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+ONLINE EXPERIENCE

ENPP1-Fc expressing AAV vector prevents ectopic tissue calcification and restores bone parameters in ENPP1 deficient mice

K. O'Brien[1], L. Flaman[1], C. Sullivan[1], J. Howe[1], S. Jungles[1], A. Lynch[1], D. Schrier[1], Z. Cheng[1], Y. Sabbagh[1]

[1] Inozyme Pharma, Boston MA

INTRODUCTION

ENPP1 Deficiency

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is the major enzyme that generates extracellular pyrophosphate (PPi), an inorganic metabolite with potent anti-calcification activity.[1-3] Loss-of-function mutations lead to a state of ENPP1 Deficiency and hypopyrophosphatemia, which is associated with extensive calcification of the arteries, organs and joints. Infants with ENPP1 Deficiency present with severe cardiovascular complications and over 50% mortality in the first 6 months of life.[5-6] Those who survive into childhood-adulthood typically develop hypophosphatemic rickets, characterized by growth plate abnormalities, bowed legs, short stature, and/or calcification of the joints and ligaments.[4-6]

AAV-ENPP1 Gene Therapy

An adeno-associated viral vector that expresses a modified human ENPP1-Fc under the control of a liver-specific promoter (AAV-ENPP1) was developed as a one-dose gene therapy to treat ENPP1 Deficiency.

We previously reported that a single intravenous injection ($2.5x10^{13}$ vg/kg) of AAV-ENPP1 in 2-week-old *Enpp1*$^{asj\text{-}2J/asj\text{-}2J}$ mice (a murine model of ENPP1 Deficiency) resulted in sustained elevation of plasma ENPP1 activity during the 10-week study.[7] It also rescued plasma PPi levels, prevented calcification in all organs analyzed, and inhibited the development of bone abnormalities.

OBJECTIVES

1. To analyze the impact of AAV-ENPP1 on prevention of bone and spinal ligament defects in *Enpp1*$^{asj\text{-}2J/asj\text{-}2J}$ mice
2. To evaluate the impact of lower dose AAV-ENPP1 therapy on PPi levels and soft tissue calcification in *Enpp1*$^{asj\text{-}2J/asj\text{-}2J}$ mice

STUDY DESIGN AND METHODS

2-week-old *Enpp1*$^{asj\text{-}2J/asj\text{-}2J}$ mice were given a single $2.5x10^{13}$ vg/kg dose of AAV-ENPP1 or vehicle, and bone microarchitecture and dynamic histomorphometry were evaluated 10 weeks after injection. Fixed cervical spines were scanned and evaluated using Scanco µCT35 (Scanco Medical, AG, Switzerland) by UMass Chan Medical School Bone Core with an X-ray energy intensity of 55kV with a current of 145mA and 400ms integration time. Quantitative analyses were carried out using IPL software (Scanco Medical, AG, Switerland). The cervical areas were scanned with 12µm voxels (1024 x 1024 pixels) and 2D images were generated using IRW Ver 4.2 software (Siemens, USA). Decalcified tibia were embedded using paraffin wax, sectioned at 6µm, and stained using Safranin O by UMass Chan Medical School Bone Core.

Prior experiments demonstrating efficacy of AAV-ENPP1 on plasma PPi and ectopic calcification 10 weeks after injection were repeated in mice treated with three lower (single) doses of AAV-ENPP1 ($1.5x10^{12}$, $5.0x10^{12}$, and $1.5x10^{13}$ vg/kg).

AAV-ENPP1 RESTORES PLASMA PPi LEVELS

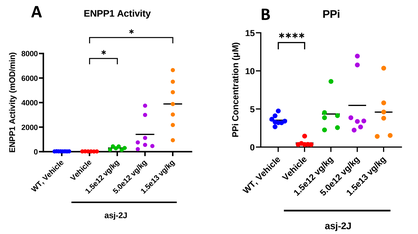

Fig 1. Plasma ENPP1 activity levels **(A)** and Plasma PPi levels **(B)**, at day 70 following a single IV dose on day 1 of ENPP1-Fc-AAV or vehicle. * p ≤ 0.05, **** p ≤ 0.0001

AAV-ENPP1 PREVENTS SOFT TISSUE CALCIFICATION

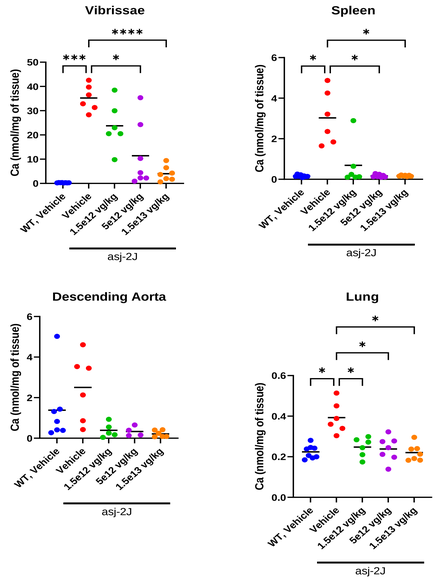

Figure 2. Terminal calcium levels of various tissues. WT and Asj-2J, vehicle treated are shown in red and blue, respectively. AAV-ENPP1-Fc treated Asj-2J groups are shown in green (1.5e12 vg/kg), purple (5.0e12 vg/kg) and orange (1.5e13 vg/kg). * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001

AAV-ENPP1 PREVENTS VERTEBRAL CALCIFICATION

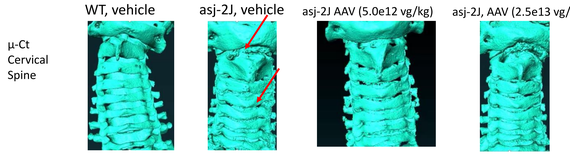

Fig 3. Representative examples of spine animations from the three groups analyzed. Red arrows highlight calcifications in the vehicle-treated asj-2J group which are more prominent compared to the ENPP1-Fc-AAV treated group

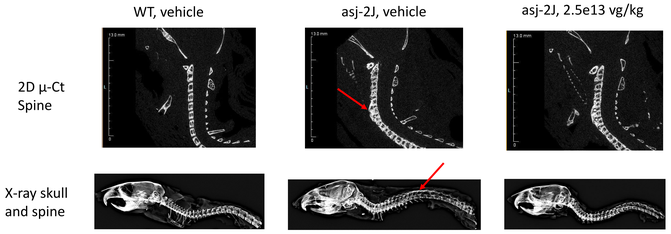

Fig 4. Representative examples of 2D µ-CT images showing head and spines of the three groups analyzed. Red arrows highlight calcifications in the vehicle-treated asj-2J group which are more prominent compared to the ENPP1-Fc-AAV treated group.

AAV-ENPP1 PREVENTS DEVELOPMENT OF GROWTH PLATE ABNORMALITIES

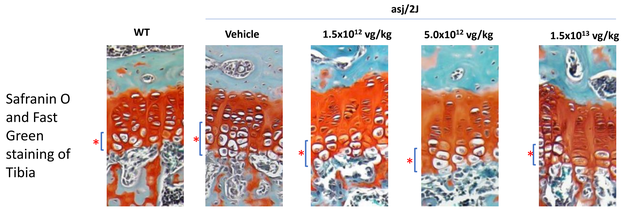

Fig 5. Representative images of Safranin O and Fast Green-stained images from the tibia from all three AAV dosed groups and controls. Red asterisk denotes hypertrophic chondrocytes.

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AAV-ENPP1 RESTORES CRITICAL BONE PARAMETERS

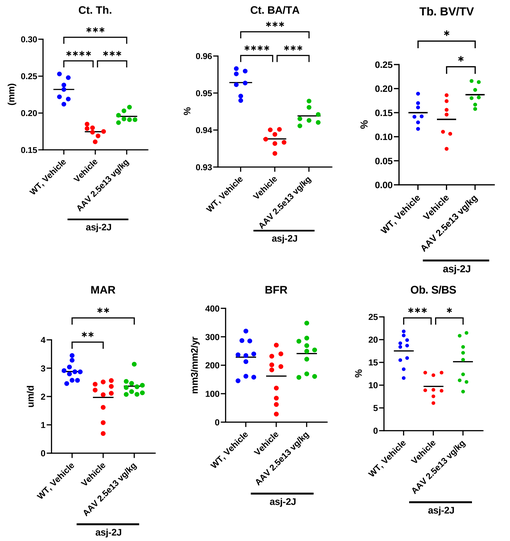

Fig 6. Static and dynamic histomorphometry from day 70 following a single intravenous injection of AAV-ENPP1 at 2.5e13 vg/kg. * p ≤ 0.05, ** p ≤ 0.01 *** p ≤ 0.001, **** p ≤ 0.0001
Cortical Thickness (Ct. Th.), Cortical Bone Area/Total Area (Ct. BA/TA), Trabecular Bone volume/total volume (Tb. BV/TV), Mineral apposition rate (MAR), Bone formation rate (BFR), Osteoblast surface/bone surface (Ob. S/BS).

CONCLUSIONS

A single 1.5e12 vg/kg intravenous dose of AAV-ENPP1 is sufficient to elevate plasma pyrophosphate to levels seen in WT animals, prevent ectopic calcification in soft tissues, and prevent the development of rickets in an ENPP1-Deficient mouse model.

A single intravenous dose of AAV-ENPP1 as low as $5.0e10^{12}$ vg/kg is sufficient to prevent vertebral calcification in ENPP1-Deficient mice. Based on these results AAV-ENPP1 could prove an effective gene therapy for disorders of vertebral calcification such as ossification of the posterior longitudinal ligament (OPLL).

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