Sexual Dimorphism in Treatment Effect of Anti-Siglec 15 in Adult Mice with Moderate-to-Severe Osteogenesis Imperfecta

Ketsia Seide[1], Holly LoTurco[1], Erin Carter[1], Jack Mulcrone[1], Priyanka Kothari[3], Sol Langermann[3], Nancy Pleshko[2], Cathleen Raggio[1]
[1]Hospital for Special Surgery, New York, NY, [2]Temple University, Philadelphia, PA, [3]Nextcure, Beltsville, MD, United States
RaggioC@hss.edu

INTRODUCTION: Osteogenesis imperfecta (OI) is a heterogeneous type 1 collagenopathy that alters either the quantity or quality of type I collagen. It is characterized by bone fragility which often leads to high fracture incidence. The *oim/oim* mouse is an established model of moderate-to-severe OI that contains a naturally occurring *COL1A2* variant that leads to proα2(I) collagen deficiency. We previously demonstrated that a monoclonal antibody targeting the sialic acid-binding immunoglobulin-like lectin 15 (Siglec 15) immunoreceptor [1], NP159 (NextCure), improves bone quality and decreases fractures in female *oim/oim* mice. We have now extended the study to evaluate efficacy in male *oim/oim* mice.

METHODS: This study was IACUC-approved. In this portion of the study, eighty male mice (20 wildtype (WT) saline, 20 WT NP159, 20 *oim/oim* saline, and 20 *oim/oim* NP159) were treated from age 14 -26 weeks with either NP159 (10mg/kg/dose weekly for the first 4 weeks and biweekly for the remaining 8 weeks) or weekly saline (equal volume to the NP159 injection). Faxitron images (UltraFocus, Faxitron Bioptics LLC) were taken in the anterior-posterior and medial-lateral planes at 14 and 26 weeks to evaluate fracture incidence and healing. Left femurs were analyzed post-sacrifice for femoral length (Image J, NIH), microcomputed tomography (micro-CT) cortical and trabecular parameters (Scanco µCT software), and biomechanical 3-point bend testing (ELF3200, Bose Corp). Harvested right tibias were embedded in poly methyl methacrylate (PMMA), sectioned at 2 microns and analyzed by Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer Spotlight 400 system) for mineral quality parameters. Statistical analysis was performed using Two-way analysis of variance (ANOVA) (SPSS 22, IBM and JMP 16, SAS institute). A $p \leq 0.05$ was considered significant. These results were compared to a previous study completed with female mice where they were treated with the same dose of NP159.

RESULTS SECTION: With NP159 treatment, 90% of male *oim/oim* versus 85% of female *oim/oim* seen earlier had no new fractures at 26 weeks, which was similar to the incidence observed in control saline mice (Figure 1). Micro-CT showed decreased trabecular separation with NP159 treatment in both *oim/oim* males and females (p=0.05) (Figure 2). Cortical porosity was normal in the male *oim/oim* treated with NP159, in contrast with females who showed increased porosity. There were no changes in the bone mineral density for male or female mice. NP159 increased stiffness of the female *oim/oim* bones such that they tended to no longer be significantly different from treated WT bones (p = 0.08). However, no changes in mechanical parameters were observed in the male *oim/oim* bone with treatment, FTIR data in females showed differences between parameters in saline WT and *oim/oim* mice, and a normalization of mineral:matrix ratio and carbonate content to that of the WT animals; in contrast the males did not show similar changes.

DISCUSSION: Overall these results show promising trends for the use of NP159 treatment for OI. Fracture incidence was reduced. Sexual dimorphism was seen with treatment and is consistent with dimorphism both in humans and previously reported treated mouse models. The observed cortical and trabecular bone changes support the premise that NP159 works as both an antiresorptive and a bone formation agent, and seems to be favorable

SIGNIFICANCE/CLINICAL RELEVANCE: Bisphosphonates, which decrease fracture incidence, are currently recommended to manage orthopedic indications in children with moderate to severe OI, but gaps persist for effective pharmacological treatment in adult patients. The differences seen i with NP159 treatment between male and female mice in this study highlight the importance of considering sex when searching for possible therapies for OI.

REFERENCES: 1.Sato, D., Takahata, M., … Iwasaki, N. Bone 2018; 116, 172–180.

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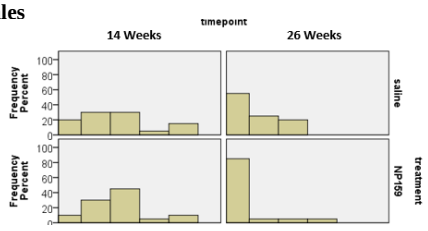
Females



Males

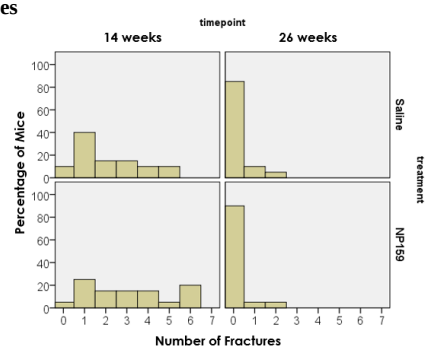


Figure 1. Graph of frequency of fractures at 14 and 26 weeks of Female and male oim/oim mice

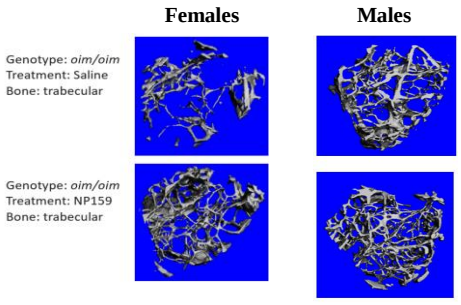


Figure 2. Micro-CT 3D images of female and male oim/oim trabecular bone