integrity without indications of toxicity. Dysferlin deficient mice receiving AAV9-341 through intravenous injection demonstrated increased rearing activity that was sustained 6 months post-injection. Consistently a trend of decreased muscle damage was observed in these mice. Our data suggest that 341, and additional hybrid dysferlin candidates under evaluation, may find relevance for the treatment of dysferlinopathy.

## 631. Rankl Knock-Out Mesenchymal Stromal Cells Have an Unexpected Osteogenic Differentiation Defect Which Is Improved by a RANKL-Expressing Lentiviral Vector

Ciro Menale[1,2], Francesca Schena[3], Lorenzo Diomede[1,2], Lucia Sergi Sergi[4], Lucia Susani[1,2], Eleonora Palagano[1,2,5], Elisabetta Traggiai[6], Cristina Sobacchi[1,2], Anna Villa[1,2]
*[1]CNR-IRGB, Milan Unit, Milano, Italy, [2]Humanitas Clinical and Research Institute, Rozzano, Italy, [3]Istituto Gaslini, Genoa, Italy, Italy, [4]San Raffaele Telethon Institute for Gene Therapy (SR-TIGET), IRCCS San Raffaele Scientific Institute, Milano, Italy, [5]BIOMETRA Dept, University of Milan, Milano, Italy, [6]Novartis Institute for Research in Biomedicine, Basel, Switzerland*

Osteoclast-poor RANKL-dependent Autosomal Recessive Osteopetrosis (ARO) is a rare bone disease characterized by an increase in bone density due to the failure of bone resorption by impaired osteoclast formation. Hematopoietic stem cell transplantation is not an effective therapy for this ARO form, since in bone RANKL is produced mainly by cells of mesenchymal origin. Therefore Mesenchymal Stromal Cells (MSC) transplantation together with a gene-therapy strategy to correct RANKL defect in MSC could represent a possible effective therapy. Of note, whether also MSC, besides the osteoclasts, are affected by RANKL deficiency is unknown. To verify this, we established and characterized bone marrow derived MSC (BM-MSC) lines from the Rankl$^{-/-}$ (KO) mouse model, which recapitulates the human disease, and from wild type (WT) mice. No differences were found between KO and WT MSC in terms of morphology, immunophenotype and proliferation capacity. However, KO MSC displayed a reduced clonogenic potential with a decrease in stemness genes expression. KO MSC were able to normally differentiate towards the adipogenic and chondrogenic lineages, while showed a significantly impaired osteogenic differentiation capacity compared to WT MSC, as demonstrated by reduced Alizarin Red staining (ARS) and expression of osteogenic genes. To confirm that this alteration was due to the lack of functional RANKL, we developed a third generation lentiviral vector expressing human soluble RANKL (hsRL) for the genetic correction of KO MSC. We first investigated lentiviral transduction in 293T cells to optimize transduction efficiency at different multiplicity of infection (MOI) ranging from 1 to 100. hsRL production increased proportionally to the MOI and was stable over time. However, the higher the MOI the higher the cytotoxicity observed. Based on these data, we performed a lentiviral hsRL transduction in KO MSC at 20 and 50 MOI, to define the optimal transduction conditions. After transduction 99.5% of MSC were GFP$^{+}$. While in Rankl$^{-/-}$ control cells the cytokine was not detected, in corrected cells hsRL production and secretion was measurable and comparable to sRL levels in WT mouse. KO MSC stably expressing hsRL showed an improved osteogenic differentiation capacity compared to untransduced KO MSC, as demonstrated by increased ARS and expression of osteogenic genes. Moreover, the expression of RANK receptor in both MSC suggested an autocrine role of sRL as possible mechanism. Our data suggest that restoration of RANKL production in lentiviral-transduced KO MSC might not only allow osteoclast differentiation in Rankl$^{-/-}$ mice upon transplantation, but also improve the osteogenic differentiation defect of KO MSC.

## 632. Alpha-1 Antitrypsin Gene Therapy Prevented Bone Loss in an Ovariectomy Induced Osteoporosis Mouse Model

Mohammad A. Akbar[1], Jay J. Cao[2], Yuanqing Lu[3], David Nardo[1], Mong-Jen Chen[1], Ahmed S. Elshikha[1], Rubina Ahamed[1], Mark Brantly[3], Lung-ji Chang[4], Lexie Shannon Holliday[5], Sihong Song[1]
*[1]Pharmaceutics, University of Florida, Gainesville, FL, [2]USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND, [3]Medicine, University of Florida, Gainesville, FL, [4]Molecular Genetics and Microbiology, University of Florida, Gainesville, FL, [5]Orthodontics, University of Florida, Gainesville, FL*

Osteoporosis is a major healthcare burden affecting mostly postmenopausal women characterized by compromised bone strength and increased risk of fragility fracture. Although pathogenesis of this disease is complex, inflammation is clearly involved in bone loss at menopause. Therefore, anti-inflammatory strategies hold great potential for the prevention of postmenopausal osteoporosis. Human alpha-1 antitrypsin (hAAT) is a multifunctional protein that has anti-inflammatory and cytoprotective properties. In this study, we investigated the protective effect of hAAT against bone loss. In vitro studies showed that hAAT significantly inhibited osteoclast formation and function in a dose-dependent manner. Treatment of hAAT inhibited M-CSF (macrophage colony-stimulating factor) induced cell surface RANK receptor expression by downregulating cFos mRNA expression. To test the protective effect of hAAT in an osteoporosis mouse model, we treated ovariectomized (OVX) mice with rAAV8-CB-hAAT, or mesenchymal stem cells (MSCs) infected with a lentiviral vector expressing hAAT (MSC-Lenti-hAAT) or phosphate buffer saline (PBS). Sham operated age-matched animals were used as controls. Eight weeks after the treatment, animals were sacrificed and subjected to μCT scanning for the evaluation of vertebral bone microarchitecture. Gene and stem cell-based hAAT therapies significantly increased bone volume density, trabecular number and decreased structure model index compared to PBS injection in OVX mice. Gene therapy also increased connectivity, density and trabecular thickness compared to PBS injection in OVX mice. We also observed that both therapies inhibited RANK gene expression in bone, which is consistent with the results of our *in vitro* study. These results demonstrate that hAAT gene and MSCs based therapies mitigate ovariectomy-induced bone loss in a mouse model, possibly through inhibition of osteoclast formation by reducing RANK gene expression. Considering the safety profile of the hAAT and rAAV vector in human, our results provide a new insight for the treatment of osteoporosis.

## 633. A Reproducible and Reliable Non-Invasive Approach to Assess the Efficacy of Gene Therapy in mdx Mice with Relevance to Clinical Trials

Yafeng Song, Mihail Petrov, Shira Rosenblum, Leon Morales, Hansell H. Stedman
*Surgery, University of Pennsylvania, Philadelphia, PA*

The mdx mouse carries a naturally occurring nonsense mutation in the dystrophin gene and has become a critical animal model for research on Duchenne Muscular Dystrophy (DMD). Despite the extensive published studies on muscle structure and function in this model, we still lack appropriate protocols for evaluation of functional rescue in mdx mice using non-invasive tests relevant to the clinically relevant symptoms of DMD. Here we present for the first time results of a complex approach for the evaluation of locomotor and behavioral patterns of mdx mice during running wheel performance in a modified open field monitoring system. These results are examined in the same mice with force grip measurements and serum enzyme biomarker