Vantictumab, an anti-FZD antibody, Rescues the Osteopetrotic Bone Phenotype in Heterozygous ADO Mice

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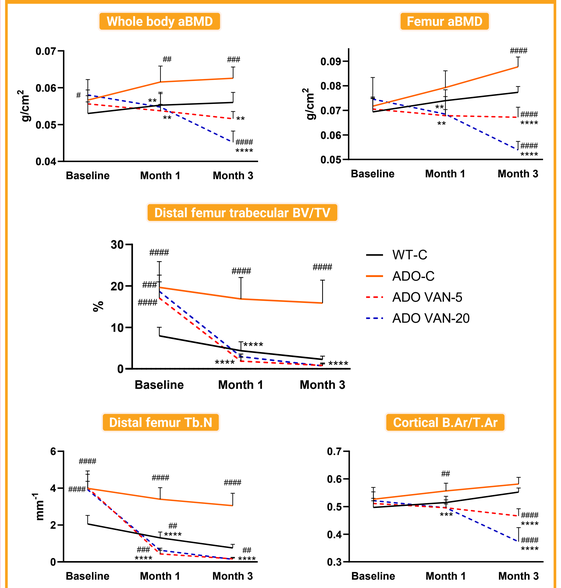
INTRODUCTION

Autosomal Dominant Osteopetrosis (ADO) is a rare bone disease of impaired osteoclastic bone resorption that usually results from heterozygous missense mutations in the chloride channel 7 (*CLCN7*) gene (1-3). Previously, we created mouse models of ADO2 by introducing a knock-in (p.G213R) mutation in the *Clcn7* gene, which is analogous to one of the common mutations (G215R) in humans (4). Vantictumab (VAN), an anti-FZD monoclonal antibody, has been previously used in clinical trials in oncology (5-7). VAN binds to several frizzled receptors (FZD1, 2, 5, 7 and 8) and thereby inhibits Wnt signaling which plays a crucial role in bone homeostasis (8). Patients receiving VAN treatment showed increased serum CTX levels indicating increased osteoclast activity. In this study, we evaluated the effects of VAN administered intraperitoneally twice weekly on bone phenotypes in 12-week-old female ADO mice in both short-term (1-month) and long-term (3 months) studies. PBS-treated wild-type and ADO mice served as controls.

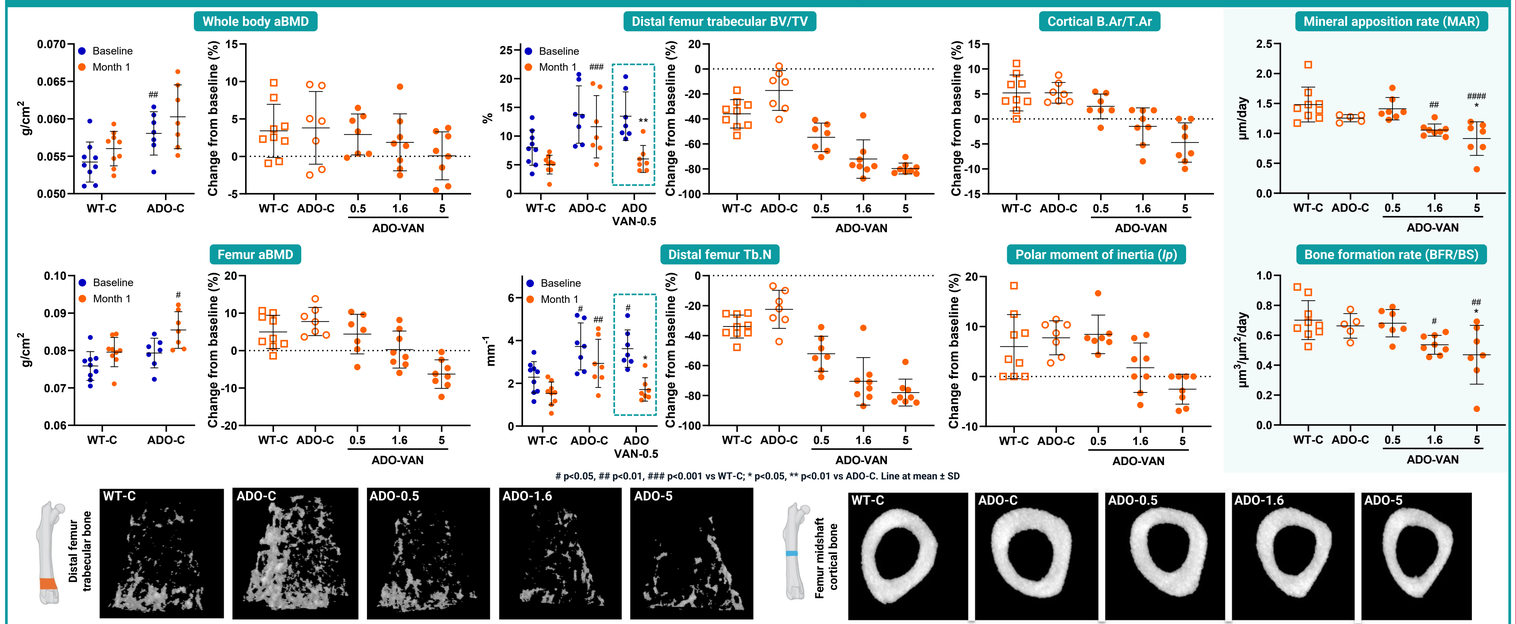
Our findings demonstrate that VAN treatment rescued the osteopetrotic bone phenotypes in ADO heterozygous mice and support clinical investigation of VAN in patients with ADO.

Long-term or 3 months study



# p<0.05, ## p<0.01, ### p<0.001, #### p<0.0001 vs WT-C; * p<0.05, **p<0.01, *** p<0.001, **** p<0.0001 vs ADO-C
Line at mean + SD

Short-term or 1 month study



# p<0.05, ## p<0.01, ### p<0.001 vs WT-C; * p<0.05, ** p<0.01 vs ADO-C. Line at mean ± SD

METHODS

Experimental Animals: ADO female mice at 12 weeks of age were administered a murine IgG1 chimera of VAN intraperitoneally twice weekly for short-term or 1-month (0.5, 1.6 and 5 mg/kg BW) and long-term or 3 months (5 and 20 mg/kg BW), whereas wild-type (WT) and ADO mice treated with PBS served as control (C) (n=5-9/group).

Bone Density and Micro-architecture: The whole body and femur areal bone mineral density (aBMD) were measured using *in-vivo* dual energy X-ray absorptiometry (DXA) (PIXImus II mouse densitometer, WI). Femurs were scanned with a high resolution *in-vivo* µCT machine (Skyscan 1176, Bruker, Belgium) with an isotropic voxel size of 9 μm^3. Trabecular bone measurements (BV/TV, Tb.N, Tb.Th, and Tb.Sp) were performed at distal femur consisting of 200 slices about 0.5 mm below the growth plate. Cortical bone parameters (B.Ar/T.Ar, Ct.Th, and Ip) were measured at femur midshaft.

Dynamic Histomorphometry: The parameters for osteoblast dynamic histomorphometry (MAR: mineral apposition rate and BFR/BS: bone formation rate) were performed at proximal femur for the short-term study.

SUMMARY AND CONCLUSIONS

1. Vantictumab (VAN) treatment in ADO mice at higher doses (5 and 20 mg/kg) for the duration of 3 months resulted in bone loss significantly lower than that of WT control mice, indicating overtreatment.
2. Whole body and femur bone mineral density (BMD) gains in ADO mice were prevented by VAN treatment in a dose dependent manner.
3. VAN treatment at the lowest dose (0.5 mg/kg) completely rescued the trabecular bone mass gain in ADO mice.
4. Cortical bone mass and strength values in the ADO mice treated with lowest dose of VAN were mostly preserved.
5. Very low dose of VAN treatment did not influence the bone formation (BFR/BS) and mineral apposition rates (MAR) in ADO mice.

Our findings indicate that vantictumab rescued the osteopetrotic bone phenotypes in ADO heterozygous mice. This study supports clinical trial of VAN in patients with ADO.

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