 months, with time steps of 1 month. Results demonstrated a clear benefit of the mechanical loading in inducing more bone formation in the scaffold as well as an important increase of its mechanical properties.

Conclusions One of the key phases of any treatment is its translational aspect. We devised a new scheme for translating the in-vivo results into clinical applications, in this case RKA. We designed our in-vivo experiment such that we have the same mechanical stimulation in scaffold in vivo as of the proposed clinical application. We measured bone formation in vivo using micro-CT imaging and we found out that loading enhances bone formation in scaffold. Using a mathematical tool that we previously developed [2], we identified the effect of loading. In the final step, we used the same model to predict bone formation in the scaffold used in RKA. The same procedure can be followed for other treatments, such as growth factor and cell therapy.

References:

1. Roshan-Ghias A, Terrier A, Bourban PE, et al. In vivo cyclic loading as a potent stimulatory signal for bone formation inside tissue engineering scaffold. Eur Cell Mater 2010; 19: 41–9.

2. Roshan-Ghias A, Vogel A, Rakotomanana L, et al. Prediction of spatiotemporal bone formation in scaffold by diffusion equation. Biomaterials 2011; 32: 7006–12.

Design of an Image Analysis Workflow to Access Bone Mineral Densities Using Different X-Ray-Based Imaging Modalities

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Introduction Obviously, HR-pQCT and DEXA systems are considered as "gold standards" for assessing bone mineral densities (BMD), but often their long acquisition time and small scan volume limit their applicability in clinical applications. On the other hand, ClinicalCT allows large volumes to be scanned in a shorter acquisition time, thus favourable for various clinical and R&D-based applications. We carried out a literature research on understanding the clinical computed tomography (CT) scanner for evaluating bone mineral density (BMD) and correlating it to its gold standards.

The aim of this work is to design an imaging and image analysis workflow to assess bone qualities (BMD) using different X-ray-based imaging modalities.

Methods The designed image analysis workflow will be addressed in individual steps. First the source volume was CT-scanned using the DEXA, ClinicalCT, HR-pQCT (XtremeCT), and VivaCT imaging modalities along with a calibration phantom. Following this 4 small bones of clinical interest, distal radius, scaphoid, capitate, and the 3rd metacarpal, were dissected and scanned. Then the desired volumes of interest (VOI) were contoured and their HU linear attenuation values were converted in mgHA/cm^3 (BMD). Lastly the bone mineral densities were analyzed on the full volume.

Results The designed image analysis workflow has been tested on 9 adult human donor hands (forearm to fingertip) with 4 bones each of clinical interest scanned and evaluated intact (gull hand) and dissected (individual bones). Evaluation is focused on 3 main aspects: correlating HU units to bone mineral density (BMD) [1], comparing quantitatively the outputs of different imaging modalities for their apparent densities (BMD), and understanding the effects of surrounding tissues while comparing the bone mineral density (BMD) of the intact bones (full hand) and dissected bones (individual bones).

Conclusion It is for the first time that the designed image analysis workflow has been implemented on the aforementioned 4 small bones of clinical interest. Linear attenuation coefficients (HU) correlate well with the "gold standard" BMD measurement from the DEXA scanner. Linear attenuation values (HU) and their corresponding BMD from the computed tomography (CT) scanner also show significant correlation with the HR-pQCT scanners and hence can be used without additional cost and time. The effects of surrounding tissues on the density evaluations are also minimal. Some challenges faced while implementing the pipeline for its BMD computations were using the Siemens Osteo tool and Amira registration techniques as these are limited in contouring and the desired VOIs have to be as close as possible to the volume being aligned. Nevertheless the available protocol has shown promising results and shows possibilities for future research.

References:

1. Schreiber JJ, Anderson PA, Rosas HG, et al. Hounsfield units for assessing bone mineral density and strength: a tool for osteoporosis management. J Bone Joint Surg Am 2011; 93: 1057–63.

Expression of TGFβ Receptors and Collagen Type II in Bovine Chondrocytes During Prolonged Monolayer Culture

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Introduction Cartilage is an avascular tissue with limited self-healing potential, hence tissue injuries require surgical attention. A possible repair strategy is the filling of the defect with autologous chondrocytes expanded in vitro. Inherent to these cells, and limiting the possibilities of their application, is their tendency to develop a fibroblast-like phenotype characterized by loss of collagen type II (COL2) and gain of COL1 expression in monolayer cultures. Moreover, the cells lose the capacity to form a cartilage-like tissue in the absence of growth factors inducing chondrogenesis, such as transforming growth factor (TGF) β_1.

Methods We characterized the expression of COL2 and components of the TGFβ-signalling pathway in primary chondrocytes during long-term monolayer culture. The expression was correlated with the cells' potential to form cartilage-like tissue in high-density micromass cultures.

Results Bovine chondrocytes were expanded for 23 population doublings (PD). After 7 PD, levels of transcripts encoding COL2 and TGFβ receptors 1 and 2 were reduced to < 1 %, 30 %, and 20 %, respectively. Levels of transcripts encoding TGFβ_1 remained unchanged. The protein levels of intracellular and secreted COL2 were highest in freshly isolated chondrocytes and decreased to undetectable levels after 7 PD. The formation of a cartilage-like tissue in the absence of TGFβ_1 was only observed from cells with less than 7–10 PD. The addition of TGFβ_1 allowed for cartilage-like tissue formation from cells with up to 23 PD, but deposition of glycosaminoglycans and COL2 in the cell-associated matrix decreased.

Conclusions The reduced expression of COL2 and TGFβ receptors by chondrocyte-lineage cells was paralleled by the decrease in their potential to form a cartilage-like tissue. It remains an open question whether a balanced and stable expression of TGFβ_1 and TGFβ receptors and the presence of COL2 are prerequisites for cartilage formation or represent a consequence of the chondrogenic phenotype.

Osteoblastic P38alpha MAPK Signalling Mediates Ovariectomy-Induced Bone Loss by Stimulating Interleukin-6 Expression

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Introduction Selective p38alpha inhibitors have been found to prevent bone loss induced by estrogen deficiency but implicated mechanisms remained to be identified. The p38 MAPK pathway has been suggested to influence bone resorption at different regulatory levels. In osteoblasts, p38alpha has been reported to stimulate the production of osteoclastogenic interleukin-6 and RANKL in response to various bone-resorptive agents in vitro. Therefore, we investigated whether p38alpha in osteoblasts may contribute to ovariectomy-induced bone loss in mice.

Methods Mice lacking p38alpha in osteoblasts (Ocn-Cre;p38alpha$^{f/f}$) and their control littermates (p38alpha$^{f/f}$) were either sham-operated or ovariectomized at 12 weeks of age. Their bone phenotypes were assessed 6 weeks after operations by DEXA, micro-CT, histomorphometry, and gene expression analyses (n = 7 per group). Data were analyzed by 2-way ANOVA and post-hoc analyses were performed using the Holm-Sidak method.

Results Ovariectomy caused a decrease in bone mineral density in the spine (–13.1 %; $p < 0.001$ versus sham) and to a lesser extent in the femur (–1.8 %; $p < 0.001$ versus sham) of control mice but not in Ocn-Cre;p38alpha$^{f/f}$ mice (+12.7 % in the spine; +8.1 % in the femur; $p < 0.01$ versus p38alpha$^{f/f}$). Ovariectomy decreased vertebral cancellous bone volume (–45.8 %; $p < 0.01$ versus sham), trabecular thickness (–16 %; $p < 0.01$), and trabecular number (–20.6 %; $p < 0.01$) in control mice but not in Ocn-Cre;p38alpha$^{f/f}$ mice, indicating that mice lacking p38alpha in osteoblasts were protected from ovariectomy-induced bone loss. Consistent with this, ovariectomy caused significant increases in osteoclast surface (4-fold), osteoclast number (3-fold), and serum level of CTX (1.4-fold) in p38alpha$^{f/f}$ mice but not in Ocn-Cre;p38alpha$^{f/f}$ mice. Moreover, ovariectomy induced a 2-fold increase in interleukin-6 expression in the long bones of p38alpha$^{f/f}$ mice ($p < 0.05$), whereas expression of this osteoclastogenic cytokine remained unchanged in Ocn-Cre;p38alpha$^{f/f}$ mice. Finally, tumour necrosis factor alpha, interleukin-1, and CD40 ligand, which are overproduced in the bone marrow under estrogen deficiency, stimulated interleukin-6 expression in control osteoblasts but not in those lacking p38alpha in vitro.

Conclusion Our findings indicate that p38alpha MAPK signalling in osteoblasts mediates ovariectomy-induced bone loss by upregulating interleukin-6 expression.

Human Serine Protease HTRA1 Is a Novel Mediator of Human Bone Marrow-Derived Stromal Cell Osteogenesis

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Introduction Human high-temperature requirement serine protease A (HTRA1) is a secreted member of the broadly conserved HTRA family of trypsin-like serine proteases. A prominent feature of this protein is its ability to degrade and modify a variety of extracellular matrix (ECM) proteins. As such, HTRA1 is now regarded as being an important mediator of musculoskeletal development and disease. In the current study, we aimed to establish a role for HTRA1 in bone formation by examining the effects of HTRA1 loss- or gain-of-function on human bone marrow-derived stromal cell (hBMSC) osteogenesis and subsequent mineral formation.

Methods Investigation of HTRA1 function in bone marrow-derived stromal cells was accomplished using 2D cell culture and 3D microtissue culture systems undergoing osteogenesis and adipogenesis. Experimental approaches included siRNA-based knock-down experiments, qPCR analysis of osteogenic and adipogenic marker genes, staining of mineralized matrix by Alizarin Red S or lipid droplet formation by Oil Red O, addition of recombinant HTRA1 protein, visualization of HTRA1 and IBSP by immunofluorescence, as well as evaluation of secretion, enzyme activity, and target specificity of HTRA1 by ELISA and Western blotting.

Results Extracellular addition of recombinant HTRA1 enhanced osteogenesis of hBMSCs as evidenced by significant alterations in several osteogenic markers including integrin-binding sialoprotein (IBSP), bone morphogenetic protein 5, and sclerostin. HTRA1 also enhanced mineral formation in differentiating osteoblasts as shown by increased Alizarin red staining and was dependent on its proteolytic activity. Conversely, matrix mineralization was completely abolished in these cultures following treatment with small interfering RNA (siRNA) specific for HTRA1. Immunofluorescence studies identified high levels of HTRA1 protein in association with IBSP at sites of newly formed bone mineral both in vitro and in vivo. Indeed, proteolytic enzyme assays confirmed IBSP as being a novel HTRA1 substrate. In contrast to its positive role in osteogenesis, HTRA1 imparted a negative effect on adipogenesis as demonstrated by its ability to significantly reduce lipid droplet formation in BMSCs undergoing adipogenic differentiation. This was confirmed by loss-of-function studies, where HTRA1 silencing resulted in a significant increase in the adipogenic potential of hBMSCs as demonstrated by increases in the expression levels of specific adipogenic markers including peroxisome proliferator-activated receptor gamma and fatty acid-binding protein 4. This differential effect of HTRA1 on BMSC differentiation was further emphasized by its secretion profile, where increases in the levels of endogenous HTRA1 protein were observed during osteogenic differentiation only.

Conclusion In summary, our data reveals HTRA1 as being an essential requirement for osteogenesis. Its dual role as both an osteogenic promoter and an adipogenic inhibitor may have significant implications in terms of its functional involvement in diseases such as osteoporosis, where deficiencies in BMSC lineage commitment are evident.

Devitalized Engineered Hypertrophic Cartilage Forms Bone Through Addition of Osteoclastic Cells

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Introduction In this study we investigated whether osteoclastic cells derived from human peripheral blood co-cultured with devitalized hyperthrophic cartilage (HC) produced in vitro by adult human mesenchymal stromal cells can prime remodelling into bone tissue.

Methods HC was devitalized by successive freeze/thaw cycles and cultured alone as control or together with freshly isolated CD14+ monocytes in the presence of M-CSF and RANK-ligand for up to 23 days. Moreover, after 1 day of in-vitro culture, samples from both groups were implanted ectopically in nude mice for up to 8 weeks. Analysis consisted of biochemistry, protein assays, histology, and microtomography.

Results In-vitro matrix degradation through significant loss of glycosaminoglycans was detected only if HC was cultured with osteoclastic cells. Supernatants of co-cultures contained significantly higher amounts of chemoattractant (MCP-1 192-fold; SDF-1 4-fold), angiogenic (IL-8 556-fold; VEGF-A 5.4-fold), and matrix degrading (MMP9 13534-fold; MMP13 8.5-fold) factors as compared to controls. In vivo only co-cultured HC generated frank bone through endochondral ossification with a 3.5-fold higher mineralized volume as compared to controls.

Conclusion The addition of osteoclastic cells on devitalized HC primed remodelling, leading to bone formation. Ongoing experiments will investigate a bioreactor-based up-scaling of this approach and

its use in an orthotopic environment. As a future streamlined and standardized process it could be suitable for clinical translation.

Regulation of Iron Transport During the Development of Osteoclasts

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Introduction Intracellular iron homeostasis is relevant in osteoclast development and activity, and aberrant regulation of iron may be involved in pathophysiological conditions of the skeleton. The mechanisms by which bone cells ensure iron homeostasis need to be further elucidated, since the involved transport molecules may serve as potential targets for the treatment of bone diseases.

Methods The expression of molecular transporters was investigated by using custom-made low-density arrays (LDA) allowing the identification of a total of 154 membrane transporters. Gene expression was assessed in developing and in actively resorbing osteoclast lineage cells. The osteoclasts derived from C57/BL6J bone marrow cells (cultured with CSF1 [30 ng/ml] and RANKL [20 ng/ml] for 3 and 5 days, respectively).

Results Transferrin receptor 1 mRNA levels were increased by a factor > 4 and levels of transcripts encoding ferroportin (SLC40A1) were decreased by > 90 %. Transcripts encoding mitochondrial iron transport molecules such as Slc25a27 and Slc25a28 were constitutively expressed during osteoclast development.

Conclusions Our data demonstrates transcriptional regulation of the components of cellular iron transport during osteoclast development, suggesting that these components and their regulators such as hepatic hormone Hepcidin might represent putative therapeutic targets in conditions of impaired bone resorption.

Denosumab verbessert bei postmenopausalen Frauen mit Osteoporose den „Trabecular Bone Score" (TBS), einen Index der trabekulären Mikroarchitektur, signifikant

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Ziel Der „Trabecular Bone Score" (TBS) ist ein neuer Strukturindex, der sich aus DXA-Messungen der Lendenwirbelsäule (LWS) ableiten lässt. Er korreliert mit den mikroarchitektonischen 3D-Parametern des trabekulären Knochens, die als Frakturprädiktoren gelten. Der TBS könnte die Identifikation von Patienten mit einem erhöhten vertebralen Frakturrisiko unabhängig von der Knochenmineraldichte (BMD) verbessern [Hans JBMR 2011; Boutroy JBMR 2010]. Denosumab (Dmab) vermindert den Knochenumbau, erhöht die BMD und vermindert vertebrale Frakturen bei postmenopausalen Frauen mit Osteoporose. Wir haben den Effekt von Dmab auf den TBS über 36 Monate und den Zusammenhang zwischen TBS und LWS-BMD bei den Frauen untersucht, für die auswertbare DXA-Messungen für den TBS aus der FREEDOM-Studie vorlagen.

Methodik Die FREEDOM-Studie war eine 3-jährige, randomisierte, doppelblinde Studie bei postmenopausalen Frauen mit einem DXA-T-Score an der LWS oder der Gesamthüfte von < –2,5, jedoch an beiden Lokalisationen nicht < –4,0. Die Frauen erhielten alle 6 Monate Placebo oder 60 mg Dmab. Ein Teil der Frauen der FREEDOM-Studie wurde in eine DXA-Substudie aufgenommen, in der DXA-Messungen der LWS zu Studienbeginn und den Monaten 1, 6, 12, 24 und 36 durchgeführt wurden. Wir haben die vorliegenden DXA-Scans retrospektiv mithilfe einer neuen Software (TBS iNsight® v1.9, Med-Imaps, Pessac, Frankreich) verblindet ausgewertet und den TBS zu Beginn sowie nach 12, 24 und 36 Monaten bestimmt. Aufgrund früherer Studien ist ein TBS > 1,35 mit einer normalen, ein TBS zwischen 1,35 und > 1,20 mit einer verschlechterten und ein TBS ≤ 1,20 mit einer weitgehend zerstörten Mikroarchitektur vereinbar.

Ergebnisse Von 285 Frauen (128 Placebo, 157 Dmab) lagen jeweils ein Ausgangs-TBS sowie ≥ 1 TBS einer Folgeuntersuchung vor. Bei diesen Frauen betrug das Durchschnittsalter 73, der mittlere BMD-T-Score an der LWS lag bei –2,79 und der mittlere TBS der LWS war 1,20. Zusätzlich zu einer stabilen BMD-Zunahme an der LWS unter Dmab (9,8 % nach 36 Monaten) zeigte sich ein konsistenter, stetiger und signifikanter Anstieg der TBS-Werte, verglichen mit Placebo und den Ausgangswerten (**Tab. I**). Die BMD erklärte nur einen sehr kleinen Teil der TBS-Varianz zu Studienbeginn ($r^2 < 0,07$). Außerdem standen die Varianzen der TBS-Änderung während der gesamten Studie größtenteils nicht in Zusammenhang mit der BMD-Änderung, unabhängig davon, ob die Werte in absoluten Zahlen oder als prozentuale Änderung angegeben wurden (alle $r^2 < 0,06$). Das deutet darauf hin, dass durch TBS BMD-unabhängige Informationen gewonnen werden.

Fazit Bei postmenopausalen Frauen mit Osteoporose verbessert Denosumab signifikant und BMD-unabhängig den TBS, einen Index der trabekulären Mikroarchitektur der LWS.

Tabelle I: Krieg MA, et al. Prozentuale Änderung für LWS-TBS und BMD über die Zeit im Vergleich zum Ausgangswert sowie Pearson-Korrelation zwischen TBS und BMD.

	Placebo (n = 128)			Denosumab (n = 157)		
Monat	**TBS-Änderung (%)**	**BMD-Änderung (%)**	**Pearson-Korrelation**	**TBS-Änderung (%)**	**BMD-Änderung (%)**	**Pearson-Korrelation**
12	0,0	–0,1	0,0036	1,4[a,b]	5,7[a,b]	0,0256
24	0,2	0,1	0,0004	1,9[a,b]	7,8[a,b]	0,0225
36	–0,7	0,0	0,0324	2,4[a,b]	9,8[a,b]	0,0529

n = Zahl randomisierter Patienten der DXA-Substudie, deren TBS zu Studienbeginn und bei ≥ 1 der Folgebesuche erfasst wurde; [a]: p ≤ 0,0144 verglichen mit Placebo; [b]: p ≤ 0,0003 verglichen mit dem Ausgangswert.

Zurück

Le traitement de longue durée avec dénosumab maintient une faible incidence fracturaire chez les femmes souffrant d'ostéoporose postménopausique âgées de ≥ 75 ans

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Objectif Lors des 3 ans de l'étude FREEDOM sur le risque fracturaire chez les femmes souffrant d'ostéoporose postménopausique, le dénosumab (Dmab) a augmenté la densité minérale osseuse (DMO) et a diminué l'incidence de nouvelles fractures vertébrales, non-vertébrales et de la hanche [Cummings 2009]. Chez les femmes présentant un haut risque fracturaire dû à l'âge, ≥ 75 ans, le Dmab a diminué le risque de fracture de la hanche de 62 % [Boonen 2011]. L'effet d'un traitement de longue durée du Dmab jusqu'à 10 ans est en cours d'étude dans l'extension de FREEDOM. L'incidence des fractures augmente avec l'âge et particulièrement l'incidence des fractures de la hanche chez les femmes âgées de ≥ 75 ans. C'est pourquoi nous avons analysé de plus près la fréquence des fractures et l'augmentation de la DMO chez les femmes de 75 ans et plus traitées avec Dmab sur une période de 6 ans.

Méthodologie Dans l'étude d'extension de FREEDOM, toutes les femmes ont reçu 60 mg de Dmab tous les 6 mois ainsi qu'un supplément quotidien de calcium et vitamine D. Nous avons analysé l'incidence fracturaire et l'augmentation de la DMO chez toutes les femmes qui ont suivi le traitement avec Dmab pendant 6 ans (groupe traité à long terme [LT]) et dans le sous-groupe de femmes qui, au début de l'étude FREEDOM, étaient âgées de ≥ 75 ans (groupe à haut risque [HR]).

Résultats Les valeurs d'entrée du groupe LT (n = 2343) et du groupe HR (n = 662) dans l'étude FREEDOM étaient comparables à l'exception du fait que les patientes du groupe HR avaient un âge moyen plus élevé (groupe LT: 72 ans; groupe HR: 78 ans), et une DMO moyenne plus faible au niveau de la hanche totale (T-score groupe LT: –1.9; T-score groupe HR: –2.1). Malgré l'âge avançant des patientes, le traitement avec Dmab au cours des années 4 à 6 était associé à un maintient de la faible incidence de nouvelles fractures vertébrales, non-vertébrales et de la hanche. De plus, l'incidence fracturaire du groupe HR entre les 4e et 6e années de thérapie était comparable à l'incidence observée chez les femmes de ≥ 75 ans lors des 3 premières années. La DMO lombaire et la DMO de la hanche totale chez les femmes de ≥ 75 ans ont augmenté de manière continue sur 6 ans, et elles étaient comparables à celles du groupe LT. Les effets indésirables (EI) et les EI graves dans le groupe HR étaient comparables au groupe HR lors de l'étude FREEDOM, malgré l'âge avancé des patientes.

Conclusion Ces résultats soulignent l'effet puissant est consistant du Dmab sur la diminution des fractures et le profil de sécurité lors d'une thérapie continue pendant 6 ans. Le dénosumab représente une option thérapeutique pour les femmes à haut risque fracturaire. Ceci est surtout valable pour les patientes de 75 ans et plus, chez qui les fractures augmentent exponentiellement (par ex. les fractures de la hanche) dues à une destruction de l'os cortical.

Zurück

7 ans de thérapie avec le denosumab chez la femme souffrant d'ostéoporose postménopausique : fractures cliniques lors des 4 premières années d'extension de l'étude FREEDOM

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Objectif La diminution du risque de fractures vertébrales et non-vertébrales ainsi que de fractures de la hanche a été démontrée par le dénosumab (DMAb) durant les 3 années de l'étude FREEDOM. L'étude d'extension menée en mode ouvert examine les effets à long terme du DMAb jusqu'à 10 ans. Nous présentons ici l'incidence des fractures cliniques (non-vertébrales et vertébrales) au cours des 4 premières années d'extension qui correspondent à une exposition de 7 ans au DMAb.

Méthodologie Toutes les participantes à l'étude d'extension FREEDOM ont reçu 60 mg de DMAb tout les 6 mois ainsi que du calcium et de la vitamine D en complément. Pour la présente analyse, les femmes du groupe DMAb lors de l'étude FREEDOM ont reçu le DMAb durant 4 ans supplémentaires qui correspondent à une durée totale de traitement de 7 ans (groupe traité à long terme) ; et les femmes du groupe placébo lors de l'étude FREEDOM ont reçu le DMAb pendant une période totale de 4 ans (groupe traité de novo). La 7e année d'étude a analysé l'incidence des fractures non-vertébrales, des fractures vertébrales cliniques, le taux des marqueurs du remodelage osseux ainsi que les effets secondaires.

Résultats 77 % des femmes éligibles, soit 4550 sur 5928, ont été incluses dans l'extension de l'étude FREEDOM (n = 2343 long terme; n = 2207 de novo). L'incidence annuelle des fractures non-vertébrales est restée faible chez les patientes traitées à long terme : 1.4 %, 1.2 %, 1.6 % et 1.5 % respectivement durant les années 4 à 7 sous traitement avec DMAb, indiquant un effet maintenu du DMAb. Dans le groupe de patientes traitées de novo, cette incidence était consistante aux résultats du groupe DMAb lors de l'étude FREEDOM : 2.5 %, 1.9 %, 2.2 % et 1.0 % respectivement durant les années 1 à 4 sous traitement avec DMAb. Le taux de fractures vertébrales cliniques était également faible dans les 2 groupes. La survenue d'effets indésirables et d'effets secondaires graves normalisée à la durée d'exposition était similaire dans les 2 groupes. On a observé une ostéonécrose de la mâchoire chez 5 individus, dont 3 traités à long terme et 2 traités de novo. Deux fractures fémorales, une dans chaque groupe, correspondant à des fractures atypiques ont également été observées.

Conclusion Une thérapie avec le dénosumab durant 7 ans reste favorable quant au rapport bénéfices/risques. De plus, cette thérapie est associée à une faible incidence de fractures cliniques (non-vertébrales et vertébrales).

Zurück

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