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Bone Marrow Transplantation as a Therapy for Autosomal Dominant Osteopetrosis Type 2 in Mice

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Abstract

Autosomal dominant osteopetrosis type II (ADO2) is a heritable bone disease of impaired osteoclastic bone resorption caused by missense mutations in the chloride channel 7 (*CLCN7*) gene. Clinical features of ADO2 include fractures, osteomyelitis of jaw, vision loss, and in severe cases, bone marrow failure. Currently, there is no effective therapy for ADO2, and patients usually receive symptomatic treatments. Theoretically, bone marrow transplantation (BMT), which is commonly used in recessive osteopetrosis, could be used to treat ADO2, although the frequency of complications related to BMT is quite high. We created an ADO2 knock-in (p.G213R mutation) mouse model on the 129 genetic background, and their phenotypes mimic the human disease of ADO2. To test whether BMT could restore osteoclast function and rescue the bone phenotypes in ADO2 mice, we transplanted bone marrow cells from 6-8 weeks old male WT donor mice into recipient female ADO2 mice. Also, to determine whether age at the time of transplant may play a role in transplant success, we performed BMT in young (12-week-old) and old (9-month-old) ADO2 mice. Our data indicate that ADO2 mice transplanted with WT marrow achieved more than 90% engraftment up to 6 months post-transplantation at both young and old ages. The *in-vivo* DXA data revealed that young ADO2 mice transplanted with WT marrow had significantly lower whole body and spine areal bone mineral density (aBMD) at month 6 post-transplantation compared to the ADO2 control mice. The old ADO2 mice also displayed significantly lower whole body, femur and spine aBMD at months 4 and 5 post-transplantation compared to the age-matched control mice. The *in-vivo* micro-CT data showed that ADO2

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Authors' roles

Study design: IA, MAK and MJE. Study conduct: IA, RLG, DA, SLH, MM, MBA, RJB, AS, EFS, MAK and MJE. Data analysis: IA, RLG, DA, MBA, RJB, MAK and MJE. Data interpretation: IA, RLG, DA, MBA, RJB, MAK and MJE. Drafting manuscript: IA, MAK and MJE. Revising manuscript content: IA, MAK and MJE. Approval of final version of manuscript: IA, RLG, DA, SLH, MM, MBA, RJB, AS, EFS, MAK and MJE.

Disclosure statement

The authors declare that they have no conflict of interest.

experimental mice transplanted with WT marrow had significantly lower BV/TV at months 2 and 4 post-transplantation compared to the ADO2 control mice at young age. In contrast, ADO2 control and experimental mice displayed similar BV/TV values for all post-transplantation time points at old age. In addition, serum CTX was significantly higher at month 2 post-transplantation in both young and old ADO2 experimental mice compared to the ADO2 control mice. Serum P1NP levels in young ADO2 experimental mice were significantly higher at baseline and month 2 post-transplantation compared to the ADO2 control mice. These data suggest that BMT may provide, at least, some beneficial effect at both young and adult ages.

Keywords

Osteopetrosis; Chloride channel 7; Osteoclasts; Bone marrow transplantation; Bone phenotypes

Introduction

Autosomal Dominant Osteopetrosis type II (ADO2) is a bone disease of impaired osteoclastic bone resorption that usually results from heterozygous missense mutations in the chloride channel 7 (*CLCN7*) gene (1-5). ADO2 is sometimes referred to as the benign form of osteopetrosis to distinguish it from the malignant or recessive form which is generally more severe. ADO2 on average has a significant disease burden, however, the symptoms and severity of ADO2 can vary greatly ranging from incidental radiological finding and bone pain to multiple fractures of the long bones, osteomyelitis (particularly of the mandible), optic nerve compression and vision loss, and in severe cases, life-treatening complications such as bone marrow failure (1,3,4,8). The disease severity of ADO2 identified in our and other studies are high rate of hip and femur fractures (16% in children and 49% in adults), significant visual impairment (5-19%), osteomyelitis (13-16%) and transfusion dependent due to bone marrow failure (3%) (4,8). To understand better the pathophysiology of ADO2 and to identify strategies to treat this disease, we developed mouse models of ADO2 by creating a knock-in (GGG to AGG; p.G213R) mutation in the *Clcn7* gene, which is analogous to one of the common mutations found in humans (6). The mutation leads to severe osteopetrosis and lethality in homozygous mice but produces substantial phenotypic variability in heterozygous mice on different genetic backgrounds that phenocopy the human disease of ADO2 (6).

Previously, high dose of calcitriol and interferon gamma (IFN-γ) were used to treat patients with recessive forms of osteopetrosis (10-12). We tested IFN-γ in our mouse model of ADO2 and found that high doses of this drug improved the osteopetrotic phenotypes, and thereby might have beneficial effect as a therapy of ADO2 (7). Based on these data, recently, we performed an open-label pilot clinical trial of IFN-γ in 12 ADO2 subjects for the duration of 14 weeks, and found that IFN-γ was poorly tolerated and failed to significantly increase markers of bone turnover, and therefore is unlikely to change bone phenotypes in these patients (13). Recently, gene silencing approach by using mutation-specific small interfering RNA (siRNA) in ADO2 mice from Teti group demonstrated the importance of potential of use of siRNA as a therapy of ADO2 in humans (14,15). Currently, the treatment for recessive forms of osteopetrosis is hematopoietic cell or bone marrow

transplantation (BMT) (16-20). As osteopetrosis results from impaired bone resorption due to osteoclast dysfunction (9,21), and osteoclasts arise from cells of hematopoietic lineage, successful transplantation of healthy hematopoietic stem cells using whole bone marrow should restore osteoclast function and with time should rescue the hematopoiesis (19,20,35). Ultimately, if this approach works, the functioning osteoclasts and normal hematopoiesis will assist in the resolution of the abnormal bone phenotype or at least decrease the disease severity of ADO2. Importantly, due to recent developments on the transplantation schemes, survival rate of patients who undergo BMT therapy improved significantly as high as 75-100% depending on the age of the recipient, use of the conditioning regimens, the degree and identity of the donor and transplantation centers where this procedure was performed (22-25). As the patients usually suffer from related complications of hematopoietic cell transplants (16,17), this still remains a critical tissue for the overall outcome of the transplantation. However, ADO2 patients who might be candidates for this risky procedure are those who are transfusion dependent or suffer from complications of multiple progressive pathological fractures, severe osteomyelitis and therefore the quality of life are severly compromised. Also, this is the only procedure which can totally correct the disease and allows long-term cure by providing functional osteoclasts when performed at young age.

The number of ADO2 patients who manifest severe disease and consequently undergo BMT therapy is very low, which severely limits our understanding of the beneficial effects this procedure offers in these patients (4). As our ADO2 mouse model on the 129 genetic background (129Sv strain) recapitulates the human phenotype, it enables us to test this procedure in mice before more ADO2 patients are subjected to future BMT treatment. In this study, we investigated whether BMT could restore osteoclast function and rescue the bone defects in ADO2 mice. As most of the symptoms in ADO2 patients get progressively worse, we also sought to test whether age at the time of transplant may play any role in the transplant success. We hypothesize that if BMT therapy is successful only at young age, further progression of the disease might be halted and longer quality of life would be experienced by these patients if it is performed earlier in life. On the other hand, if BMT is found to be similarly successful in both young and old ages, the patients might be advised to wait until disease burden is very high before considering this approach. In addition, if BMT does not work either at young or old age, this high mortality procedure should not be considered in ADO2 patients. Thus, we performed BMT in both young (12-week-old) and old (9-month-old) ADO2 mice and evaluated bone phenotypes by *in-vivo* DXA and micro-CT as well as analyzed serum bone biomarkers at baseline and up to 6 months post-transplantation. Our data suggest that BMT may provide, at least, some long-term beneficial effects for both young and old recipients.

Materials and Methods

Experimental animals

For young (12-week-old) BMT study, we used 18 WT female and 19 ADO2 female heterozygous recipient mice. Mice were randomly assigned into WT>WT control (WT mice transplanted with WT bone marrow; n=10), ADO2>ADO2 control (ADO2 mice

transplanted with ADO2 bone marrow; n=9), ADO2>WT control (WT mice transplanted with ADO2 bone marrow; n=8) and WT>ADO2 experimental (ADO2 mice transplanted with WT bone marrow; n=10) groups. For older (9-month-old) BMT study, we used 17 WT female and 19 ADO2 female heterozygous recipient mice including WT>WT control (n=8), ADO2>ADO2 control (n=9), ADO2>WT control (n=9) and WT>ADO2 experimental (n=10) mice. The donor mice for both studies were male mice between 6-8 weeks of age. All mice were generated and maintained at Indiana University. Mice were housed in polycarbonate cages in a vivarium maintained on a 12-h light and 12-h dark cycle and were fed a regular diet and water ad libitum. The procedures performed throughout the experiment followed the guidelines of the Indiana University Animal Care and Use committee (IACUC).

Bone marrow transplantation (BMT)

Recipient female mice at either 12 weeks or 9 months of age were irradiated with split dose of 1000 centi-gray (cGy) (650 cGy followed by 350 cGy 4 hours later) of total body irradiation with a 137Cs source (MARK I Model 68A, Shepherd & Associates, San Fernando, CA). The following day the recipient mice were transplanted with 5 X 10^6 bone marrow cells obtained from the femurs and tibias of 6-8 weeks old donor male mice of different genotypes through tail vein injections.

Euthanasia and specimen collection

Young BMT mice were euthanized 6 months post-transplantation whereas old BMT mice were euthanized 5 months post-transplantion as they developed some age related complications such as tumor and generalized poor body condition by that time. The lower limbs were dissected from these animals, the femora were stripped of muscle, transferred to 70% ethyl alcohol and stored at 4°C for futher analyses. In addition, serum was collected and stored at −80°C until analysis.

In-vivo dual energy X-ray absorptiometry (DXA)

The whole body, femur and lumbar vertebrae 1 through 5 (L1-5) vertebrae of the control and experimental mice at baseline (12 weeks or 9 months), 2 and 4 months post-transplantation and terminal (6 months for young mice and 5 months for old mice) were scanned using DXA (PIXImus II mouse densitometer; Lunar Corp., Madison, WI, USA) with ultra-high resolution (0.18 × 0.18 mm/pixel) as described previously (6). The global window for the whole body was defined as the whole body image minus calvarium, mandible and teeth. After completion of the scan of each bone, mutually exclusive region of interest (ROI) boxes were drawn around the bone from which femur and lumbar vertebrae areal bone mineral density (aBMD, g/cm^2) and bone mineral content (BMC, g) measurements were obtained.

In-vivo micro-computer tomography (μCT) analysis

The femurs of the control and experimental mice at baseline (12 weeks or 9 months), 2 and 4 months post-transplantation and terminal (6 months for young mice and 5 months for old mice) were scanned using μCT scanner (Skyscan 1176, Bruker, MA) with an isotropic voxel size of 9 $μm^3$. The settings for each *in-vivo* scan included voxel size 9 $μm^3$, X-ray

peak potential 50 kVp, X-ray intensity 500 uA and image resolution of 4000 X 2672 pixels. In brief, from the scout-view, the growth plate location was identified and trabecular bone measurements consisting of 200 slices (1.8 mm) was completed from about 1 mm below the growth plate. Finally, 3D and 2D morphometric evaluations were performed for the trabecular bone from each scan, and bone volume over tissue volume (BV/TV, %) and structural parameters (trabecular number, Tb.N, 1/mm; trabecular thickness Tb.Th, mm; trabecular separation, Tb.Sp, mm) were determined. The use of the nomenclature, symbols and units were followed as described in Dempster et al. (26).

Serum biochemistry and biomarkers analysis

Serum levels of mouse pro-collagen 1 intact N-terminal (P1NP, ng/mL), carboxy-terminal collagen crosslinks (CTX, ng/mL) and tartrate-resistant acid phosphatase 5, isoform b (TRAcP5b, U/L) of control and experimental mice at baseline (12 weeks or 9 months), 2 and 4 months post-transplantation and terminal (6 months for young mice and 5 months for old mice) were measured by ELISA kits (Immunodiagnostics Systems, AZ, USA and Biomedical Technologies Inc., MA, USA) according to the manufacturers' instructions. Serum calcium (Ca, mg/dL), phosphorus (Phos, mg/dL), blood urea nitrogen (BUN, mg/dL), creatinine (CREA, mg/dL) and alkaline phosphatase (ALP, IU/L) were measured at terminal (6 months for young mice and 5 months for old mice) using the Randox Rx kit (Daytona Analyzer, WV, USA).

Analysis of donor chimerism

To determine the donor chimerism blood from facial vein was obtained at 1 and 3 months post-transplantation for both young and old mice. Blood was also collected at 6 months post-transplantation for young mice and at 5 months post-transplantation for old mice, and blood from WT and ADO2 mice without BMT was obtained as controls. Serum was prepared from blood and genomic DNA was extracted using kit (QIAamp DNA Blood Mini Kit, Qiagen, CA). Real-time PCR detection of sex-determining region Y (Sry) on the Y-chromosome was performed using SYBR green PCR master mix (Thermo Fisher Scientific, MA) using the following PCR parameters - 50°C for 2 minutes, 95°C for 10 minutes, followed by 39 cycles of 95°C for 15 seconds, 60°C for 1 minute with 65°C to 95°C melt curve analysis and normalized to the house-keeping gene Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). The mouse Sry primers (Forward: GCTGGGATGCAGGTGGAAAA and reverse: CCCTCCGATGAGGCTGATATT) and mouse Gapdh primers (Forward: CGTGGGGCTGCCCAGAACAT and reverse: TCTCCAGGCGGCACGTCAGA) were obtained from Sigma-Aldrich, Inc, (St. Louis, MO). The percent engraftment (% of *Sry* expressing male cells in the female recipient mice transplanted with different donor bone marrow cells) was subsequently determined using male to female genomic DNA ratio using this formula: (expression level of *Sry* in female BMT recipient WT or ADO2 mice / expression level of *Sry* in male WT or ADO2 mice without BMT) X 100 = % engraftment in the female recipient mice.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to identify mean differences for variables among control

and experimental groups at baseline, 2, 4 and 6/5 months post-transplantation. The Fisher protected least significant difference (PLSD) was used for all pairwise post-hoc comparisons. All statistical analysis was performed using the statistical software package StatView (Abacus Concepts, Inc., Berkeley, CA). The level of significance was set at $p < 0.05$.

Results

Percent engraftment of male donor cells in the female recipient mice

Early engraftment data at 1 month post-transplantation in young mice demonstrated complete engraftment of male donor cells into the female recipient mice (110 ± 10 %, 122 ± 21 %, 115 ± 11 %, 116 ± 10 % in WT>WT control, ADO2>ADO2 control, WT>ADO2 experimental and ADO2>WT control mice, respectively; n = 8/10 mice per group). The percent engraftment at 3 months post-transplantation showed more than 90% engraftment and at 6 months post-transplantation complete (100%) engraftment in young mice (Figure 1A). In older mice, the overall sample numbers at 1 month post-transplantation were low (n=4/group) for all groups of BMT recipient mice and we could not obtain enough good quality samples for ADO2>ADO2 control group at this time point. However, we observed engraftment rate of 117 ± 9 %, 127 ± 31 % and 118 ± 8 % in WT>WT control, WT>ADO2 experimental and ADO2>WT control mice, respectively, in old mice. In addition, 100% engraftment of male donor cells was achieved at both 3 and 5 months post-transplantation (Figure 1B) in old mice. These data indicates that successful long-term engraftment of donor bone marrow cells was obtained in both young and old recipients.

BMT at young age prevented the whole-body and spine bone mass gain in ADO2 mice

As expected, the whole body areal BMD was significantly higher in ADO2>ADO2 control mice at 2 ($p<0.05$), 4 ($p<0.005$) and 6 ($p<0.005$) months post-transplantation compared to the WT>WT control mice at young age (Figure 2A). Also, the whole body BMC was significantly higher ($p<0.05$) in ADO2>ADO2 control mice at 6 months post-transplantation compared to the WT>WT control mice (Figure 2B). WT>ADO2 experimental mice displayed significantly lower ($p<0.05$) whole body aBMD only at month 6 post-transplantation compared to the ADO2>ADO2 control mice (Figure 2A). At 6 months post-transplantation, the ADO2>WT control mice showed significantly higher ($p<0.05$) whole body aBMD compared to the WT control mice (Figure 2A).

Femur aBMD values were significantly higher ($p<0.005$) in ADO2>ADO2 control group at months 4 and 6 in young mice compared to WT>WT control group (Figure 2C). A slight but non-significant decrease of femur aBMD, but significantly lower ($p<0.05$) femur BMC was observed for WT>ADO2 experimental group in young mice at month 6 post-transplantation compared to ADO2>ADO2 control group (Figure 2D).

Spine (lumbar 1-5) aBMD values were significantly higher ($p<0.05$) at months 2, 4 and 6 in young BMT mice in ADO2>ADO2 control group compared to WT>WT control group. WT>ADO2 experimental group had significantly lower ($p<0.05$) spine aBMD at months 4 and 6 post-transplantation (Figure 2E) and significantly lower ($p<0.05$) BMC at month 6

post-transplantation (Figure 2F) compared to ADO2>ADO2 control group in young mice. These data indicate that BMT prevented whole-body and spine bone mass gain at 4 months post-transplantation onward in young ADO2 mice that received bone marrow from the WT donor mice.

BMT attenuated the whole body, femur and spinal bone mass gain in old ADO2 mice

BMT in older mice at 9 months of age revealed that whole body areal BMD were significantly ($p<0.05$) higher in ADO2>ADO2 control mice at baseline, months 2, 4 and 5 compared to the WT>WT control mice (Figure 3A). WT>ADO2 experimental mice displayed significantly lower ($p<0.05$) whole body aBMD at months 4 and 5 post-transplantation compared to the ADO2>ADO2 control mice (Figure 3A). The whole body BMC values were similar for all BMT groups in older mice at months 2, 4 and 5 post-transplantation (Figure 3B).

Femur aBMD values were significantly higher ($p<0.05$) at months 2, 4 and 5 in old mice in ADO2>ADO2 control group compared to WT>WT control group (Figure 3C). WT>ADO2 experimental group had significantly lower ($p<0.05$) femur aBMD at months 4 and 5 post-transplantation (Figure 3C) and significantly lower ($p<0.05$) femur BMC at month 5 post-transplantation (Figure 3D) compared to the ADO2>ADO2 control group in old mice.

Spine (lumbar 1-5) aBMD values were significantly higher at baseline ($p<0.005$), months 4 ($p<0.05$) and 5 ($p<0.05$) in old BMT mice in ADO2>ADO2 control group compared to WT>WT control group (Figure 3E). At month 2, the aBMD for ADO2>ADO2 mice was higher but non-significant ($p=0.09$) compared to the WT>WT control mice. WT>ADO2 experimental group had significantly lower ($p<0.05$) spine aBMD at months 4 and 5 post-transplantation (Figure 3E) and significantly lower ($p<0.05$) spine BMC values at month 4 post-transplantation (Figure 3F) compared to the ADO2>ADO2 control group in old BMT mice. These data indicate that BMT attenuated whole-body, femur and spine bone mass gain at 4 months post-transplantation onward in old ADO2 mice that received bone marrow from the WT donor mice.

BMT ameliorated the trabecular bone mass gain at distal femur in young ADO2 experimental mice

Representative micro-CT images at distal femur of young recipient mice transplanted with different donor bone marrow cells at baseline, months 2, 4 and 6 post-transplantation (Figure 4A). Compared to the WT>WT control mice, trabecular BV/TV, Tb.Th and Tb.N values obtained from distal femur were significantly higher ($p<0.005$) at baseline, months 2 and 4 and significantly higher ($p<0.05$) at month 6 post-transplantation in ADO2>ADO2 control mice at young age (Figures 4A, 4B, supplemental figure 1). WT>ADO2 experimental mice displayed significantly lower ($p<0.05$) BV/TV at months 2 and 4 (Figures 4A, 4B), significantly lower ($p<0.05$) Tb.Th at month 4 (Supplemental figure 1) and significantly lower ($p<0.05$) Tb.N at month 2 (Supplemental figure 1) post-transplantation compared to the ADO2>ADO2 control mice. In addition, a trend of lower BV/TV, Tb.Th and Tb.N were observed in the WT>ADO2 experimental mice at month 6 post-transplantation compared to the ADO2>ADO2 control mice (Figures 4A, 4B, supplemental figure 1). Tb.Sp values

were significantly lower at baseline ($p<0.005$), months 2 ($p<0.05$) and 6 ($p<0.005$) post-transplantation in the ADO2>ADO2 control group compared to their WT>WT counterparts, however, no significant differences were observed between WT>ADO2 experimental and ADO2>ADO2 control mice at baseline and any post-transplantation time points (Supplemental figure 1). Further, at 6 months post-transplantation, the ADO2>WT control mice showed significantly higher ($p<0.05$) BV/TV, Tb.Th and Tb.N values but significantly lower ($p<0.05$) Tb.Sp compared to the WT>WT control in young BMT mice (Figures 4A, 4B, supplemental figure 1). These data indicate that BMT ameliorated much of the trabecular bone mass gain in ADO2 mice that received bone marrow from the WT donor mice at young age.

BMT did not reverse the trabecular bone mass gain at distal femur in old ADO2 experimental mice

Representative micro-CT images at distal femur of old recipient mice transplanted with different donor bone marrow cells at baseline, months 2, 4 and 5 post-transplantation (Figure 5A). BMT in old mice at 9 months of age showed that trabecular BV/TV, Tb.Th and Tb.N values were significantly higher ($p<0.005$) at baseline and month 2, and significantly higher ($p<0.05$) at month 4 post-transplantation in ADO2>ADO2 control mice compared to the WT>WT control mice (Figures 5A, 5B, supplemental figure 2). All of these trabecular micro-architectural parameters were similar between ADO2>ADO2 control and WT>ADO2 experimental mice except ADO2 experimental mice displayed significantly lower ($p<0.05$) Tb.Th at month 2 post-transplantation compared to the ADO2>ADO2 control mice (Figures 5A, 5B, supplemental figure 2). In addition, higher BV/TV, Tb.N and Tb.Th values were observed at month 5 post-transplantation in all mice (Figures 5A, 5B, supplemental figure 2) without any significant differences among different groups. Tb.Sp values were significantly lower ($p<0.05$) only at baseline in the ADO2>ADO2 control group compared to their WT>WT counterparts, and no significant differences were observed between WT>ADO2 experimental and ADO2>ADO2 control mice at baseline and any post-transplantation time points (Supplemental figure 2). These data indicate that BMT at old age did not reverse the trabecular bone mass gain up to 4 months post-transplantation in ADO2 experimental mice.

Serum levels of bone formation and resorption markers were slightly changed in ADO2 mice undergoing BMT at young age

Serum CTX and CTX/TRAP levels were similar between WT>WT and ADO2>ADO2 control mice at baseline, months 2 and 6 post-transplantation in young BMT mice and at baseline, months 2, 4 and 5 in mice receiving BMT at 9 months of age (Table 1). Serum TRAP levels were significantly higher in ADO2>ADO2 control mice at baseline ($p<0.05$) and month 6 ($p<0.005$) post-transplantation in young BMT mice and significantly higher at baseline ($p<0.05$) and month 2 ($p<0.05$) post-transplantation in old BMT mice compared to the age-matched WT>WT control groups (Table 1). Serum CTX was significantly higher ($p<0.05$) in WT>ADO2 experimental mice at month 2 post-transplantation in both young and old BMT mice compared to the age-matched ADO2>ADO2 control groups (Table 1). In addition, serum P1NP levels were significantly higher ($p<0.05$) in the WT>ADO2 experimental group at baseline compared to ADO2>ADO2 control mice and at month 2 post-transplantation compared to the WT>WT and ADO2>ADO2 control groups in

young BMT mice whereas P1NP level was significantly higher ($p<0.05$) in WT>ADO2 experimental mice compared to WT>WT control mice at month 2 post-transplantation in old BMT mice (Table 1). Further, higher P1NP levels were observed at months 4 and 5 post-transplantation in all mice compared to baseline values (Table 1); however, no significant differences were observed among different groups. These data suggest that BMT somewhat influenced both bone formation and resorption markers only in young recipient mice.

Serum biochemistry varied among ADO2 experimental and control mice in young and old ages

At month 6 post-transplantation, serum levels of calcium (Ca), phosphorus (P), creatinine (CREA) and blood urea nitrogen (BUN) were similar in all groups of mice receiving BMT at young age. However, the serum level of alkaline phosphatase (ALP) was significantly higher ($p<0.05$) in WT>ADO2 experimental mice compared to the other control groups (ADO2>ADO2, WT>WT and ADO2>WT) (Table 2). In older mice, serum levels of Ca, P, CREA and BUN were significantly higher ($p<0.05$) in ADO2>ADO2 control group compared to the other groups (WT>ADO2, WT>WT and ADO2>WT), however, serum ALP levels were similar in all groups receiving BMT at old age (Table 2).

Discussion

In this study, we found that ADO2 mice transplanted with WT bone marrow prevented the DXA derived whole body, femur and spine bone mass gain at 4 months post-transplantation onward in both young and old recipient mice. In addition, the trabecular bone mass gain at distal femur (measured by microCT) was attenuated up to 4 months post-transplantation in young ADO2 mice. Further, higher serum bone formation and resorption markers were observed up to 2 months post-transplantation in young ADO2 mice that received WT bone marrow. In contrast, trabecular bone mass and all serum bone biomarkers parameters were similar between old ADO2 mice transplanted with WT bone marrow and control ADO2 mice. Collectively, these data suggest that BMT in our ADO2 mouse model provides, at least, some beneficial effects to both young and old mice.

With respect to therapeutic strategies in human, IFN-γ has been administered in patients with recessive forms of osteopetrosis (11,12), and it has also been used as a bridge therapy to control the disease until a donor could be found for bone marrow transplantation. Recent pre-clinical findings also suggest the potential of use of siRNA as a therapy of ADO2 (14,15). However, hematopoietic cell transplantation is the only current treatment option for severe recessive forms of osteopetrosis without neurodegeneration and in most cases, BMT improves but does not fully rescue the bone phenotypes. Also, the patients undergoing BMT suffer complications like graft versus host disease, pulmonary hypertension, hepatic veno-occlusive disease and hypercalcemia (16,17,27, 28). The symptoms and severity of ADO2 can vary greatly from asymptomatic and sudden radiological discovery to life-threatening complications such as bone marrow failure in severe cases. Therefore, the risky procedure like BMT will be beneficial to those ADO2 patients who suffer from complications of multiple progressive pathological fractures, severe osteomyelitis and recurrent infections or

are transfusion dependent. This could also correct the disease and allows long-term cure if performed in early life.

Hematopoietic reconstitution or recovery of donor cell population is a critical factor for successful bone marrow transplantation. In this study, we adopted a split radiation dose of 1000 cGy (650 cGy followed by 350 cGy 4 hours later) based on the dose tested in mice previously (29), which was tolerated well by all mice for the duration of experiment and might have helped reduce radiation-induced tissue damage as it has been reported before (30). Adverse events in the early stage of post-transplantation especially infection is an important factor for the outcome of BMT. To minimize infection, we provided acidified water and administered antibiotics (doxycycline) via water for at least 1 week before and 2 weeks after transplantation. We did not observe any significant adverse events other than the BMT recipient mice were slight sluggish in appearance for a couple of days immediately after the transplantation. These recipient mice also did not show any indications of failure to thrive such as lower body weight, less food consumption etc. for the entire post-transplantation duration in this study. Although the number of donor cells and the survival of recipient mice vary among studies (31-33), we injected 5 million donor cells per recipient. Our percent engraftment data showed that more than near complete engraftment of male donor cells established into the female recipient mice as early as 1 month post-transplantion and 100% engraftment of the donor cells by 6 months post-transplantation in both young and old mice.

Patient age at the time of transplantation might play an important role in the transplant success. The mean age of ADO2 patients in our cohort under 18 years old was 8.9 ± 4.5 (9 months – 17 years) and above 18 years old was 47.1 ± 16.4 (22 – 79 years) (4). Subjects from this cohort who received BMT and later died from complications of hematopoietic cell transplants included both young and old ADO2 patients (4). Limited data suggest that ADO2 disease severity worsens with age (4) and BV/TV values increase with age in our mouse model (6). Early death of ADO2 patients is rare but disease progression is an important factor for ADO2 and some patients experience a very poor quality of life. Hematological problems in ADO2 patients vary from mild to transfusion dependent anemia with extramedullary hematopoiesis and hepatosplenomegaly (4,5). In our original cohort, we reported bone marrow failure in 3% of ADO2 patients who were anemic and required frequent blood transfusions (4). Recently, we observed anemia in 58% severly affected ADO2 patients (13), highlighting the serious and life-threatening nature of this disease. Our mouse model of ADO2 do not show significant abnormalites related to blood cell counts (6) and deficit in erythroid, lymphoid and myeloid lineages or marrow fibrosis compared to WT mice at both young and old age (unpublished data). Work in recessive osteopetrosis showed that the older patients receiving BMT experienced more graft rejection or autologous reconstitution, even when an haploidentical donor source was used (28). In addition, toxicity for the conditioning regimen and hypercalcemia increased in older patients (28). In this study, we investigated whether the age play any roles for the outcome of BMT in our mouse model of ADO2. We found that trabecular bone mass were significantly improved up to 4 months post-transplantation of WT bone marrow only in young ADO2 recipient mice.

Our previous studies showed that ADO2 mice on 129 background display high whole body aBMD and BV/TV at distal femur with increased number of poorly resorbing osteoclasts (6,7). In addition, we observed both WT and ADO2 mice displayed similar bone density values up to 8 weeks of age and then their bone phenotypes started to diverge and aroud month 3 the differences become significant between the two genotypes (6,7). Therefore, in the young mice, the bone density values were similar at baseline but at month 2 post-transplantation onward the ADO2 mice displayed significantly higher density values compared to the WT control mice. Compared to the wild-type mice, the ADO2 mice have 2-fold higher BV/TV at 12 weeks of age on this background (6) and in concert with human disease, the phenotype is more pronounced with age, as we observed 4-7 fold increase of BV/TV in the distal femur in these mice at 18 months of age (data not shown). We chose 12 week-old mice as they represent young adult between 20 -25 years of age, and 9 month-old mice as they represent approximately 40-45 year-old human (34). The DXA data demonstrated that ADO2 experimental mice transplanted with WT marrow had significantly lower whole body and spinal aBMD compared to ADO2 control group in young mice at month 6, whereas there was a trend for lower whole body aBMD at month 4 ($p=0.16$) and femur aBMD at month 6 ($p=0.06$) in the young experimental ADO2 mice. The older ADO2 experimental mice also displayed significantly lower whole body, femur and spine aBMD at months 4 and 5 post-transplantation compared to the age-matched ADO2 control mice. These data indicate that bone marrow transplantation offers long-term beneficial effects on DXA-derived appendicular and axial bone phenotypes at both young and old ages. Further, trabecular bone mass data at distal femur by microCT showed significantly lower BV/TV at months 2 and 4 in young ADO2 experimental group compared to the ADO2 control mice. Although BV/TV values in the young ADO2 experimental mice at month 6 did not gain as much as the values to the control ADO2 mice, this difference was not significant between these 2 groups at this time point. In contrast, trabecular bone mass at distal femur did not change significantly in the old ADO2 experimental group at any post-transplantation time periods compared to the control mice, suggesting long-term beneficial effect of BMT on trabecular bone mass depends on age when the transplantation is performed. Serum bone turnover markers data showed that young experimental ADO2 mice had significantly higher CTX and P1NP levels at month 2 and TRAP level at month 6 post-transplantation compared to ADO2 control mice, whereas the level of CTX, TRAP and P1NP remained unchanged in old ADO2 experimental mice at all post-transplantation time points, suggesting effect of BMT on the bone formation and resorption markers also depends on early versus late transplantation age. Overall, these data indicate that BMT offers limited beneficial effects in ADO2 mice at both young and adult ages.

This study has several limitations. We noticed significantly higher BV/TV, Tb.N, Tb.Th values and significantly higher P1NP levels in old mice at the end of the study. Whether this is due to the effect of BMT at older age was unclear as we did not include control mice without BMT in this study. We could not obtain enough good quality samples for serum bone biomarkers analysis at month 4 post-transplantaion in young mice. We also observed some age-related complications such as tumor development and generalized poor body condition etc. in older BMT mice particularly at the end of the study; and therefore, we had to terminate our study at month 5 post-transplantation in the older mice. Also, we

tested BMT only in female ADO2 recipient mice as male Y chromosome was used for engraftment monitoring. We did not investigate the effect of BMT during growth phase in ADO2 recipient mice and did not monitor the change of bone marrow micro-environment after BMT in the recipient mice. Further, we used only young donor cells in this study; however, in the clinical setting donor age could be variable, therefore, whether donor cells at different ages will impact the BMT success deserves further evaluation. Also, a more clinically relevant conditioning strategy such as busulfan can be tested in future for its efficiency in ADO2 mice. Finally, we did not explore whether extra-skeletal phenotypes due to altered CIC-7 function in the ADO2 mice could be rescued by BMT in this study.

In conclusion, our data suggest that bone marrow transplantation may provide, at least, some beneficial effects for both young and adult ADO2 patients. However, since bone marrow transplant procedure has been documented to have high rate of complications, our study underscores the importance of comprehensive risk-benefit analysis of this procedure before it should be performed in ADO2 patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Data Availability Statement

The data that support the findings of this study are available in the methods and/or supplementary material of this article.

Abbreviations

aBMD	Areal bone mineral density
ADO2	Autosomal dominant osteopetrosis type II
ALP	Alkaline phosphatase
ANOVA	One-way analysis of variance
BMC	Bone mineral content
BMT	Bone marrow transplantation
BUN	Blood urea nitrogen
BV/TV	Bone volume over tissue volume
Ca	Calcium

cGy	Centi-gray
CLCN7	Chloride channel 7
CREA	Creatinine
CTX	Carboxy-terminal collagen crosslinks
DXA	Dual energy X-ray absorptiometry
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase
IFN-γ	Interferon gamma
μCT	Micro-computer tomography
P1NP	Pro-collagen 1 intact N-terminal
PCR	Polymerase chain reaction
Phos	Phosphorus
PLSD	Fisher protected least significant difference
ROI	Region of interest
SD	Standard deviation
siRNA	Small interfering RNA
Sry	Sex-determining region Y
Tb.N	Trabecular number
Tb.Th	Trabecular thickness
Tb.Sp	Trabecular separation
TRAcP5b	Tartrate-resistant acid phosphatase 5, isoform b
WT	Wild type

References

1. Waguespack SG, Koller DL, White KE, et al. Chloride Channel 7 (CICN7) Gene Mutations and Autosomal Dominant Osteopetrosis, Type II. JBMR, 2003. 18(8): p. 1513–1518.
2. Johnston CC Jr., et al. , Osteopetrosis. a clinical, genetic, metabolic, and morphologic study of the dominantly inherited, benign form. Medicine, 1968. 47: p. 149–167. [PubMed: 4871758]
3. Cleiren E, et al. , Albers-Schonberg disease (autosomal dominant osteopetrosis, type II) results from mutations in the ClCN7 chloride channel gene. Hum Mol Genet, 2001. 10(25): p. 2861–7. [PubMed: 11741829]
4. Waguespack SG, Hui SL, DiMeglio L, Econs MJ. Autosomal Dominant Osteopetrosis: Clinical Severity and Natural History of 94 Subjects with Chloride Channel 7 (C1CN7) Gene Mutations. J Clinical Endocrinol Metab. 92(3):771–8, 2007. [PubMed: 17164308]
5. Weber DR, Econs MJ and Levine MA. Osteopetrosis: pathogenesis, management and future directions for research. IBMS BoneKey. 11:520, 2014.

6. Alam I, Gray AK, Chu K et al. Generation of the first autosomal dominant osteopetrosis type II (ADO2) disease models. Bone 2013. Nov 1:59C:66–75.
7. Alam I, Gray AK, Acton D, et al. Interferon gamma but not calcitriol improves the osteopetrotic phenotypes in ADO2 mice. J Bone Miner Res, 2015 May 5. Doi: 10.1002/jbmr.2545.
8. Bénichou OD, Laredo JD, de Vernejoul MC. Type II autosomal dominant osteopetrosis (Albers-Schönberg disease): clinical and radiological manifestations in 42 patients. Bone. 2000 Jan;26(1):87–93. [PubMed: 10617161]
9. Chu K, Snyder R, and Econs MJ, Disease status in autosomal dominant osteopetrosis type 2 is determined by osteoclastic properties. J Bone Miner Res, 2006. 21(7): p. 1089–97 [PubMed: 16813529]
10. Key L, Carnes D, Cole S, et al. Treatment of congenital osteopetrosis with high-dose calcitriol. N Engl J Med. 1984;310(7):409–15. [PubMed: 6546410]
11. Key LL Jr, Ries WL, Rodriguiz RM, Hatcher HC. Recombinant human interferon gamma therapy for osteopetrosis. J Pediatr 1992;121:119–124. [PubMed: 1320672]
12. Key LL Jr, Rodriguiz RM, Willi SM, et al. Long-term treatment of osteopetrosis with recombinant human interferon gamma. N Engl J Med. 1995;332(24):1594–9. [PubMed: 7753137]
13. Imel EA, Liu Z, Acton D, Coffman M, Gebregziabher N, Tong Y, Econs MJ. Interferon Gamma-1b Does Not Increase Markers of Bone Resorption in Autosomal Dominant Osteopetrosis. J Bone Miner Res. 2019 Aug;34(8):1436–1445. doi: 10.1002/jbmr.3715. Epub 2019 May 13 [PubMed: 30889272]
14. Maurizi A, Capulli M, Patel R, Curle A, Rucci N, Teti A. RNA interference therapy for autosomal dominant osteopetrosis type 2. Towards the preclinical development. Bone. 2018 May;110:343–354. [PubMed: 29501587]
15. Capulli M, Maurizi A, Ventura L, Rucci N, Teti A. Effective Small Interfering RNA Therapy to Treat CLCN7-dependent Autosomal Dominant Osteopetrosis Type 2. Mol Ther Nucleic Acids. 2015 Sep 1;4(9):e248. [PubMed: 26325626]
16. Driessen GJ, Gerritsen EJ, Fischer A, Fasth A, Hop WC, Veys P et al. Long-term outcome of haematopoietic stem cell transplantation in autosomal recessive osteopetrosis: an EBMT report. Bone Marrow Transplant 2003;32:657–663. [PubMed: 13130312]
17. Steward CG. Hematopoietic stem cell transplantation for osteopetrosis. Pediatr Clin North Am 2010;57:171–180. [PubMed: 20307717]
18. Eapen M, Davies SM, Ramsay NK, Orchard PJ. Hematopoietic stem cell transplantation for infantile osteopetrosis. Bone Marrow Transplant. 1998 Nov;22(10):941–6. [PubMed: 9849690]
19. Abinun M, Pieniazek P. Successful haematopoietic stem cell transplantation for osteopetrosis due to TCRIG1 mutation. Arch Dis Child. 2010 Dec;95(12):984. [PubMed: 20870624]
20. Maximova N, Zennaro F, Gregori M, Boz G, Zanon D, Mbalaviele G. Hematopoietic stem cell transplantation-induced bone remodeling in autosomal recessive osteopetrosis: Interaction between skeleton and hematopoietic and sensory nervous systems. Bone. 2020 Jan;130:115144. [PubMed: 31706050]
21. Henriksen K, et al. , Characterization of osteoclasts from patients harboring a G215R mutation in ClC-7 causing autosomal dominant osteopetrosis type II. Am J Pathol., 2004. 164(5): p. 1537–45. [PubMed: 15111300]
22. Zhu G, Wei A, Wang B, Yang J, Yan Y, Wang K, Jia C, Luo Y, Li S, Zhou X, Wang T, Zheng H, Qin M. Haploidentical haematopoietic stem cell transplantation for malignant infantile osteopetrosis and intermediate osteopetrosis: a retrospective analysis of a single centre. Orphanet J Rare Dis. 2021 Jul 15;16(1):314. [PubMed: 34266467]
23. Penna S, Villa A, Capo V. Autosomal recessive osteopetrosis: mechanisms and treatments. Dis Model Mech. 2021 May 1;14(5).
24. Stepensky P, Grisariu S, Avni B, Zaidman I, Shadur B, Elpeleg O, Sirin M, Hoenig M, Schuetz C, Furlan I, Beer M, von Harsdorf S, Bunjes D, Debatin KM, Schulz AS. Stem cell transplantation for osteopetrosis in patients beyond the age of 5 years. Blood Adv. 2019 Mar 26;3(6):862–868. [PubMed: 30885997]

25. Shadur B, Zaidman I, NaserEddin A, Lokshin E, Hussein F, Oron HC, Avni B, Grisariu S, Stepensky P. Successful hematopoietic stem cell transplantation for osteopetrosis using reduced intensity conditioning. Pediatr Blood Cancer. 2018 Jun;65(6):e27010. [PubMed: 29469225]

26. Dempster DW, Compston JE, Drezner MK, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 2013; 28(1):2–17. [PubMed: 23197339]

27. Martinez C, Polgreen LE, DeFor TE, Kivisto T, Petryk A, Tolar J et al. Characterization and management of hypercalcemia following transplantation for osteopetrosis. Bone Marrow Transplant 2010;45:939–944. [PubMed: 19802031]

28. Schulz AS, Moshous D, Steward CG, Villa A, Sobacchi C. Osteopetrosis. Consensus Guidelines for Diagnosis, Therapy and Follow-Up (2015).

29. Duran-Struuck R, Hartigan A, Clouthier SG, Dyson MC, Lowler K, Gatza E, Tawara I, Toubai T, Weisiger E, Hugunin K, Reddy P, Wilkinson JE. 2008. Differential susceptibility of C57BL/6NCr and B6.Cg-Ptprca mice to commensal bacteria after whole-body irradiation in translational bone marrow transplant studies. J Transl Med 6:10. [PubMed: 18307812]

30. van Os R, Thames HD, Konings AW, Down JD. 1993. Radiation dose-fractionation and dose-rate relationships for long-term repopulating hemopoietic stem cells in a murine bone marrow transplant model. Radiat Res 136:118–125. [PubMed: 8210327]

31. Ema H, Morita Y, Yamazaki S, Matsubara A, Seita J, Tadokoro Y, Kondo H, Takano H, Nakauchi H. 2006. Adult mouse hematopoietic stem cells: purification and single-cell assays. Nat Protoc 1:2979–2987. [PubMed: 17406558]

32. Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ. 2005. SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell 121:1109–1121. [PubMed: 15989959]

33. Morrison SJ, Qian D, Jerabek L, Thiel BA, Park IK, Ford PS, Kiel MJ, Schork NJ, Weissman IL, Clarke MF. 2002. A genetic determinant that specifically regulates the frequency of hematopoietic stem cells. J Immunol 168:635–642. [PubMed: 11777956]

34. Dutta S, Sengupta P. Men and mice: Relating their ages. Life Sci. 2016; May 1;152:244–8. [PubMed: 26596563]

35. Palagano E, Slatter MA, Uva P, Menale C, Villa A, Abinun M, Sobacchi C. Hematopoietic stem cell transplantation corrects osteopetrosis in a child carrying a novel homozygous mutation in the FERMT3 gene. Bone. 2017 Apr;97:126–129. [PubMed: 28095295]

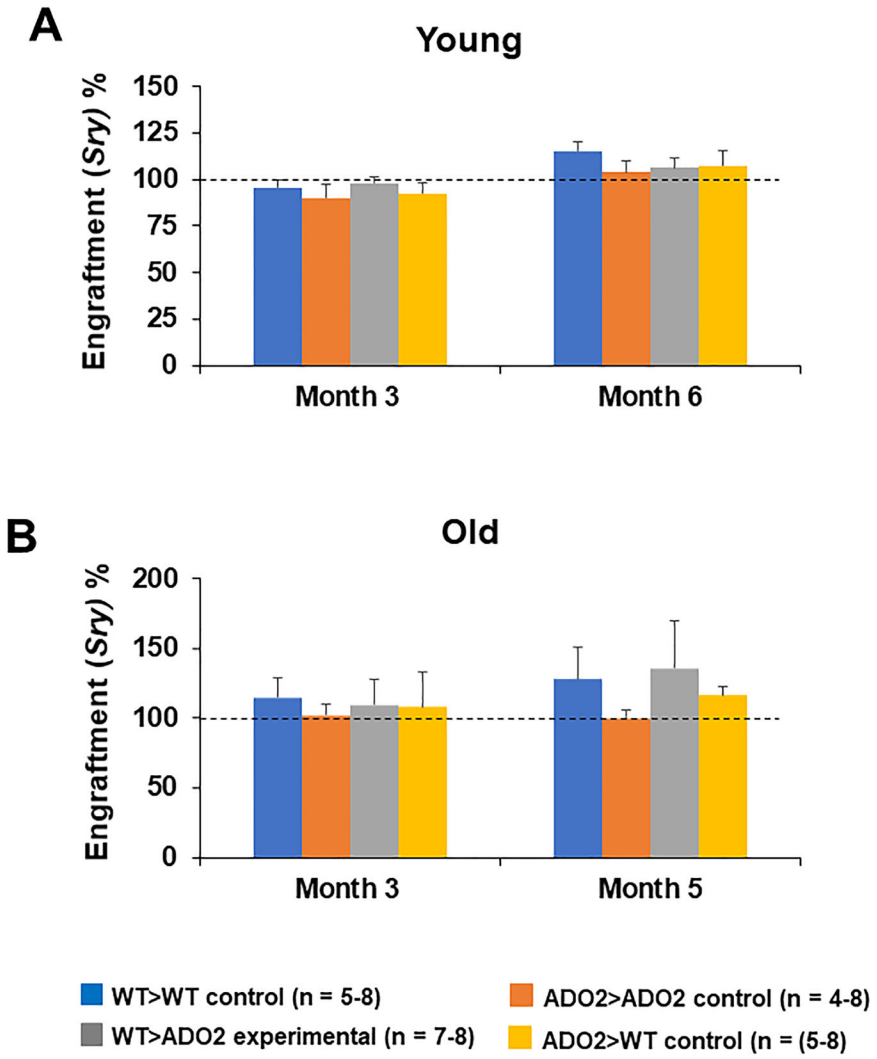

Fig. 1.

Donor chimerism determined using *Sry* gene on the Y chromosome. A. Female BMT recipient mice at young age displayed more than 90% engraftment of male donor cells at 3 months and complete (100%) engraftment at 6 months post-transplantation. B. 100% engraftment of male donor cells was achieved in old recipient mice at both 3 and 5 months post-transplantation. WT mice received WT bone marrow (WT>WT control), ADO2 mice received ADO2 bone marrow (ADO2>ADO2 control), ADO2 mice received WT bone marrow (WT>ADO2 experimental), WT mice received ADO2 bone marrow (ADO2>WT control). *Sry:* sex-determining region Y

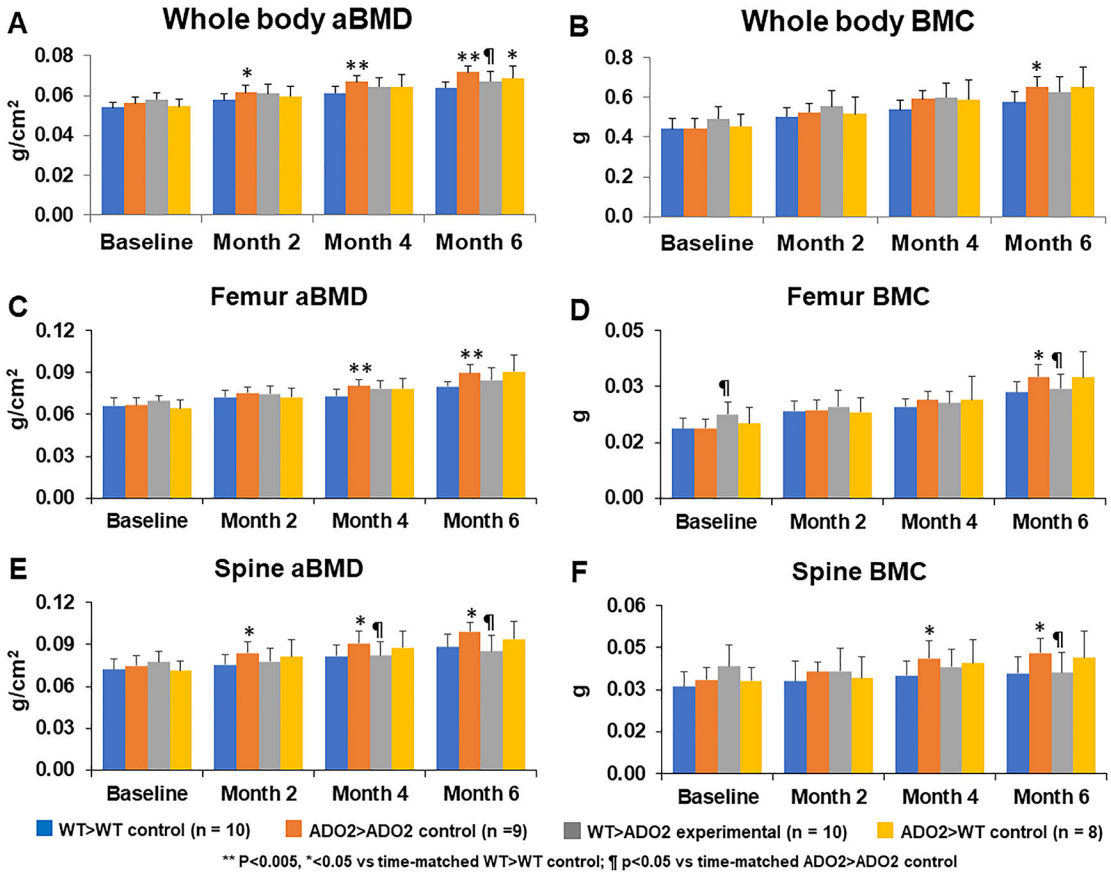

Fig. 2.

Whole body, femur and spine (L1-5) aBMD and BMC measured by *in-vivo* DXA in young recipient mice. Female mice at 12 weeks of age were transplanted with 6-8 weeks old male donor bone marrow cells. Measurements of aBMD and BMC were performed *in-vivo* at baseline, months 2, 4 and 6 post-transplantation. WT mice received WT bone marrow (WT>WT control), ADO2 mice received ADO2 bone marrow (ADO2>ADO2 control), ADO2 mice received WT bone marrow (WT>ADO2 experimental), WT mice received ADO2 bone marrow (ADO2>WT control). Values are given as mean ± SD ** p<0.005 and * p<0.05 vs. time-matched WT>WT control. ¶ p<0.05 vs. time-matched ADO2>ADO2 control. aBMD: areal bone mineral density, BMC: Bone mineral content

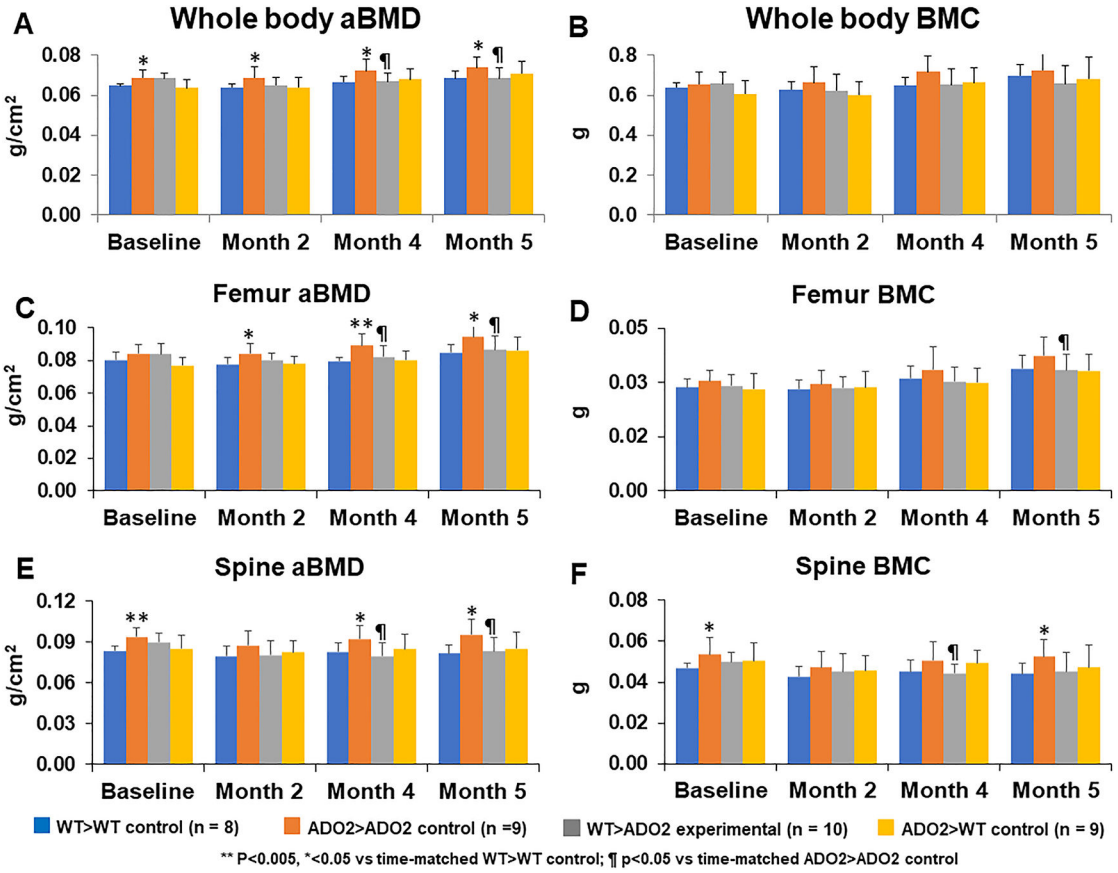

Fig. 3.

Whole body, femur and spine (L1-5) aBMD and BMC measured by *in-vivo* DXA in old recipient mice. Female mice at 9 months of age were transplanted with 6-8 weeks old male donor bone marrow cells. Measurements of aBMD and BMC were performed *in-vivo* at baseline, months 2, 4 and 5 post-transplantation. WT mice received WT bone marrow (WT>WT control), ADO2 mice received ADO2 bone marrow (ADO2>ADO2 control), ADO2 mice received WT bone marrow (WT>ADO2 experimental), WT mice received ADO2 bone marrow (ADO2>WT control). Values are given as mean ± SD ** $p<0.005$ and * $p<0.05$ vs. time-matched WT>WT control. ¶ $p<0.05$ vs. time-matched ADO2>ADO2 control. aBMD: areal bone mineral density, BMC: Bone mineral content

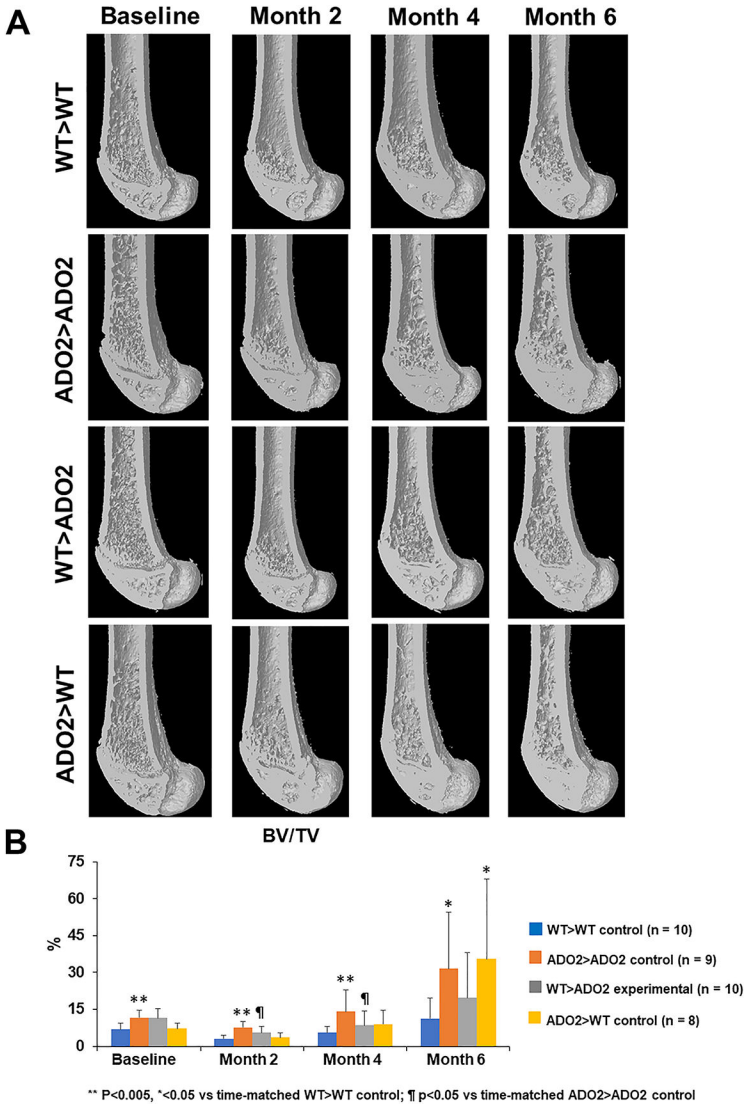

Fig. 4.

A. Representative micro-CT images at distal femur of young recipient mice transplanted with different donor bone marrow cells at baseline, months 2, 4 and 6 post-transplantation. B. Trabecular bone morphometry at distal femur measured by *in-vivo* micro-CT in young recipient mice. Female mice at 12 weeks of age were transplanted with 6-8 weeks old male donor bone marrow cells. Measurements of trabecular BV/TV at distal femur were performed *in-vivo* at baseline, months 2, 4 and 6 post-transplantation. WT mice received WT bone marrow (WT>WT control), ADO2 mice received ADO2 bone marrow (ADO2>ADO2 control), ADO2 mice received WT bone marrow (WT>ADO2 experimental), WT mice received ADO2 bone marrow (ADO2>WT control). Values are given as mean ± SD ** p<0.005 and * p<0.05 vs. time-matched WT>WT control. ¶ p<0.05 vs. time-matched ADO2>ADO2 control. BV/TV: bone volume over tissue volume

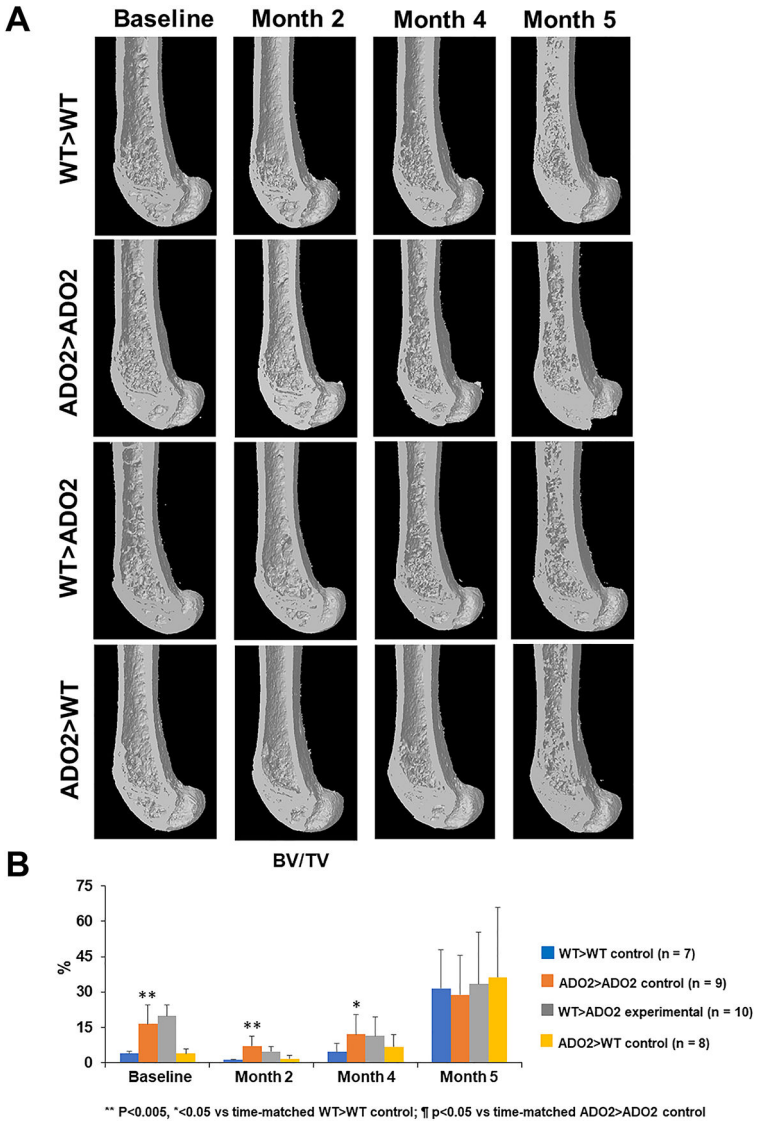

Fig. 5.

A. Representative micro-CT images at distal femur of old recipient mice transplanted with different donor bone marrow cells at baseline, months 2, 4 and 5 post-transplantation. B. Trabecular bone morphometry at distal femur measured by *in-vivo* micro-CT in old recipient mice. Female mice at 9 months of age were transplanted with 6-8 weeks old male donor bone marrow cells. Measurements of trabecular BV/TV at distal femur were performed *in-vivo* at baseline, months 2, 4 and 5 post-transplantation. WT mice received WT bone marrow (WT>WT control), ADO2 mice received ADO2 bone marrow (ADO2>ADO2 control), ADO2 mice received WT bone marrow (WT>ADO2 experimental), WT mice received ADO2 bone marrow (ADO2>WT control). Values are given as mean ± SD ** p<0.005 and * p<0.05 vs. time-matched WT>WT control. ¶ p<0.05 vs. time-matched ADO2>ADO2 control. BV/TV: bone volume over tissue volume

Table 1.

Comparison of bone formation and resorption markers in mice received bone marrow transplantation at young and old age

	Young (12 weeks old) mice			Old (9 months old) mice			
	Baseline	**Month 2**	**Month 6**	**Baseline**	**Month 2**	**Month 4**	**Month 5**
CTX (ng/mL)							
WT>WT control	49.1 ± 24.7	25.3 ± 18.9	25.0 ± 12.8	18.4 ± 2.5	13.5 ± 5.9	23.4 ± 9.1	22.7 ± 13.6
ADO2>ADO2 control	41.6 ± 27.1	16.7 ± 11.0	24.8 ± 9.7	21.3 ± 7.8	11.9 ± 5.4	22.0 ± 10.1	29.6 ± 15.5
WT>ADO2 experimental	34.4 ± 15.3	**36.0 ± 20.5** [c]	32.6 ± 18.8	17.3 ± 12.8	**19.3 ± 3.4** [a c]	22.3 ± 5.1	30.8 ± 27.7
ADO2>WT control	49.6 ± 21.2	26.1 ± 14.9	19.5 ± 10.8	18.2 ± 6.5	20.8 ± 10.2	25.8 ± 7.7	24.9 ± 20.6
TRAP (U/L)							
WT>WT control	21.4 ± 7.8	15.1 ± 4.6	22.9 ± 7.5	29.4 ± 18.3	27.7 ± 14.5	51.6 ± 12.8	83.5 ± 35.6
ADO2>ADO2 control	**29.8 ± 5.7** [a]	16.6 ± 4.8	**35.5 ± 6.0** [b]	**46.0 ± 13.2** [a]	**49.4 ± 29.4** [a]	47.6 ± 17.3	98.9 ± 45.2
WT>ADO2 experimental	26.7 ± 10.0	**19.8 ± 3.1** [a]	**28.3 ± 6.5** [c]	42.8 ± 22.1	32.5 ± 13.5	65.6 ± 33.9	100.3 ± 60.0
ADO2>WT control	20.2 ± 6.3	16.9 ± 6.6	23.5 ± 7.1	24.3 ± 8.0	26.6 ± 11.2	53.8 ± 18.6	121.1 ± 71.0
CTX/TRAP							
WT>WT control	2.3 ± 1.0	1.7 ± 1.0	1.2 ± 0.7	0.8 ± 0.3	0.4 ± 0.4	0.5 ± 0.2	0.4 ± 0.3
ADO2>ADO2 control	1.4 ± 0.9	1.1 ± 1.0	0.7 ± 0.3	0.5 ± 0.3	0.3 ± 0.3	0.5 ± 0.1	0.3 ± 0.1
WT>ADO2 experimental	1.3 ± 0.6	1.8 ± 1.0	1.2 ± 0.7	**0.4 ± 0.3** [a]	0.6 ± 0.4	0.4 ± 0.2	0.5 ± 0.5
ADO2>WT control	1.6 ± 0.9	2.5 ± 0.7	0.9 ± 0.4	0.9 ± 0.5	0.9 ± 0.6	0.5 ± 0.3	0.5 ± 0.6
P1NP (ng/mL)							
WT>WT control	33.7 ± 15.3	28.8 ± 21.8	45.2 ± 32.7	21.4 ± 13.9	29.3 ± 13.7	87.8 ± 37.2	93.9 ± 50.5
ADO2>ADO2 control	23.4 ± 12.0	17.6 ± 17.0	24.4 ± 14.6	15.9 ± 6.2	51.7 ± 33.7	55.7 ± 27.1	74.1 ± 30.5
WT>ADO2 experimental	**40.6 ± 14.3** [c]	**51.7 ± 15.2** [a c]	46.1 ± 26.1	26.6 ± 16.5	**63.5 ± 46.2** [a]	86.0 ± 26.0	118.6 ± 53.8
ADO2>WT control	39.1 ± 20.9	24.6 ± 10.8	30.9 ± 29.8	19.3 ± 13.5	44.0 ± 30.3	81.4 ± 42.1	122.5 ± 57.4

[a] $p<0.05$ versus time-matched WT>WT control

[b] $p<0.005$ versus time-matched WT>WT control

[c] $p<0.05$ versus time-matched ADO2>ADO2 control in young or old mice

CTX: carboxy-terminal collagen, TRAP: tartrate-resistant acid phosphatase 5, isoform b, P1NP: pro-collagen 1 intact N-terminal

Table 2.

Comparison of serum biochemistry in mice received bone marrow transplantation at young and old age

	Calcium (mg/dL)	Phosphorus (mg/dL)	Creatinine (mg/dL)	BUN (mg/dL)	ALP (IU/L)
	Month 5/6[a]	**Month 5/6[a]**	**Month 5/6[a]**	**Month 5/6[a]**	**Month 5/6[a]**
Young (12 weeks old) mice					
WT>WT control	9.50 ± 0.4	5.77 ± 1.0	0.36 ± 0.10	16.70 ± 2.9	200.9 ± 58.3
ADO2>ADO2 control	9.32 ± 1.0	5.20 ± 1.0	0.44 ± 0.15	17.74 ± 3.5	184.1 ± 69.5
WT>ADO2 experimental	9.19 ± 0.4	5.13 ± 0.8	0.39 ± 0.07	17.49 ± 3.8	**298.0 ± 101.4**
ADO2>WT control	9.44 ± 0.4	6.07 ± 1.9	0.38 ± 0.04	19.19 ± 4.6	189.2 ± 92.9
Old (9 months old) mice					
WT>WT control	6.92 ± 0.5	3.75 ± 0.6	0.23 ± 0.03	14.66 ± 2.5	292.7 ± 118.0
ADO2>ADO2 control	**9.81 ± 0.5**	**7.85 ± 2.8**	**0.35 ± 0.08**	**18.71 ± 2.1**	278.9 ± 108.3
WT>ADO2 experimental	7.51 ± 1.4	5.39 ± 1.5	0.28 ± 0.06	14.48 ± 2.0	280.0 ± 105.2
ADO2>WT control	6.92 ± 1.2	4.96 ± 0.9	0.20 ± 0.06	17.00 ± 6.3	264.7 ± 131.6

Bold represents significant difference versus time-matched values for all other groups in young or old mice

[a]Month 5 for old mice and month 6 for young mice