

Safety and efficacy of denosumab in children with osteogenesis imperfecta - a first prospective trial

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Abstract

Objectives: Osteogenesis imperfecta (OI) is a rare hereditary disease leading to bone fragility. Denosumab as a RANK ligand antibody inhibiting osteoclast maturation has been approved for osteoporosis treatment in adults. Aim of this study was a 48-week, open-label, pilot study of the safety and efficacy of denosumab in 10 children with OI. **Methods:** Ten patients (age range: 5.0-11.0 years; at least two years of prior bisphosphonate treatment) with genetically confirmed OI were studied. Denosumab was administered subcutaneously every 12 weeks with 1 mg/kg body weight. Primary endpoint was change of areal bone mineral density (aBMD) using dual energy x-ray absorptiometry of the lumbar spine after 48 weeks. Safety was assessed by bone metabolism markers and adverse event reporting. **Results:** Mean relative change of lumbar aBMD was +19 % (95%-CI: 7-31%). Lumbar spine aBMD Z-Scores increased from -2.23 ± 2.03 (mean \pm SD) to -1.27 ± 2.37 ($p=0.0006$). Mobility did not change (GMFM-88 $+2.72 \pm 4.62\%$ ($p=0.16$); one-minute walking test $+11.00 \pm 15.82$ m ($p=0.15$). No severe side effects occurred. **Conclusions:** On average, there was a significant increase in lumbar spine aBMD percent change after 48 weeks of denosumab. There was no change in mobility parameters and no serious adverse events. Further trials are necessary to assess long-term side effects and efficacy.

Keywords: Denosumab, Osteogenesis Imperfecta, Bone Mineral Density, Mobility, Prospective Trial

Introduction

Osteogenesis imperfecta (OI) is a rare disease leading to fractures, skeletal deformities, scoliosis and a reduced bone mass due to an impaired production of collagen type 1 in most cases¹. Pathological fractures are the most severe symptom. More than 85% of patients are affected by mutations in *COL1A1* or *COL1A2* impairing quantity and quality of collagen². Although OI is caused by a dysfunction of osteoblasts, children have been treated with antiresorptive drugs (e.g. bisphosphonates) to reduce osteoclastic activity for more than 10 years³. For severely affected children, it has been shown that intravenous bisphosphonates increases bone mass, reduces fracture rates and increases mobility^{4,6}.

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One main concern regarding the use of bisphosphonates is the possibility of long term side effects. Recently, first results demonstrating an increased risk of pathologic femur neck fractures in woman treated with bisphosphonates have been published⁷. The major concern is the fact that bisphosphonates, once given, will bind to the bone for years. In 2010, denosumab as a fully human IgG2 antibody that binds to RANK ligand was approved to treat osteoporosis in postmenopausal women⁸. By inhibiting the interaction of RANK ligand to its receptor RANK, denosumab is a potent anti-resorptive agent, decreasing the differentiation of pre-osteoclasts and survival of osteoclasts, and therefore reducing bone resorption^{9,10}. The beneficial effect (reducing bone resorption) is comparable to a therapy with bisphosphonates in postmenopausal women, but the subcutaneous application is more convenient and the potential risk of long term side effects might be reduced due to the complete degradation of the antibody after a few months¹¹.

Denosumab is neither approved in OI nor in children. Rare case reports about applications in children with various skeletal diseases revealed severe side effects in some cases¹²⁻¹⁷.

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The aim of this phase-II-trial was to investigate safety and efficacy of osteoclast inhibition with denosumab in children with OI caused by mutations in *COL1A1* and *COL1A2*.

Materials and methods

Study participants were eligible if they fulfilled the following inclusion criteria: diagnosis of OI by mutations in *COL1A1* or *COL1A2*; male or female subjects between 5 years and 10 years of age; prior treatment with bisphosphonates for at least 2 years; willingness to discontinue bisphosphonate therapy for a wash out phase of 6 months. Exclusion criteria were: hypocalcemia (<1.03 mmol/l ionized Calcium); reduced renal function (estimated glomerular filtration rate (Schwartz formula) <30 ml/min/1.73m²; current treatment with other osteoanabolic or antiresorptive drugs.

The study was designed as a 48 weeks single-arm, open-label phase-2-trial with 10 participants lasting 48 weeks (drug administration 36 weeks, follow up 12 weeks). After screening and baseline examinations, participants subcutaneously received denosumab (Prolia®, Amgen Inc., Thousand Oaks, CA) 1mg per kg body weight four times in an interval of 12 weeks (+/-7days). The dose per injection was chosen based on protocols used for women with osteoporosis who receive a total of 60 mg per injection. Every patient received post injection (p.i.) weight adjusted oral calcium and vitamin D supplementation to ensure serum calcium levels in the normal range:

- <15 kg body weight (from 0 to 14 days p.i.): 2 x 250 mg/day Ca (from 15 to 28 days p.i.) 1 x 250 mg/day Ca and (from 0 to 28 days p.i.) 500 international units Vit D
- 15-30 kg body weight (from 0 to 14 days p.i.): 2 x 500 mg/day Ca, (from 15 to 28 days p.i.): 1 x 500 mg/day Ca and (from 0 days to 28 days p.i.) 500 international units Vit D
- >30 kg body weight (from 0 to 14 days p.i.): 2 x 1000 mg/day C- a, (from 15 to 28 days p.i.): 1 x 1000 mg /day Ca, and (from 0 to 28 days p.i.): 1000 international units Vit D

All patients continued their regular physiotherapeutic training according to national standards once or twice per week. Frequency and intensity of physiotherapy and occupational therapy has not changed during or shortly before entering the trial. Elective orthopedic surgery to correct long bone deformities was performed prior to start of treatment with denosumab. In two cases in which fractures occurred during the trial period Fassier-Duval telescopic rods have been replaced by similar rods of same length and diameter.

Primary objective was to investigate the relative change of areal bone mineral density (aBMD) of the lumbar spine (L2-L4) after treatment with denosumab at week 48 compared to baseline. aBMD, vertebral height and bone mineral content (BMC) were assessed using a GE Lunar iDXA densitometer (GE Ultraschall GmbH, Germany) and Encore software version 13.6. aBMD results were transformed to age-specific Z-scores using reference data provided by the manufacturer¹⁸. Quality checks are performed at least weekly based on the local authority requirements and revealed a precision variability of 0.23% between the phantom measurements. To reduce radiation dos-

age DXA scans were performed at baseline and week 48. An additional retrospective analysis in the context of our prospective trial was performed after end of the trial. Therefore patient's charts were evaluated and DXA results available within 10 to 13 months prior study entry in children assessed on the same GE Lunar iDXA densitometer (GE Ultraschall GmbH, Germany) and Encore software version 13.6.

Secondary objectives were changes of bone metabolism markers at each visit and between the visits. We used urinary deoxypyridinoline/creatinine ratio (DPD/crea) to monitor bone resorption measured with High-Performance-Liquid-Chromatography with age matched reference data. Additionally, serum n-telopeptides (NTX) were measured by enzyme immune assay and matched to age adapted reference data. Osteocalcin (Enzyme-immuno-Assay, reference ranges 10-100 ng/ml), Parathyroid hormone (Modular E-Modul, Roche Diagnostics, Germany, reference range 12-72 ng/l), 25-OH-Vitamin D (Modular E-Modul, Roche Diagnostics, Germany, reference range 30-70 µg/l) and total serum calcium (Modular P-Modul, Roche Diagnostics, Germany, reference range 2.2-2.7 mmol/l) were measured in the serum by our central laboratory.

Radiographs (Philips Optimus 65 Bucky Diagnostic TH and VT Philips Healthcare, The Netherlands) of the lumbar and thoracic spine were taken at baseline and week 48 in a lateral direction in a spine dedicated technique. Spine morphometry was evaluated according to Sumnik et al 2004 and based on the semi-quantitative score described by Koerber et al 2011. This numeric score include compression of vertebrae of the thoracic and lumbar spine separately, as well as the shape of deformities (e.g. fish-shape or wedge shape) in these regions and the kyphosis of the whole spine. The score was developed to quantify impairments of the vertebrae in a semiquantitative way allowing the detection of smaller changes of morphometry compared to other more generalized scores like the Genant grading¹⁹⁻²³. Especially for follow-up examinations, the underlying concept of the "Severity Classification" is extended to a much more detailed "Severity Score". This uses a larger range of numbers (1-138) describing the overall severity more detailed, allowing a further refined assessment of the actual status and occurring changes during treatment.

Mobility was evaluated using the gross motor function measurement (GMFM) at baseline, week 24 and week 48²⁴. The one minute and six minute walking distance was assessed by a standardized walking course on a flat floor.

Skeletal pain was evaluated at every visit by a visual analog "Wong-Baker-Scale" ranging from 1 to 10²⁵. Height and weight were measured at baseline, week 24 and week 48. Height was measured either using a stadiometer or lying on a bench for children not able to stand. All patients were measured with the same method throughout the trial. Body weight was measured using a sitting scale.

Peripheral fractures were assessed by telephone interviews and at every visit. Parents were instructed in case of sudden pain / trauma and suspected fracture to inform the study center directly and perform an x-ray at the local hospital to confirm or exclude a fracture. X-rays were transferred to our center to reassess the radiological findings.

For safety assessment laboratory examinations including repeated calcium level determination were performed. In addition to standard reporting of all adverse events, data were collected by telephone interviews between the visits.

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee (approval number: 12-283). Written informed consent was obtained from each subject’s parent(s) or legal guardian(s) and children before study-related procedures. The trial was registered on February 24, 2013 (NCT01799798) at the ClinicalTrials.gov Protocol Registration System (<http://clinicaltrials.gov>).

Statistics

A target sample size of n=10 was calculated based on our previous experiences with children receiving bisphosphonates.

All analyses were conducted using the full intention-to-treat set including all enrolled patients. Individual and mean changes over time in the various outcome variables were displayed graphically. The mean (absolute and relative) change in lumbar bone mineral density at 48 weeks and the mean change in aBMD Z-score were calculated with a 95% confidence interval and tested for significance using the paired t-test. Analogous methods were employed for secondary outcome variables as appropriate. Cumulative lists of adverse events and serious adverse events were presented descriptively. If a patient could not perform a mobility test based on a fracture, he was excluded a-priori from the analyses. Due to the small sample size no subgroup analysis of the gender groups was performed. P-values <0.05 were considered significant. Statistical analyses were conducted using SAS version 9.4.

Results

Ten children with a genetically confirmed OI (7 children with COL1A1 and 3 children with COL1A2 mutation) were included in the study between July 2013 and February 2014. The study cohort included 7 males and 3 females with a mean age (±SD) of 7.0 years (±2.12) with a caucasian ethnicity. A synopsis of baseline characteristics is given in Table 1.

All patients received denosumab four times as planned. 11 children were screened for participation. One patient was excluded before the first denosumab application based on deterioration of general clinical and psychological condition.

All participants completed the 48 weeks course of trial participation. Mean Height (±SD) increased from 105.0 cm (±20.2) to 108.9 cm (±21.2); p=0.002; (Z-scores -4.6±3.7 vs. -4.6±3.6; p=0.70) during study participation. Four patients sustained a fracture within the study (tibia after traumatic injury, femur in two subjects after traumatic injury, clavicle after a mild trauma; 4 fractures in 10 children within the trial period). Bone pain did not change during denosumab treatment (p=0.07).

Primary objective

Bone mineral density lumbar spine

All patients were included in the intention-to-treat analysis. Absolute aBMD increased from 0.507±0.187 g/cm² to 0.612±0.229 g/cm² (mean±SD; p<0.001) between baseline

| | |
|---|--------------------------------------|
| Participants n | 10 |
| Male n (%) | 7 (70) |
| Age Mean [years] (range) | 7.0 (5.0 – 11.0) |
| Height Mean [cm] (range) Z-Scores ± SD | 105.0 (66.0 – 134.0) -4.64 ± 3.72 |
| Weight Mean [kg] (range) | 19.3 (7.8 – 27.3) |
| BMI Mean [kg/m ²] (range) | 17.6 (13.1 – 33.0) |
| OI Type I/IV n (%) Able to walk (GMFM item 69) n (%) | 8 (80) 7 (70) |
| OI Type III n (%) Able to walk (GMFM item 69) n (%) | 2 (20) 0 (0) |
| Causative gene COL1A1 n (%) COL1A2 n (%) | 7 (70) 3 (30) |

Abbreviations: BMI (body mass index); GMFM (gross motor function measurement); COL1A1, COL1A2 (Collagen genes: Collagen 1A1, Collagen 1A2).

Table 1. Baseline characteristics of the study cohort.

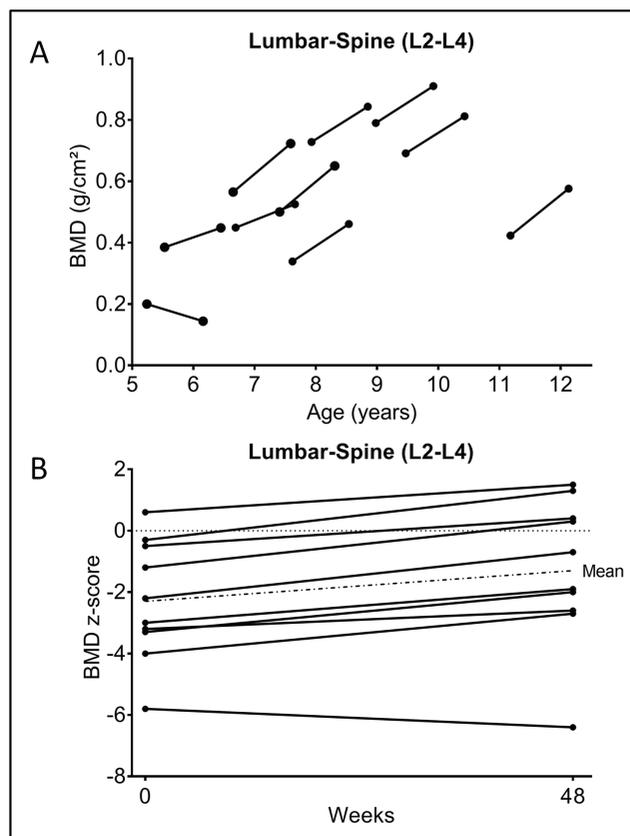


Figure 1. A. Presents the individual absolute lumbar spine areal bone mineral density values (L2-L4) plotted against age at trial entry (baseline) and week 48. **B.** Age-adjusted Z-scores and their changes are shown individually and as mean for 10 patients between baseline and week 48.

| Parameter | Number of patients | Baseline | Week 48 | Change baseline – week 48 [95% CI] | p-value |
|--|--------------------|--------------------|--------------------|---------------------------------------|---------|
| aBMD lumbar vertebrae L2-L4 (g/cm ²) | 10 | 0.5070 ± 0.1868 | 0.6118 ± 0.2294 | + 0.10 [0.06-0.15] | < 0.001 |
| aBMD lumbar vertebrae L2-L4 Z-score | 10 | -2.230 ± 2.0281 | -1.270 ± 2.366 | + 0.96 [0.597-1.323] | < 0.001 |
| Morphometry Spine Anterior-posterior index (1-ah/ph)*100 | 8 | -16.18 ± 41.46 | -4.475 ± 14.67 | - | 0.30 |
| Morphometry Spine Concavity index (1-mh/ah)*100 | 8 | 5.436 ± 25.7 | 7.322 ± 23.96 | - | 0.92 |
| Morphometry score x-ray spine (mean score) [range] | 10 | 24.7 [1 – 105] | 23.2 [1 – 109] | +1.5 | 0.63 |

aBMD = areal bone mineral density, L2 – L4 = Lumbar vertebrae 2 - 4, SD = standard deviation; CI = confidence interval.

Table 2. Changes of areal bone mineral density and spine morphometry between baseline and week 48 of the trial.

and week 48 (Table 2). Z-scores increased from -2.23 ± 2.03 (mean \pm SD) to -1.27 ± 2.37 ($p=0.0006$). Mean relative change of lumbar aBMD was + 19 % (95%-CI: 7-31%). Figure 1A presents individual aBMD data at baseline and week 48 plotted against age of patients. In one patient a decrease of aBMD was seen. In this patient the height Z-score did not change from -12.5 at study entry to -12.4 at study end. BMC values for lumbar spine (L2-L4) in that patient also decreased from 1.66 g at study start to 0.36 g at study end. Probably these values result out of a measurement error at the beginning of the trial.

Individual and mean age- adjusted Z-scores for aBMD of all patients are shown in Figure 1B. In an additional retrospective analysis lumbar aBMD data of eight children in the period of 10-13 months before trial entry were available. The increase of individual Z-scores was significantly higher under denosumab treatment compared to the earlier period under bisphosphonates (mean Z-score difference \pm SD before trial period = $+0.3125 \pm 0.512$ vs. $+1.15 \pm 0.316$ within the trial period; $p=0.016$; $n=8$; raw data shown in Figure 2).

Mean lumbar (L2-L4) vertebral height increased in the trial period significantly from 1.94 ± 0.35 cm to 2.0 ± 0.38 cm; $p=0.03$.

Secondary objectives

Changes of bone metabolism markers

DPD levels decreased within eight days after each application in all patients. Calcium levels decreased in parallel after each application in all children. DPD and Calcium levels are depicted exemplarily after the first application in Figure 3A/B. Over the entire treatment period, a downward drift of Osteocalcin and parathyroid hormone levels was detectable, whereas NTX and serum Calcium levels showed a tendency to rise. Mean levels are shown for visit 1-6 in Figure 3C/D/E/F. Vitamin D levels were analysed at every visit: In 14 out of 60 analyses a vitamin D insufficiency with a level between 10 and 20 $\mu\text{g/l}$ (25-50 nmol/l) was observed, in one analysis a deficiency with a level of 8.2 $\mu\text{g/l}$ (20.5 nmol/l) at start) was detected. A secondary hyperparathyroidism was not observed.

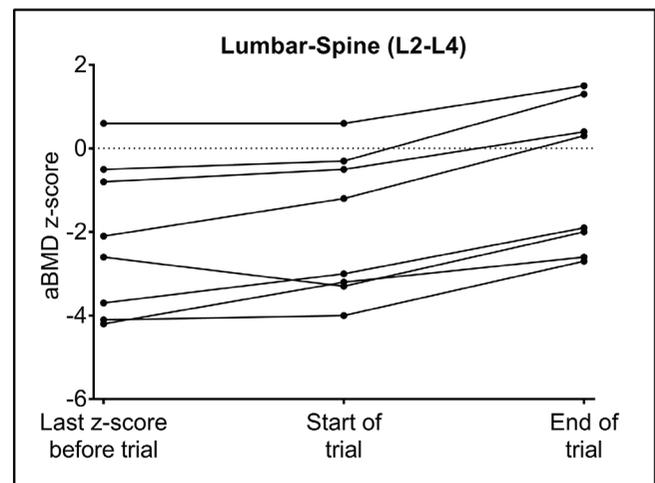


Figure 2. Presents individual courses of the lumbar spine areal bone mineral density Z-scores between last DXA evaluation before start of the trial, at start of the trial and end of the trial. The last available comparable Z-score before trial entry was retrospectively available in 8 out of 10 study participants (in the period of 10-13 months before trial entry).

Spine morphometry

Radiologic examinations of the spine revealed no new vertebral compression fractures. Evaluation of morphometry indices of the spine (L2/3/4) according to Sumnik et al did not reveal significant changes (anterior-posterior index $p=0.30$; concavity index $p=0.92$). Changes of the anterior-posterior index and concavity index are shown in Table 2. Using the “Koerber-score” the mean change of morphometry score was +1.5 points, $p=0.63$. In 6 out of 10 children no change of the score was observed (Table 2).

Mobility

Mobility changed not significantly in the trial cohort. One participant sustained a traumatic femur fracture before end of trial

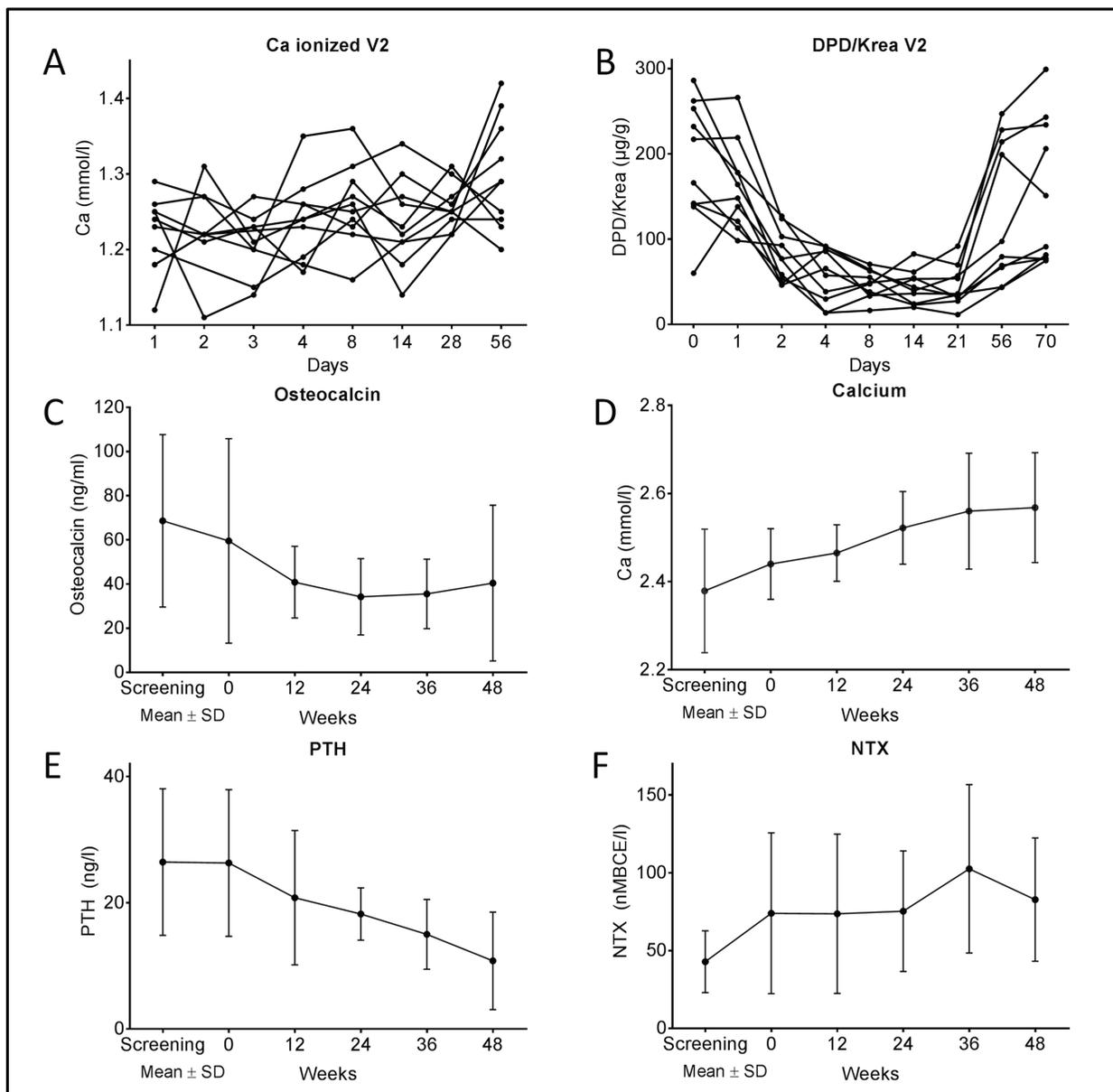


Figure 3. A/B. presents exemplarily the individual courses in ten patients of ionized serum calcium (Ca ionized) and urinary desoxypyridinoline/creatinine (DPD/Krea) levels after the first application of denosumab at trial week 0. Presented are all blood and spot urine controls from the day of application (day 0) to day 70 post first denosumab injection (one trial interval of 12 weeks). The next dosage was given at day 84 (not shown here). C-F. Mean levels \pm SD of the bone metabolism markers Osteocalcin (C), total serum calcium (D), parathyroid hormone (PTH), and serum N-Telopeptides (NTX) at the different visits (Screening, baseline, week 12, week 24, week 36, and week 48; whole trial intervals!) are demonstrated.

and therefore was not tested at week 48. Mobility results are presented in Table 3. Individual changes in mobility between baseline and week 24/48 are presented graphically in Figure 4 A/B.

A mean increase of motor function of 2.95% (GMFM-88 score $77.58 \pm 31.64\%$ to $80.30 \pm 31.06\%$; $p=0.16$) between baseline and week 48 was seen but was not significant. Two patients presented with a full score (100 percent) at start, thus no improvement was possible. A relative change of one-minute walking distance of +12.7% (absolute change from $86.6m \pm 26.83$ to $97.6 \pm 18.0m$;

$n=7$; $p=0.14$) was detected. Six-minute walking distance could be evaluated in 6 patients. An increase from $486.5m \pm 166.5$ to $535.2m \pm 159.8$; $p=0.06$ (mean change of 10.01%) could be seen between baseline and week 48. The changes of walking distances were not significant.

Safety

Subcutaneous application of denosumab was well tolerated. Children reported local pain while receiving the injections.

| Parameter | Number of patients | Baseline | Week 24 | Week 48 | Change baseline – week 48 [95% CI] | p-value |
|-----------------------------|--------------------|---------------|---------------|---------------|------------------------------------|---------|
| GMFM [%] | 9 | 77.58 ± 31.64 | 79.69 ± 31.33 | 80.3 ± 31.06 | 2.722 [-0.8253-6.27] | 0.16 |
| Walking 1 Min [m] | 7 | 86.57 ± 26.83 | 93.0 ± 15.28 | 97.57 ± 18.0 | 11 [-3.633-25.63] | 0.14 |
| Walking 6 Min [m] | 6 | 486.5 ± 166.5 | 530.2 ± 164.5 | 535.2 ± 159.8 | 48.7 [18.561-78.773] | 0.06 |
| Height Mean [cm] (SD) | 10 | 105.0 ± 20.2 | - | 108.9 ± 21.2 | 3.9 [2.98-4.82] | 0.002 |
| Height Mean [Z-Scores] (SD) | 10 | -4.64 ± 3.72 | - | -4.62 ± 3.58 | 0.024 [-0.3- 0.3483] | 0.70 |

GMFM = Gross motor function measurement, SD = standard deviation CI = confidence interval.

Table 3. Changes of mobility and height between baseline, week 24 and week 48 of the trial.

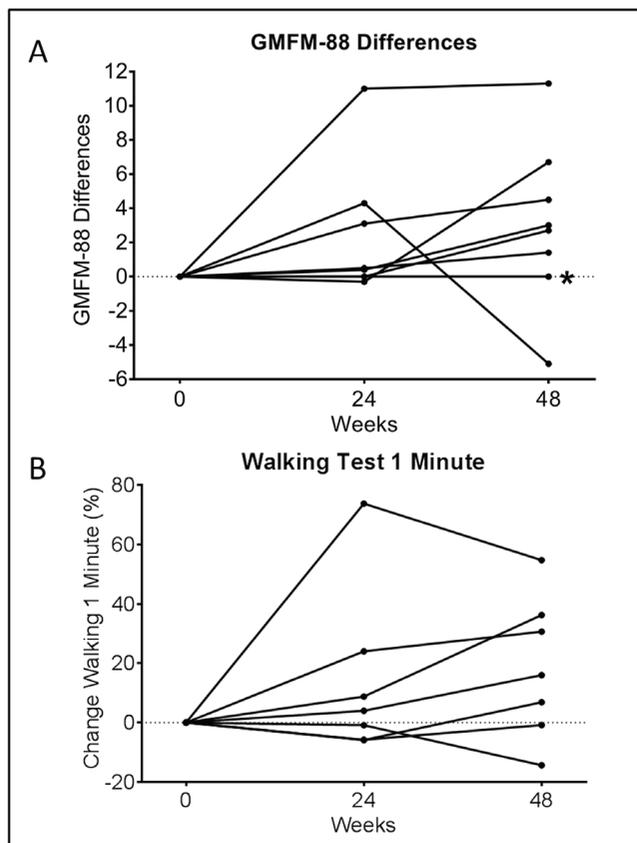


Figure 4. **A.** shows the individual absolute differences of 9 study participants in the GMFM-88 assessments at baseline, week 24 and 48. 2 children started with a maximum of 100% in the GMFM-88 and maintained their mobility levels until week 48. Therefore no changes are detectable in these 2 children (marked by the asterisk) lying on the dotted line which marks the line of no difference. **B.** The individual percent changes of walking distance are presented between week 0, week 24 and week 48 in the 7 children who were able to walk at trial entry.

There were no discontinuations of trial medication application due to adverse events. Two serious adverse events were reported based on planned hospitalization (elective rod surgery after two traumatic fractures of the femur). In summary, 76 adverse events were reported. 60 of these were declared as not related to denosumab representing common childhood illnesses. The 16 events assessed as possible related were: slight hypocalcaemia and general arthralgia (listed in Table 4). 60 adverse events revealed a Common Toxicity Criteria grade 1, 15 a Grade 2, and one a Grade 3 (flue with fever >38.5°C).

Two patients reported generalized joint pain after the second, third and last application of denosumab. Pain resolved within 14 days and was controlled by oral analgetic therapy. In one child a mild hypocalcemia was reported on day 14 after denosumab application and after an episode of vomiting and therefore skipping one dosage. But the patient did not present with a clinical relevant hypocalcemia and therefore did not need any additional substitution. The observed side effects possibly related to denosumab were those cited in the investigators brochure.

Discussion

The aim of this phase-2-study was to investigate safety and efficacy of denosumab in children with OI.

Denosumab on average significantly increases lumbar aBMD (absolute values and z-zcores) based on a suppression of bone resorption. In contrast to the current standard treatment approach with bisphosphonates, the increase of the lumbar aBMD was comparable, with a mean percent change of 18.4% compared to 15% in the third year of neridronate treatment trial, and 16.3% in the risedronate trial^{6,26}. Retrospective comparison of the available lumbar aBMD data before trial entry and within the trial period showed a significant higher increase of Z-scores under denosumab treatment underlining the efficacy of denosumab. Regarding the patient with a drop in Z-score there is no clear explanation. He is the youngest trial patient,

| Description of side effects | Common Toxicity Criteria grade | Relationship to denosumab | Frequency (n) |
|--------------------------------------|--------------------------------|---------------------------|---------------|
| Hypocalcemia | 1 | certain | 1 |
| Arthralgia | 1 | possible | 7 |
| Arthralgia | 2 | possible | 3 |
| Aphthous lesion soft palate | 1 | possible | 1 |
| Muscle pain | 1 | possible | 3 |
| Pain left thoracic side between ribs | 1 | possible | 1 |

Table 4. List of side effects possible attributed to the drug.

with a very severe form of OI, wheelchair dependent. Unfortunately we are not able to compare his data with data before trial entry because no DXA of the lumbar spine was performed before. His mobility increased in the trial period and radiographic morphometry evaluation of the spine did not reveal a deterioration of his lumbar vertebral shape (thoracic spine was not evaluated based on overlap of lung tissue). In previous trials in children with OI an increase of aBMD was always interpreted as a positive effect of the therapy. Up till now it is not clear what the ideal aBMD for children with OI is and if the bones will get sclerotic after long time antiresorptive therapy. Therefore an increase of aBMD above the normal range might not be beneficial for the patient. This is currently an unsolved problem in patients treated with bisphosphonates and it applies to all interventions with antiresorptive drugs in these children. Additionally in this population the short stature of the children has to be considered when using age matched reference data. Due to their short stature they might have already a sclerotic skeleton even if the Z-scores are still low. Based on the earlier bisphosphonate trials one would have expected beside an increase of aBMD that treatment with denosumab avoids new vertebral fractures. Data of morphometry and vertebral fracture assessment revealed that denosumab might have comparable effects on vertebral shape as bisphosphonates. No incidental fractures at the spine have been detected within the trial period.

The growth velocity comparable to healthy children (no change of Z-scores) should result also in an increase of vertebral height. Since a significant change of lumbar vertebrae (L2-L4) mean height was detected, increases of aBMD Z-scores were not artificially caused by crush-fracture-related decreases in vertebral-body size. The percent change of 3.21% seems to be comparable to the results from Gatti et al⁶ during the first year of neridronate treatment (increase of 2.64% in the first 12 months).

Additionally, radiographs showed evidence of new bone formation as published recently¹⁵.

It should be kept in mind that “efficacy” was assessed primarily in terms of densitometry changes. We could not assess significant changes regarding bone pain and peripheral fractures. It is known from prior trials e.g. with oral alendronate in OI that despite significant changes in aBMD, there was not a significant

effect on the important clinical endpoints of fracture and bone pain²⁷. These aspects should be evaluated in further trials.

Reports on denosumab application in OI are rare. Additionally, no information is available yet about application intervals and body weight adjusted dosing in children. Treatment intervals and dosing were chosen on the basis of our first experiences with denosumab in the rare OI type VI^{14,17}. Analysis of osteoclastic activity by urinary DPD levels repeatedly showed a prompt decrease of DPD excretion within 4-8 days after application. From one visit to the next, a slow increase of DPD was observed in all patients. This phenomenon is comparable to the pharmacokinetic data of the phase 1 and 2 trials performed in adult women with postmenopausal osteoporosis, in which an inverse relationship between serum denosumab levels and NTX levels was observed⁸. Therefore we concluded that the efficacy of denosumab is declining within the treatment intervals of 12 weeks, since we observed an increase of DPD between the application visits compared to the pre-drug levels. In previous bisphosphonate trials a continuous reduction of bone turnover markers has been described^{3,28}. To the best of our knowledge, an intermittent regain of osteoclast activity is essential to avoid a state of bone metabolism imitating osteopetrosis. The decrease of osteocalcin levels and increase of NTX levels over the whole trial period suggest that suppression of bone metabolism outweighs bone formation. However, growth velocity data, measurements of vertebral height and extremity x-rays taken during treatment revealed constant growth in our participants¹⁵. Therefore one might argue that bone formation was adequate in the trial period and no growth arrest appeared.

Mobility levels did not change in the study cohort. Several bisphosphonate-trials have failed to demonstrate differences in gross motor abilities or quality of life measures^{26,27,29,30}. Nevertheless, clinicians have the strong impression that bisphosphonate treatment results in pain reduction and subsequently in increased mobility and functionality. This anecdotal impression is comparable to our impression concerning denosumab treatment, but with the benefit of a more rapid and potent effect on aBMD increase of denosumab compared to bisphosphonates.

Safety: Our trial suggests that denosumab is well tolerated even in children. No deterioration of growth was seen. The most important concomitant effect of denosumab is alteration of the

calcium homeostasis (hypocalcemia after injection followed by a rebound hypercalcemia at the end of each treatment interval). A continuous oral body weight adjusted calcium and vitamin D substitution is mandatory to avoid severe hypocalcemia within the first 14 days after injection as it was reported in adults and children treated with denosumab³¹. A clinical significant hypercalcemia was recently reported after cessation of denosumab treatment in a boy with fibrous dysplasia and two children with giant cell tumor and interpreted as a rebound effect^{13,32,33}. At the end of our trial, only a slight hypercalcemia without clinical significance was observed in our patients. The previous bisphosphonate treatment may have attenuated this rebound effect in our patients.

In our patients no increase of skeletal problems as bone pain were reported by the patients and their parents. Earlier bisphosphonate treatment might have had controlled bone pain before trial entry. After this one-year period of application, the benefits seem to outweigh the risks of treatment if a frequent monitoring of calcium homeostasis is guaranteed. In the long term, denosumab might offer an advantage in various disorders with increased bone resorption because of its high efficacy, its complete degradation within 3-6 months compared to bisphosphonates which are stored in the bone persistently^{34,35}. Last but not least, the possibility of subcutaneous injections instead of an intravenous treatment could simplify the care and might lead to greater acceptance in children (whose venous status is very poor in some cases) and their parents.

Limitations: An important limitation of this trial is that only one-year data of ten children with OI are available. This study was designed as a phase II trial due to the rarity of OI and the novelty employing denosumab in children. The study was not powered to look at fractures and bone pain. The small sample size of this monocentric pilot trial severely limits the precision of estimates and the power to detect changes over time. Possible protective effect of 2 or more years of prior bisphosphonates in preventing new fractures and bone pain cannot be excluded. Even if retrospective intra-individual data show that there was a significant higher increase in lumbar spine aBMD percent change after 48 weeks of denosumab treatment compared to the earlier bisphosphonate treatment period, further randomized controlled trials are necessary to compare the presented effects with those of bisphosphonates. Recently, several case reports about the short-time use of denosumab in children with different conditions became available, but there are no long-term data on its use in children.

The patients have to be monitored for possible long-term effects in order to evaluate the risk-benefit ratio more precisely, as e.g. allergic reactions or antibodies targeting denosumab may require time to develop.

Most of these limitations are a consequence of the fact that evaluating efficacy and safety of a new drug in the field of pediatrics and for a rare disease requires a maximum of safety precautions to gain excellent data in accordance with the good clinical practice guidelines.

Conclusion

In summary, this first prospective clinical trial of denosumab application in OI children gives evidence:

- That on average denosumab leads to significant changes in areal bone mineral density at the lumbar spine,
- That children under denosumab treatment show an increase in absolute height,
- That denosumab appears to suppress bone resorption in children over 10-12 weeks,
- That denosumab seems to be safe in a one-year treatment course if a sufficient calcium and vitamin D substitution is provided.

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References

1. Rauch F, Glorieux FH. Osteogenesis imperfecta. *Lancet* 2004;363:1377-85.
2. Rauch F, Lalic L, Roughley P, Glorieux FH. Relationship between genotype and skeletal phenotype in children and adolescents with osteogenesis imperfecta. *J Bone Miner Res* 2010;25:1367-74.
3. Glorieux FH, Bishop NJ, Plotkin H, Chabot G, Lanoue G, Travers R. Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. *N Engl J Med* 1998;339:947-52.
4. Land C, Rauch F, Montpetit K, Ruck-Gibis J, Glorieux FH. Effect of intravenous pamidronate therapy on functional abilities and level of ambulation in children with osteogenesis imperfecta. *J Pediatr* 2006;148:456-60.
5. Phillipi CA, Remington T, RD S. Bisphosphonate therapy for osteogenesis imperfecta. *Cochrane Database Syst Rev* 2008;(4):CD005088, 2008.
6. Gatti D, Antoniazzi F, Prizzi R, et al. Intravenous neridronate in children with osteogenesis imperfecta: a randomized controlled study. *J Bone Miner Res* 2005;20:758-63.
7. Yoon RS, Hwang JS, Beebe KS. Long-term bisphosphonate usage and subtrochanteric insufficiency fractures: a cause for concern? *J Bone Joint Surg Br* 2011;93:1289-95.
8. McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med* 2006;354:821-31.
9. Lacey DL, Timms E, Tan HL, et al. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998;93:165-76.

10. Udagawa N, Takahashi N, Yasuda H, et al. Osteoprotegerin produced by osteoblasts is an important regulator in osteoclast development and function. *Endocrinology* 2000;141:3478-84.
11. Brown JP, Prince RL, Deal C, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: a randomized, blinded, phase 3 trial. *J Bone Miner Res* 2009;24:153-61.
12. Boyce A, Chong W, Yao J, et al. Denosumab treatment for fibrous dysplasia. *J Bone Miner Res* 2012;27:1462-70.
13. Grasemann C, Schundeln MM, Hovel M, et al. Effects of RANKL-ligand antibody (denosumab) treatment on bone turnover markers in a girl with juvenile Paget's disease. *J Clin Endocrinol Metab* 2013;98:3121-6.
14. Hoyer-Kuhn H, Netzer C, Koerber F, Schoenau E, Semler O. Two years experience with denosumab for children with Osteogenesis imperfecta type VI. *Orphanet J Rare Dis* 2014;9:145.
15. Hoyer-Kuhn H, Semler O, Schoenau E. Effect of denosumab on the growing skeleton in osteogenesis imperfecta. *J Clin Endocrinol Metab* 2014;99:3954-5.
16. Karras NA, Polgreen LE, Ogilvie C, Manivel JC, Skubitz KM, Lipsitz E. Denosumab treatment of metastatic giant-cell tumor of bone in a 10-year-old girl. *J Clin Oncol* 2013; 31:e200-2.
17. Semler O, Netzer C, Hoyer-Kuhn H, Becker J, Eysel P, Schoenau E. First use of the RANKL antibody denosumab in Osteogenesis Imperfecta Type VI. *J Musculoskelet Neuronal Interact* 2012;12:183-8.
18. Fan B, Shepherd JA, Levine MA, et al. National Health and Nutrition Examination Survey Whole-Body Dual-Energy X-Ray Absorptiometry Reference Data for GE Lunar Systems. *J Clin Densitom* 2013;17:344-77.
19. Sumnik Z, Land C, Rieger-Wettengl G, Korber F, Stabrey A, Schoenau E. Effect of pamidronate treatment on vertebral deformity in children with primary osteoporosis. A pilot study using radiographic morphometry. *Horm Res* 2004;61:137-42.
20. Koerber F, Schulze Uphoff U, Koerber S, Schonau E, Maintz D, Semler O. Introduction of a new standardized assessment score of spine morphology in osteogenesis imperfecta. *Rofo* 2012;184:719-25.
21. Koerber F, Semler O, Demant AW, Koerber S, Schonau E, Lackner KJ. [Standardized x-ray reports of the spine in osteogenesis imperfecta]. *Rofo* 2011;183:462-9.
22. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. *J Bone Miner Res* 1993;8:1137-48.
23. Crans GG, Genant HK, Krege JH. Prognostic utility of a semiquantitative spinal deformity index. *Bone* 2005; 37:175-9.
24. Ruck-Gibis J, Plotkin H, Hanley J, Wood-Dauphinee S. Reliability of the gross motor function measure for children with osteogenesis imperfecta. *Pediatr Phys Ther* 2001;13:10-7.
25. Wong DL, Hockenberry-Eaton M, Wilson D, Winkelstein ML, Ahmann E, DiVito-Thomas PA. *Whaley and Wong's Nursing Care of Infants and Children*. 6 ed. St. Louis: Copyrighted by Mosby; 1999.
26. Bishop N, Adami S, Ahmed SF, et al. Risedronate in children with osteogenesis imperfecta: a randomised, double-blind, placebo-controlled trial. *Lancet* 2013;382:1424-32.
27. Ward LM, Rauch F, Whyte MP, et al. Alendronate for the treatment of pediatric osteogenesis imperfecta: a randomized placebo-controlled study. *J Clin Endocrinol Metab* 2011;96:355-64.
28. Arikoski P, Silverwood B, Tillmann V, Bishop NJ. Intravenous pamidronate treatment in children with moderate to severe osteogenesis imperfecta: assessment of indices of dual-energy X-ray absorptiometry and bone metabolic markers during the first year of therapy. *Bone* 2004;34:539-46.
29. Bishop N, Harrison R, Ahmed F, et al. A Randomised Controlled Dose-Ranging Study of Risedronate in Children with Moderate and Severe Osteogenesis Imperfecta. *J Bone Miner Res* 2009;25:32-40.
30. Letocha AD, Cintas HL, Troendle JF, et al. Controlled trial of pamidronate in children with types III and IV osteogenesis imperfecta confirms vertebral gains but not short-term functional improvement. *J Bone Miner Res* 2005;20:977-86.
31. Kendler DL, Roux C, Benhamou CL, et al. Effects of denosumab on bone mineral density and bone turnover in postmenopausal women transitioning from alendronate therapy. *J Bone Miner Res* 2010;25:72-81.
32. Gossai N, Hilgers MV, Polgreen LE, Greengard EG. Critical hypercalcemia following discontinuation of denosumab therapy for metastatic giant cell tumor of bone. *Pediatr Blood Cancer* 2015;62:1078-80.
33. Setsu N, Kobayashi E, Asano N, et al. Severe hypercalcemia following denosumab treatment in a juvenile patient. *J Bone Miner Metab* 2015;[published ahead of print] DOI 10.1007/s00774-015-0677-z.
34. Bekker PJ, Holloway DL, Rasmussen AS, et al. A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. *J Bone Miner Res* 2004;19:1059-66.
35. Whyte MP, Wenkert D, Clements KL, McAlister WH, Mumm S. Bisphosphonate-induced osteopetrosis. *N Engl J Med* 2003;349:457-63.