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Interferon gamma-1b does not increase markers of bone resorption in autosomal dominant osteopetrosis

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Abstract

In autosomal dominant osteopetrosis type 2 (ADO2) *CLCN7* mutations cause impaired osteoclast function. Severe consequences include skeletal fragility despite high bone mass, osteomyelitis, osteonecrosis, bone marrow failure, and severe cranial nerve impingement. There is no effective medical treatment for ADO2.

We recruited subjects with ADO2 into a 14-week, open-label, pilot clinical trial of interferon gamma-1b. Doses were titrated based on tolerability and if fasting serum C-telopeptide (CTX) was <25% above baseline at week 8, targeting doses of 100 mcg/m^2 three times a week. The primary outcomes were change from baseline in CTX and N-telopeptide/creatinine ratio (NTX/Cr) at week 14. Secondary outcomes included changes in urine calcium/creatinine ratio, bone formation markers and tolerability.

Nine adults and 3 children were recruited. Severe manifestations of ADO2 included histories of fractures (100%), osteomyelitis (16.7%), vision loss (50%), and anemia (58.3%). Baseline CTX and NTX/Cr were generally low-normal. Procollagen type I N-terminal propeptide was elevated or in the upper-normal range in 11/12 (91.6%) subjects. Elevations of AST and LDH were common.

One subject withdrew due to rash. Five subjects achieved doses of 50 ug/m^2 three days a week, while 6 reached the full dose of 100 ug/m^2 three days a week. Only 3/11 (27.3%) completing subjects achieved the primary outcome of increasing CTX ≥25% above baseline at week 14. The mean change from baseline in CTX at week 14 was +2.2% (SD 43.2%, p=0.86). Likewise, there was no significant change in NTX/Cr (mean change −2.1%, p=0.81). Interferon gamma-1b was poorly tolerated. Most subjects had adverse events, and the Mental Health and Mental Component Scales of the SF-36v2 declined slightly (p<0.05).

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Over 14 weeks, interferon gamma-1b failed to significantly increase bone turnover markers in ADO2 and was poorly tolerated. Consequently, interferon gamma-1b is unlikely to be effective for decreasing bone mass in ADO2.

Keywords

osteopetrosis; clinical trials; osteoclasts; other therapeutics

Introduction:

Autosomal dominant osteopetrosis type 2 (ADO2), also known as Albers- Schönberg disease, is caused by heterozygous mutations in the *CLCN7* gene[1,2]. While most mutations are missense mutations, occasional mutations causing amino acid deletion, frameshifts, splicing defects or premature stop codons are described. *CLCN7* mutations result in decreased osteoclast function, but osteoclast generation remains normal, and high numbers of osteoclasts are seen on bone histology[3]. ADO2 has an incidence of about 5.5 per 100,000 persons. Despite the dominant inheritance pattern, the penetrance is only approximately 66%, suggesting other genetic or environmental modifiers[2,4].

ADO2 is sometimes referred to as a “benign” osteopetrosis to distinguish it from severe life-threatening (“malignant”) infantile recessive osteopetrosis. However, “benign” is an inappropriate designation, as ADO2 patients may still suffer severe and sometimes life-threatening complications of their disease[4]. These complications include fragility fractures, despite elevated bone mineral density, as well as bone marrow failure, severe vision loss or other cranial nerve impairment, osteonecrosis, and osteomyelitis. Patients also have high rates of complications after orthopedic surgeries[5].

In culture, osteoclasts from affected ADO2 patients resorb bone (or dentin) poorly compared to osteoclasts from healthy controls[6]. However, osteoclasts from non-penetrant carriers of *CLCN7* mutations demonstrate normal resorption. This suggests that an osteoclast specific defect determines whether a patient is affected or a carrier with mutations in this gene.

Efforts to treat osteopetrosis medically, have included attempts to increase osteoclast activity using very high doses of calcitriol[7–9] or interferon gamma-1b[10,11] to increase osteoclastic resorption. The response to calcitriol in case reports was limited, though some change in bone turnover markers were reported. In small studies, of mostly infantile recessive osteopetrosis, 18 months of treatment with interferon gamma-1b increased bone turnover markers, and decreased trabecular bone area and areal bone density[10,11]. Interferon gamma-1b is FDA approved for management of recessive osteopetrosis, typically being used as a bridge to bone marrow transplant. However the effectiveness of these agents in ADO2 has not been established.

Until recently, lack of a mouse model hindered attempts at developing therapies. We generated a “knock-in” mouse model of the G213R mutation in *CLCN7*, which is syntenic to the human G215R mutation identified in several ADO2 kindreds[2,3,12]. While homozygous mice have severe osteopetrosis and die by 4 weeks of age, the heterozygous

mice survive, but have high areal bone mineral density (BMD), with high bone volume/total volume (BV/TV) on microCT, and increased numbers of poorly resorbing osteoclasts on bone histology. This mouse model was used to test both calcitriol and interferon gamma-1b effect in ADO2[13]. Treating 6-week old ADO2 mice for 8 weeks with interferon gamma-1b increased the ratio of serum C-telopeptide (CTX) /Tartrate resistant acid phosphatase 5b (TRAP5b) (representing osteoclast function and number respectively) and decreased BV/TV, suggesting possible benefit for managing this disease[13].

We conducted a pilot clinical trial of interferon gamma-1b in humans with ADO2 to test the hypothesis that interferon gamma-1b can stimulate bone resorption markers and is tolerable in ADO2.

Methods

Design.

This was a single center, open-label, dose-escalation study evaluating the efficacy, as defined by biochemical markers of bone resorption, and safety profiles of interferon gamma-1b (ACTIMMUNE) in ADO2 subjects. The primary outcomes were defined as change in fasting serum C-telopeptide (CTX) and N-telopeptide/creatinine ratio (NTX/Cr) from baseline after 14 weeks of treatment. Secondary outcomes included changes in other biochemical markers and tolerability.

Subjects: Eligible subjects were patients age 3–65 years having clinical features of ADO2, including increased bone mass, and classic radiographic features (such as rugger jersey spine, “endo-bone” and Ehrlenmeyer flask deformities, etc.). Genetic confirmation was not required. Key exclusion criteria were serum calcium >10.6 mg/dL at initial screening; estimated glomerular filtration rate (eGFR) <35 mL/min/1.73m^2; nephrocalcinosis of Grade 3 or higher on screening ultrasound; history of hepatitis C, alcoholism, or intravenous drug abuse; presence of moderate or severe renal disease or liver disease at screening, or any unstable illness likely to influence successful completion or subject risk based on the discretion of the primary investigator. Subjects were recruited from 2016–2017.

Intervention: After screening, subjects entered a dose escalation protocol to minimize side effects and improve tolerance, starting with a low dose of interferon gamma-1b[14]. Subjects were taught self administration via subcutaneous injection, dosing three days out of each week with at least two days separating each dose (for example, Monday, Wednesday, Friday). From Day 0 to 6, subjects received 15 μg/m^2 three days a week; from Day 7 to 14, subjects received 30 μg/m^2 SC three days a week; from day 15–56 subjects received 50 μg/m^2 SC three days a week. Titration was then based on fasting serum CTX at day 56 (week 8). If CTX had increased by ≥25% above the mean of the screening and baseline visit values, the interferon gamma-1b dose remained at 50 μg/m^2 three days a week through week 14. If CTX was <25% above the mean of screening and baseline visit values, then the interferon gamma-1b dose was increased to 100 μg/m^2 three days a week through week 14. The target doses were based on the approved dose of interferon gamma-1b for severe recessive osteopetrosis and the animal studies[13]. Doses were decreased on a case by case

basis to manage subsequent drug-related adverse events. Study drug was stopped at the week 14 visit, and subjects attended a final followup visit 4 weeks after ceasing study drug.

Role of the sponsor: Interferon gamma-1b (ACTIMMUNE) and funding for the trial were provided by Horizon Pharmaceuticals. The study and analysis were conducted independently by the investigators.

Human subjects approvals.—This study was conducted in accordance with the Declaration of Helsinki and was approved by the Indiana University Institutional Review Board. Prior to enrollment, all subjects provided written consent after appropriate discussion of risks and benefits of participation. Children over age 7 provided written assent in accordance with local IRB procedures. The trial was listed on ClinicalTrials.gov identifier NCT02584608.

Assessments.—All laboratory specimens were collected in the fasting state for blood and urine. Primary efficacy and safety laboratories were measured at Covance Inc., Analytical Services Central Laboratory (Indianapolis, Indiana). Serum CTX was measured using the Beta-Cross-Laps assay (Elecsys electrochemiluminescence immunoassay, Roche Diagnostic). Additional assays included urine NTX ELISA (Osteomark, Ostex International Inc., Seattle, Wa); procollagen type I N-terminal propeptide (P1NP; Electrochemiluminescent immunoassay, Roche Diagnostic), Trap5b ELISA (Quidel, San Diego, Ca), 1,25-dihydroxyvitamin D was measured by LC-MS/MS (AB Sciex QTRAP 5500), and bone specific alkaline phosphatase (Ostase EIA; Immunodiagnosticsystems, Gaithersburg, MD). Biochemistries were measured using standard clinical analyzers (Roche Cobas 8000, Indianapolis, IN). Complete blood counts with differential were assessed on standard clinical platforms. Samples were analyzed sequentially as the samples were collected, since treatment decisions were determined by values at certain time points (specifically CTX, and safety laboratory tests). As a secondary quality check, at the end of the study, samples from each subject were analyzed concurrently on the same assay in the investigator's laboratory using an ELISA for serum CTX (Immunodiagnosticsystems Serum CrossLaps (CTX-I) ELISA, Gaithersburg, MD) and EIA for serum NTX ((Alere Scarborough, Inc. OSTEOMARK NTx Serum (EIA) Scarborough, ME). Presense of interfering anti-Interferon gamma-1b antibodies were assessed in serum using ELISA by Intertek (Manchester, UK). Bone density of the lumbar spine (L1-L4) was measured at baseline using dual energy x-ray absorptiometry (DXA) using the GE Lunar Prodigy Advance (Madison, WI, USA) and Z-scores were calculated using age and sex normative data. The planned 14 week duration of treatment was considered too short to be likely to detect changes in bone density; thus, serial densitometry was not performed.

Patient reported outcomes were collected using SF-36v2 at baseline and at week 14. The SF-36v2 includes 36 questions addressing 8 concepts for health related quality of life: Physical Functioning, Role Limitations due to Physical Health, Bodily Pain, General Health Perceptions, Vitality, Social Functioning, Role Limitations due to Emotional Problems, and Mental Health. Normative based standardized scores are calculated. Scores less than 50 are considered below the mean for the US population (mean is 50 and SD 10). Concepts are also grouped into a Physical Component Score and a Mental Component Score.

Statistics.—Descriptive statistics were calculated for all variables and reported as means (standard deviations) or median (minimum, maximum) values for continous variables and frequencies (percentages) for categorical variables. Percent changes in bone markers (CTX and NTX/Cr) from baseline were summarized as means and standard deviations and tested for significance using Student's t-test. Median change for CTX was also evaluated using Wilcoxon rank sum test. Longitudinal changes in measured variables were evaluated using a linear mixed effect model with time as a categorical variable. Additionally, the proportions of subjects obtaining changes in bone markers ≥25% from baseline were estimated with their 95% Agresti-Coull confidence intervals. CTX and NTX/Cr measurements during dose escalation, at peak dose, and at study end were analyzed by repeated measure analysis of variance to evaluate dose response and to determine whether bone resorption markers are stable once final dose therapy is achieved. Paired T-tests were used to compare the results of SF36-v2 domain results from baseline and week 14.

Power was calculated for a planned enrollment of 12 subjects in this pilot study, providing a power of 80% at a p value of 0.05 to detect change in serum CTX ≥25% from baseline with a standard deviation of 28.1% using Student's t-test. With the 11 subjects that completed the full protocol, the resulting power was 76%. Endpoints were assessed on all subjects tolerating at least 30 ug/m^2 three days a week. For all analyses, p-values < 0.05 were considered significant.

Results

Study population.

All 12 of the subjects screened were enrolled, and 11 subjects completed the full protocol. Baseline clinical characteristics are shown in Table 1. Three subjects were children (ages 8.1, 9.6 and 15.9 years). All subjects had classic radiographic characteristics consistent with the diagnosis of ADO2. Lumbar spine Z-scores, as measured by DXA, ranged from +4.7 to +15.5. Patients were mostly from families with confirmed mutations in CLCN7; 4 subjects were from families with G215R, and 6 subjects were from families with R286W. Genotype was not available for 2 adult subjects, one of whom had an affected daughter consistent with dominant inheritance, while the other subject had no affected relatives.

As a group, the enrolled subjects had severe manifestations of ADO2 including histories of fractures (100%), multiple fractures (91.7%), osteomyelitis (16.7%), vision loss attributable to ADO2 (50%), and anemia (58.3%). Two children had a history of intracranial decompression surgeries of the optic nerve canal to attempt to preserve vision. Three patients were legally blind in one or both eyes. One child and one adult were transfusion dependent due to anemia. Two subjects had fractured during the 2 years prior to baseline, and 2 subjects fractured during the trial. The clinical features and disease severity of the three children were similar to those of the adults. Patients were not taking calcitriol during the trial, though 4 (33.3%) subjects had previously attempted treatment with calcitriol, all > 6 years prior to enrollment.

Baseline laboratory values are shown in Table 2 (with reference ranges in Supplemental Table S1). All subjects had normal serum calcium, phosphorus and total alkaline

phosphatase for age. Blood urea nitrogen and serum creatinine were normal. Two subjects (16.7%) had elevated 1,25-dihydroxyvitamin D concentrations, including the one subject with elevated PTH. Urine calcium/creatinine ratio was <0.2 mg/mg in all subjects, and < 0.1 mg/mg in 10 (83%).

Baseline Bone turnover markers: CTX was normal for age in all subjects. Urine NTX/creatinine ratio was normal, except for one subject with a low value. However, most subjects were in the lower end of the age appropriate normal range for one or both tests. TRAP5b was elevated for age in all subjects. Total alkaline phosphatase was normal for age. Bone specific alkaline phosphatase was low for age in two pediatric subjects (ages 8.1 and 15.9 years), but normal in the rest of the subjects. However, for most subjects P1NP was either above normal (7/12 or 58.3%) or near the upper end of the normal range (4/12 or 33.3%). Consistent with the normal age appropriate differences in bone turnover markers, the children had higher bone formation and resorption markers than the adults.

Mild elevations of AST were present in 8 (75%) subjects, including all three children, but only one subject had mild elevation of ALT. The two youngest subjects (both boys) had slight elevations in GGT. LDH was above normal in 10/12 (83%) subjects. Total creatine kinase was elevated for age in 7/12 (58.3%) subjects.

Blood cell counts provided evidence indicating bone marrow impairment in studied subjects. Seven (58.3%) were anemic, including one who had received a recent transfusion prior to screening. Four (33.3%) subjects had low white blood cell counts, and 3 (25%) were neutropenic. Two (16.7%) subjects had platelet counts less than 100 $\times 10^3$/ uL. No subjects had immature neutrophils (bands) or elevated neutrophil counts detected at baseline. Hepatomegaly or splenomegaly was not noted on physical examination during the study in any patients.

Response to treatment.

At week 8, 4 subjects (33.3%) had increased serum CTx ≥25% from baseline, and continued on 50 ug/m^2 three days a week according to protocol. Due to tolerability of symptoms, an additional subject remained at 50 ug/m^2 instead of dose escalating at week 8. One subject discontinued the study drug after 6 weeks due to a rash, while taking 50 ug/m^2 three days a week. Thus, six subjects achieved the full dose of 100 ug/m^2 (three days a week), while 6 subjects only achieved a dose of 50 ug/m^2. No subject decreased their dose from the highest dose achieved.

Individual subject plots of serum CTX and urine NTX/creatinine ratio are shown in Figure 1. The percent changes in serum CTX, urine NTX/creatinine ratio and urine calcium/creatinine ratio are shown in Figure 2. At week 14, only 3/12 (25%) of the subjects achieved the primary outcome of CTX ≥25% above baseline, though 50% of subjects increased CTX ≥25% at some point in the study, without sustained increases. The mean change in serum CTX at week 14 was only +2.2% (SD 43.2%, p=0.86), while the median change was actually a decrease (−14.5%, p=0.85 by Wilcoxon rank sum test). Table 3 shows the percent change in serum CTX and urine NTX/creatinine ratio by study visit. Supplemental Table S2 shows the proportions of subjects at each time point with increases ≥25% bone resorption

marker changes from baseline. The 3 subjects that had CTX ≥25% above baseline at week 14 were adults, one with G215R, one with R286W and one whose genotype was not tested. Two were receiving 50 mcg/m^2, while one was receiving 100 mcg/m^2. There were not differences between these 3 subjects and the other subjects for assessed variables.

Similarly, the mean change in urine NTX/creatinine ratio at week 14 was not significant [−2.1% (SD 29.1%), p=0.81]. At week 14 only 17% had urine NTX/creatinine ratio ≥25% above baseline. Although 58% of subjects had an increase in urine NTX/creatinine ratio ≥25% above baseline at some point in the trial, there were not consistent increases. Trap5b and CTX/Trap5b ratio also did not change significantly during the course of treatment.

In a mixed effects model using time as a categorical variable, changes in bone resorption markers remained nonsignificant. There was not a significant dose or time relationship.There was considerable variability among subjects. Some subjects had increases in one resorption marker and decreases in the other. Notably the subject with the largest percent increase in serum CTX, also had a decrease in urine NTX/creatinine ratio.

In general similar proportions of subjects had decreases in serum CTX ≥25% (7/12, 58%) as had increases ≥25% (6/12, 50%) at some point during treatment. Likewise, similar proportions of subjects had decreases in urine NTX/creatinine ≥25% (5/12, 42%) as had increases ≥25% (7/12, 58%) at any point.

To ensure that a potential therapeutic effect on bone resorption markers was not missed by having samples run on separate days at the central laboratory, we also measured serum CTX and serum NTX on stored samples, batching all of an individual subject’s samples on the same assay kit. These assays confirmed no significant change from baseline in serum CTX (p=0.84) or serum NTX (p=0.65).

Serum calcium was unchanged during the study. Fasting urine calcium/creatinine ratio was assessed on therapy as a potential marker of calcium resorption from bone. There was no significant increase in urine calcium/creatinine ratio over time using mixed effect models (p=0.78). Urine calcium/creatinine ratio increased to 0.25 mg/mg in one subject at week 4 and another at week 14. There were no occurrences of symptomatic kidney stones during the trial. There were also not significant changes in other mineral metabolism parameters during the trial, including P1NP, BSAP, serum phosphorus, or PTH. Interfering antibodies to interferon gamma-1b were not detected in any subject.

SF36 results.

We assessed patient reported QOL using the SF36v2 short form (Table 4). Except for the Mental Health score, at baseline all subscale mean scores were below the US population mean, though all were within 1SD. The Physical Component Scale score was also below the US population mean, though the Mental Component Scale score was not. When assessed at week 14, the Mental Health Scale and Mental Component Scale declined significantly from baseline, but remained similar to US norms. All other scales did not significantly change during treatment.

Adverse events.

All subjects (100%) had one or more treatment emergent adverse events. Most adverse events were mild or moderate in severity. The most common treatment emergent adverse events were headache (83%), fatigue (75%), nausea (75%), flu-like symptoms (67%), myalgia (58%), decreased white blood cell count (50%), arthralgia (42%), diarrhea (42%), vomiting (42%), back pain (33%), injection site reaction (33%), and pain in extremities (33%). One subject discontinued the protocol after 6 weeks due to urticarial rash. The median (minimum, maximum) number of moderate or severe adverse events was 6 (1, 27) among patients that had decreases in mental health scores, and 5 (2, 8) among subjects that did not have decreases in mental health scores.

No subject developed an elevated neutrophil count. White blood cell count and absolute neutrophil count decreased during the trial with a nadir at week 11, but the decreases over time were not statistically significant. However two subject developed anemia (17%), five had decrease in neutrophil count (42%), and three had decreased platelet count (25%). Two subjects developed severely low neutrophil count (defined as $<1.0 \times 10^3$/ uL; one subject with 0.58×10^3/ uL and another with 0.83×10^3/ uL), which recovered. There was no evidence during the study of increased immature neutrophils. One subject had detectable bands of 0.02×10^3/ uL (1% of their total white blood cells) at 4 weeks, and another subject had 0.05×10^3/ul (2% of their total white blood cells) at 14 weeks, which were near the lower end of the normal range ($0 – 0.27 \times 10^3$/ul, or 0–3% of total white blood cells). No other subjects had measurable bands during the study. Three subjects had 2 infections each during the course of treatment. There were no serious adverse events.

Discussion

In this open label clinical trial, fourteen weeks of treatment with interferon gamma-1b, failed to significantly and consistently increase biochemical markers of bone resorption in patients with severe clinical manifestations of ADO2. There was no dose or time relationship to the pattern of bone resorption markers. To our knowledge this is the largest trial to evaluate the effects of interferon gamma-1b in a cohort of patients with ADO2.

Interferon gamma-1b was used to increase bone resorption in small trials of patients with osteopetrosis[10,11]. Although not genetically defined, most of the subjects in those trials were young children and likely had recessive forms of osteopetrosis, though two adults were included that may have had dominant osteopetrosis[10,11]. The authors reported increases in urinary hydroxyproline, NTX and urine calcium by 6 and 18 months of therapy. On bone biopsy, they found a corresponding decrease in trabecular bone area, despite no change in osteoclast number. Six of those patients had bone mineral density testing which indicated decreases in lumbar spine BMD[11]. Inteferon gamma-1b is FDA approved for management of recessive osteopetrosis, often being used as a bridge to bone marrow transplant[11].

Our group recently generated a mouse model of ADO2 due to mutations in *CLCN7*, which enabled testing of therapies for ADO2. After 8 weeks of low (1.5 ug/kg three times a week), medium (7.5 ug/kg three times a week), or high (37.5 ug/kg three times a week), doses of Interferon gamma-1b young (6 week old) ADO2 mice demonstrated improvements in

osteoclast resorption markers, and in BV/TV[13]. Areal BMD and BMC measured by DXA were attenuated and micro-CT measurements indicated reduced BV/TV and trabecular thickness. The low doses used in this animal study were still somewhat higher than doses tolerated by humans, while the medium and high doses were several times higher than that used in humans. Based on the skeletal improvements even at the lower dose level mice, we anticipated positive results in this human trial.

In the current pilot trial patients with ADO2, all had severe manifestations of their disease, including evidence of bone marrow impairments, or frank marrow failure. The reason for lack of increased resorption markers could include reduced sensitivity of osteoclasts to the effect of interferon gamma-1b compared to mice, different genotypes compared to the prior study of mostly recessive osteopetrosis, or insufficient duration of treatment. Prior studies in primarily recessive osteopetrosis did not report bone turnover markers at earlier time points than 6 or 18 months for comparison[10,11]. Administering interferon gamma-1b to human granulocyte-macrophage cultures from osteopetrotic patients results in measurable differences in cell activity reported from interferon gamma-1b within 5 days[15]. The assumption in the design of this trial was that effects on osteoclastic bone resorption would be detectable using bone resorption markers before changes in bone density would be measurable.

Perhaps the doses acheivable were not high enough to have similar effect in humans as in the mouse model. However, our doses were consistent with doses used in other human diseases, and were at the limit of general tolerability regarding side effects. Most patients had significant drug-related symptoms repeatedly with dosing. We used a higher maximum dose (100 mcg/m^2) than those earlier osteopetrosis trials (50 mcg/m^2) and measured multiple bone turnover markers, but still did not demonstrate increased bone resorption. Another possibility is that we did not identify the most optimal biomarker for early effect of interferon gamma-1b. However, osteoclastic activity would be necessary to increase bone resorption, and hopefully improve bone quality as well as increase marrow space. Thus resorptive markers would be expected to increase if having an effect to decrease bone mass. Of note, we also saw no significant changes in any measured bone formation marker. Notably, in a case report of severe recessive osteoporosis, interferon gamma-1b treatment for 17 weeks also failed to increase urine calcium excretion or urine hydroxyproline, leading to discontinuation[8].

This study highlights some additional features of ADO2. We confirmed prior report of elevations in Trap5b and creatine kinase[16], and also in AST and LDH among ADO2 patients[17]. We also noted general baseline elevations in the bone formation marker P1NP, which were not previously described. Notably elevated P1NP was not recapitulated in the mouse model. Serum calcium, phosphorus, alkaline phosphatase and P1NP concentrations were similar in the WT and ADO2 mice bred to several different mouse strains, though TRAP was higher and CTX/TRAP ratio was lower in the ADO2 mice[12]. Similar or higher P1NP levels in the setting of impaired osteoclast activity, suggests an uncoupling of bone formation and resorption, allowing the continued production of bone, even while failing to resorb and remodel appropriately. This may explain a lack of apparent growth impairment, and the normal sized, though abnormally shaped, bones in ADO2. One possible explanation

is that osteoblasts may still receive various signals in support of bone formation from the excessive numbers of poorly resorbing osteoclasts present in patients with CLCN7 mutations. Similarly other models of impaired osteclast activity with preserved osteoclast numbers such as Cathepsin K knockout or inhibition, also demonstrate preservation of bone formation[18,19].

We also noted 2 subjects with elevated 1,25-dihyroxyvitamin D level. This has been previously reported in various osteopetrotic models[20,21], and in some osteopetrotic children. 1,25-dihydroxyvitamin D stimulates osteoclast activity, and increases osteoclast number in some recessive osteopetrotic models, though some studies have also suggested resistance of osteopetrotic osteoclasts to 1,25-dihydroxyvitamin D[21,22]. The use of calcitriol (1,25-dihyroxyvitamin D) has been attempted in humans with osteopetrosis, but published data of successful management is limited in recessive osteopetrosis, and frankly lacking in ADO2. In fact, ADO2 mice treated with high dose calcitriol actually increased areal BMD and BV/TV[13], suggesting that this approach is likely to harm patients skeletally, in additions to the risks of associated hypercalciuria. Because of these mouse findings, we have abandoned the practice of treating ADO2 patients with calcitriol.

We also highlight the severe nature of ADO2, which has erroneously been refered to in the past as a “benign” disorder. Most of our patients had evidence of marrow failure and fractures, and half had various degrees of vision impairment related to ADO2.

This is the first study to our knowledge to assess patient reported quality of life outcomes in ADO2. Although subjects had quality of life scores on the SF-36v2 within 1SD of the US population mean, the Mental Health Scale and Mental Component Scales decreased significantly during the course of treatment. Declines in mental health quality of life may be due to the symptoms caused by administering interferon gamma-1b, or their perception of their overall disease burden. Patients having decreases in the mental health scores from baseline generally had more adverse events that were moderate or severe than those without decreases in mental health scores.

Strengths of our study include robust attempts to confirm effects on bone resorption biomarkers, which are generally expected to reflect substantial changes in bone turnover, even though smaller changes might not be detected. Despite significant symptoms, most patients completed the protocol, emphasizing the motivation of these patients to find a better treatment. Limitations include that due to the short duration of the study, indices of bone biopsy, or bone density were not assessed. Had a bone resorption signal been identified, we would have extended the trial to a duration sufficient to assess bone density changes. While our study could have missed a longer term effect on osteoclast resorption, given the poor tolerability and frequent systemic symptoms induced by interferon gamma, a longer treatment course was not considered warranted, without evidence for short term alteration in bone resorption markers.

Conclusion:

Interferon gamma-1b failed to significantly increase markers of bone resorption in this 14 week trial in ADO2. Thus a long term effect on bone mass is not anticipated. Given the significant side effects with low tolerability experienced by the subjects, and the current lack of any preliminary indicator of benefit, treatment with interferon gamma-1b should not be pursued in ADO2. There is a need for novel, effective therapies for this condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Cleiren E, Benichou O, Van Hul E, Gram J, Bollerslev J, Singer FR, et al. Albers-Schonberg disease (autosomal dominant osteopetrosis, type II) results from mutations in the ClCN7 chloride channel gene. Hum Mol Genet. 12 01 2001;10(25):2861–7. [PubMed: 11741829]
2. Waguespack SG, Koller DL, White KE, Fishburn T, Carn G, Buckwalter KA, et al. Chloride channel 7 (ClCN7) gene mutations and autosomal dominant osteopetrosis, type II. J Bone Miner Res. 8 2003;18(8):1513–8. [PubMed: 12929941]
3. Alam I, Gray AK, Chu K, Ichikawa S, Mohammad KS, Capannolo M, et al. Generation of the first autosomal dominant osteopetrosis type II (ADO2) disease models. Bone. 2 2014;59:66–75. [PubMed: 24185277]
4. Waguespack SG, Hui SL, Dimeglio LA, Econs MJ. Autosomal dominant osteopetrosis: clinical severity and natural history of 94 subjects with a chloride channel 7 gene mutation. J Clin Endocrinol Metab. 3 2007;92(3):771–8. [PubMed: 17164308]
5. Benichou OD, Laredo JD, de Vernejoul MC. Type II autosomal dominant osteopetrosis (Albers-Schonberg disease): clinical and radiological manifestations in 42 patients. Bone. 1 2000;26(1):87–93. [PubMed: 10617161]
6. Chu K, Snyder R, Econs MJ. Disease status in autosomal dominant osteopetrosis type 2 is determined by osteoclastic properties. J Bone Miner Res. 7 2006;21(7):1089–97. [PubMed: 16813529]
7. Key L, Carnes D, Cole S, Holtrop M, Bar-Shavit Z, Shapiro F, et al. Treatment of congenital osteopetrosis with high-dose calcitriol. N Engl J Med. 2 16 1984;310(7):409–15. [PubMed: 6546410]
8. Kubo T, Tanaka H, Ono H, Moriwake T, Kanzaki S, Seino Y. Malignant osteopetrosis treated with high doses of 1 alpha-hydroxyvitamin D3 and interferon gamma. J Pediatr. 8 1993;123(2):264–8. [PubMed: 8345424]
9. van Lie Peters EM, Aronson DC, Everts V, Dooren LJ. Failure of calcitriol treatment in a patient with malignant osteopetrosis. Eur J Pediatr. 10 1993;152(10):818–21. [PubMed: 8223784]
10. Key LL, Jr., Ries WL, Rodriguiz RM, Hatcher HC. Recombinant human interferon gamma therapy for osteopetrosis. J Pediatr. 7 1992;121(1):119–24. [PubMed: 1320672]
11. Key LL Jr., Rodriguiz RM, Willi SM, Wright NM, Hatcher HC, Eyre DR, et al. Long-term treatment of osteopetrosis with recombinant human interferon gamma. N Engl J Med. 6 15 1995;332(24):1594–9. [PubMed: 7753137]

12. Alam I, McQueen AK, Acton D, Reilly AM, Gerard-O'Riley RL, Oakes DK, et al. Phenotypic severity of autosomal dominant osteopetrosis type II (ADO2) mice on different genetic backgrounds recapitulates the features of human disease. Bone. 1 2017;94:34–41. [PubMed: 27746321]
13. Alam I, Gray AK, Acton D, Gerard-O'Riley RL, Reilly AM, Econs MJ. Interferon Gamma, but not Calcitriol Improves the Osteopetrotic Phenotypes in ADO2 Mice. J Bone Miner Res. 11 2015;30(11):2005–13. [PubMed: 25943708]
14. Devane JG, Martin ML, Matson MA. A short 2 week dose titration regimen reduces the severity of flu-like symptoms with initial interferon gamma-1b treatment. Curr Med Res Opin. 6 2014;30(6): 1179–87. [PubMed: 24576196]
15. Key LL Jr., Ries WL. Osteopetrosis. The pharmaco-physiologic basis of therapy. Clin Orthop Relat Res. 9 1993(294):85–9.
16. Waguespack SG, Hui SL, White KE, Buckwalter KA, Econs MJ. Measurement of tartrate-resistant acid phosphatase and the brain isoenzyme of creatine kinase accurately diagnoses type II autosomal dominant osteopetrosis but does not identify gene carriers. J Clin Endocrinol Metab. 5 2002;87(5):2212–7. [PubMed: 11994366]
17. Whyte MP, Kempa LG, McAlister WH, Zhang F, Mumm S, Wenkert D. Elevated serum lactate dehydrogenase isoenzymes and aspartate transaminase distinguish Albers-Schonberg disease (Chloride Channel 7 Deficiency Osteopetrosis) among the sclerosing bone disorders. J Bone Miner Res. 11 2010;25(11):2515–26. [PubMed: 20499337]
18. Pennypacker B, Shea M, Liu Q, Masarachia P, Saftig P, Rodan S, et al. Bone density, strength, and formation in adult cathepsin K (−/−) mice. Bone. 2 2009;44(2):199–207. [PubMed: 18845279]
19. Duong LT, Pickarski M, Cusick T, Chen CM, Zhuo Y, Scott K, et al. Effects of long term treatment with high doses of odanacatib on bone mass, bone strength, and remodeling/modeling in newly ovariectomized monkeys. Bone. 7 2016;88:113–24. [PubMed: 27126999]
20. Zerwekh JE, Marks SC Jr., McGuire JL. Elevated serum 1,25-dihydroxyvitamin D in osteopetrotic mutations in three species. Bone Miner. 5 1987;2(3):193–9. [PubMed: 3504730]
21. Safadi FF, Hermey DC, Popoff SN, Seifert MF. Skeletal resistance to 1,25-dihydroxyvitamin D3 in osteopetrotic rats. Endocrine. 12 1999;11(3):309–19. [PubMed: 10786828]
22. Popoff SN, McGuire JL, Zerwekh JE, Marks SC, Jr. Treatment of congenital osteopetrosis in the rabbit with high-dose 1,25-dihydroxyvitamin D. J Bone Miner Res. 2 1989;4(1):57–67. [PubMed: 2718779]

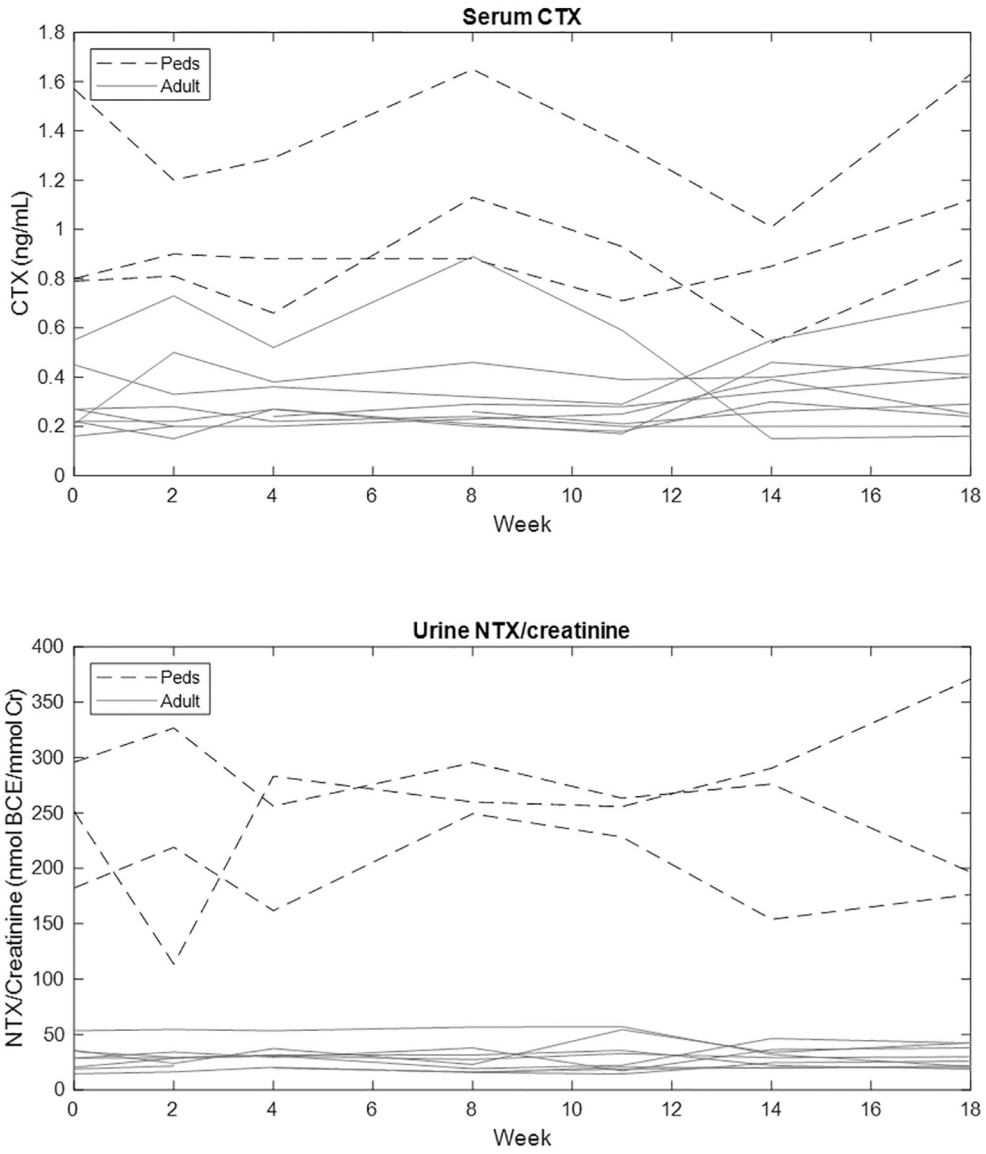

Figure 1.

Bone resorption markers during treatment with Interferon gamma-1b: serum CTX and urine NTX/creatinine ratio. Children are marked in dashed black lines and adults in solid gray lines.

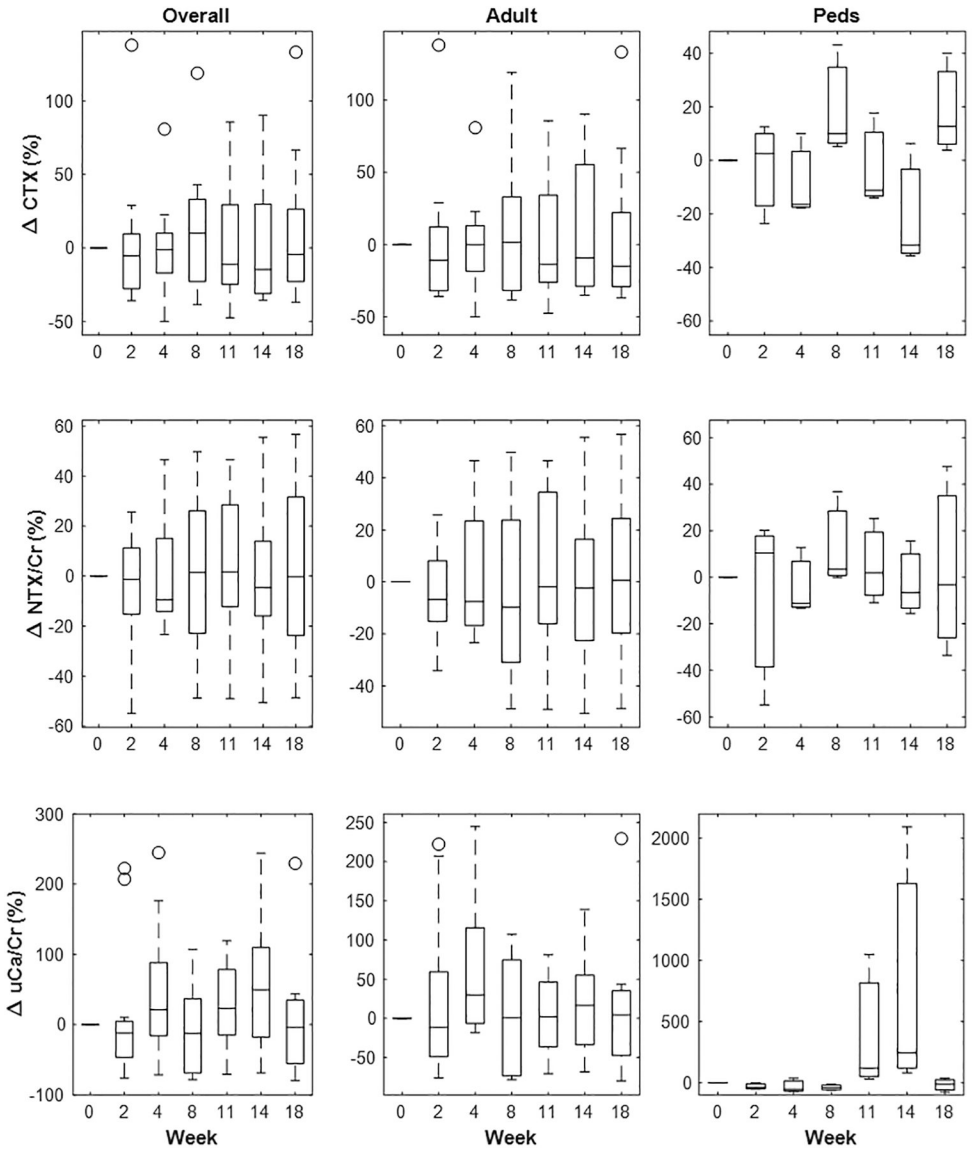

Figure 2.

Percentage changes from baseline in serum CTX, urine NTX/creatinine ratio and fasting urine calcium/creatinine ratio. Some data of percent change in urine Ca/Cr for pediatric subject 007 were deleted from the overall plot due to extreme outliers (from a very low baseline value 0.011mg/mg, he had percent increases of +1049.26 at week11 and +2093.91 at week14, but his highest Urine Ca/Cr was only 0.24 mg/mg). However there were no occurrences of hypercalciuria.

Table 1.

Baseline clinical characteristics

	Full cohort n= 12
Subject number	
Age, years	27.4 (8.1, 50.8)
Race	11W, 1 B
Female/Male	6/6
Fracture history	
Any fracture	12 (100%)
Multiple fractures	11 (91.7%)
Osteomyelitis history	2(16.7%)
Vision loss	6 (50%)[a]
Anemia	7 (58.3%)
Transfusion history	2 (16.6%)[b]
Lumbar spine (L1–4) Z-score	+11.9 (+4.7, +15.5)

[a]Two children had history of optic nerve decompression surgery, Three subjects were legally blind in one or both eyes;

[b]Two subjects required recurrent transfusions due to bone marrow failure. Summary values are given as n (%) or as median (minimum, maximum). W white, B black

Table 2.

Baseline Laboratory values

	Child						Adult							
	Mean	**Std Dev**	**Median**	**Min**	**Max**		**Mean**	**Std Dev**	**Median**	**Min**	**Max**		**Below normal**	**Above normal**
Calcium (mg/dL)	9.5	0.5	9.5	9.0	10.0		9.3	0.3	9.4	8.7	9.6		0	0
Phosphorus (mg/dL)	5.3	0.2	5.4	5.0	5.4		4.1	0.4	4.1	3.5	4.7		0	0
Magnesium (mg/dL)	2.2	0.1	2.3	2.1	2.3		2.2	0.2	2.2	1.9	2.5		0	2
Serum Bicarbonate (mEq/L)	20.4	1.5	20.2	19.1	22.0		19.9	2.5	20.2	15.3	24.4		1	0
Urea Nitrogen (mg/dL)	13.0	1.7	14.0	11.0	14.0		14.3	3.9	16.0	8.0	19.0		0	0
Serum Creatinine (mg/dl)	0.4	0.0	0.4	0.4	0.4		0.7	0.2	0.7	0.4	1.0		0	0
1,25-dihydroxyvitamin D (pg/mL)	85.0	13.8	82.0	72.9	100.1		70.1	38.2	54.9	38.7	144.4		0	2
iPTH (pg/mL)	77.5	60.8	52.3	33.4	146.9		43.7	21.0	47.6	11.8	75.2		1	2
Beta CrossLaps-CTX (ng/mL)	1.1	0.4	0.8	0.8	1.6		0.3	0.1	0.3	0.2	0.6		0	0
NTX/Creatinine nmol BCE/mmol Cr	243.1	57.3	251.2	182.2	296.0		29.4	9.9	32.0	14.5	46.4		1	0
TRAP 5b (units/liter)	162.7	8.5	164.0	153.6	170.4		97.4	37.7	96.1	47.3	151.0		0	12
Urine Calcium/Creatinine (mg/mg)	0.0	0.0	0.0	0.0	0.0		0.1	0.0	0.1	0.0	0.2		0	0
Alkaline Phosphatase (U/L)	141.3	18.6	139.0	124.0	161.0		61.1	16.3	56.0	37.0	88.0		0	0
Bone Specific Alkaline Phosphatase (ug/L)	30.0	10.5	26.5	21.7	41.8		11.1	3.6	10.3	7.4	18.6		2	0
P1NP (ng/mL)	926.6	295.2	1029.0	593.9	1157.0		88.5	49.7	83.0	25.6	205.5		0	7
ALT (SGPT) (U/L)	14.3	0.6	14.0	14.0	15.0		21.9	10.6	23.0	9.0	44.0		0	1
AST (SGOT) (U/L)	91.7	32.5	76.0	70.0	129.0		35.1	13.3	37.0	16.0	49.0		0	8
LDH (U/L)	775.3	152.2	693.0	682.0	951.0		289.1	74.0	298.0	187.0	380.0		0	10
Albumin (g/dL)	4.3	0.5	4.4	3.8	4.7		4.3	0.3	4.5	3.7	4.7		0	0
GGT (U/L)	30.3	6.8	28.0	25.0	38.0		17.4	4.6	17.0	11.0	25.0		0	2
Creatine Kinase (U/L)	538.7	39.0	540.0	499.0	577.0		276.9	176.6	248.0	87.0	684.0		0	7
WBC (x 10^3/uL)	4.6	1.7	4.6	2.8	6.3		4.8	1.3	5.4	3.2	6.4		4	0
Neutrophils (x 10^3/uL)	2.2	1.3	2.0	1.0	3.6		2.9	1.0	3.2	1.6	4.5		3	0
Hemoglobin (g/dL)[a]	10.9	0.7	11.1	10.1	11.4		12.0	1.9	12.9	8.7	13.9		7	0
Platelets (x 10^3/uL)	219.7	131.7	262.0	72.0	325.0		192.2	60.4	179.0	76.0	272.0		2	0

Values below normal or above normal are summarized for the total group using age- and sex-appropriate normal ranges from the reference laboratory (See supplemental Table 1).

a One child had transfusion within the 2 weeks before visit.

Table 3.

Change in bone resorption markers.

	Week	N	Mean	SD	Median	Min	Max	P value
Percent change CTX (%)								0.8611
	0	12	0.0	0.0	0.0	0.0	0.0	
	2	12	2.9	47.2	−5.4	−35.9	138.1	
	4	12	−0.8	33.6	−1.1	−50.0	81.0	
	8	11	13.2	44.8	10.0	−38.5	119.0	
	11	11	1.8	38.0	−11.3	−47.5	85.7	
	14	12	2.2	43.2	−14.5	−35.7	90.5	
	18	12	10.0	49.4	−4.5	−37.0	133.3	
Percent change NTX/creatinine (%)								0.8081
	0	12	0.0	0.0	0.0	0.0	0.0	
	2	12	−4.6	23.1	−1.3	−54.8	25.7	
	4	12	1.6	23.6	−9.4	−23.3	46.5	
	8	11	0.6	31.4	1.5	−48.8	49.8	
	11	11	4.2	28.5	1.7	−49.0	46.6	
	14	12	−2.1	29.1	−4.5	−50.5	55.5	
	18	12	3.2	34.3	−0.2	−48.6	56.7	

Table 4.

Summary of normalized SF36 Scales and comparison between baseline and week 14. US norms have mean 50, SD 10. P values are for change from baseline; * p< 0.05.

Scale	Week	Mean	SD	Median	Min	Max	25th percentile	75th Percentile	p-value from Paired t-test
Physical Functioning Scale	**0**	43.3	12.9	41.8	23.8	57.7	33.8	56.7	0.6912
	14	41.8	14.3	37.8	19.8	57.7	30.8	57.7	
Role Physical Scale	**0**	45.6	12.6	52.5	26.9	57.1	32.0	57.1	0.3802
	14	42.4	13.4	44.4	22.3	57.1	31.6	54.8	
Bodily Pain Scale	**0**	40.7	8.2	40.1	25.3	55.7	34.3	46.7	0.7334
	14	41.4	12.1	38.0	21.2	62.3	32.1	51.2	
General Health Scale	**0**	45.4	12.5	48.6	26.5	62.6	32.4	56.8	0.1126
	14	43.6	13.6	43.9	26.5	62.6	30.1	56.8	
Vitality Scale	**0**	47.8	9.8	48.8	33.7	70.0	39.7	51.8	0.2742
	14	44.2	14.8	41.2	27.6	70.0	30.6	53.3	
Social Functioning Scale	**0**	47.6	12.8	54.2	19.9	56.9	38.4	56.9	0.0584
	14	41.0	14.9	46.3	14.6	56.9	27.8	54.2	
Role Emotional Scale	**0**	49.6	10.0	56.1	33.9	56.1	39.4	56.1	0.1123
	14	44.4	15.0	54.3	11.7	56.1	33.9	56.1	
Mental Health Scale	**0**	52.6	9.8	55.3	36.3	63.5	45.8	60.8	0.0256*
	14	47.4	11.9	48.5	28.1	63.5	37.6	58.0	
Health Transition Item	**0**	2.9	1.2	3.0	1.0	5.0	2.5	3.5	0.7227
	14	3.0	1.0	3.0	2.0	5.0	2.0	3.5	
Physical Component Scale	**0**	40.9	10.6	38.3	27.0	56.6	32.0	50.6	0.9414
	14	41.1	13.0	36.0	24.5	57.7	29.7	55.2	
Mental Component Scale	**0**	53.0	12.3	58.3	32.9	66.5	39.4	62.9	0.0403*
	14	46.3	13.9	49.2	20.1	64.7	37.2	57.1	