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# Therapeutic applications of ENPP1-Fc prevent muscle calcification following cardiotoxin-induced muscle damage in *Abcc6*$^{-/-}$ mice

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## INTRODUCTION

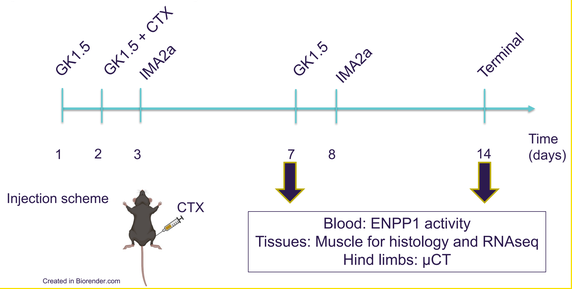
Ectopic mineralization (EM) from burns, blast injury, spinal cord injury, or surgical trauma range from dystrophic calcifications to heterotopic ossification. No effective therapeutic intervention exists, apart from surgical excision of affected regions, which can prolong or exacerbate recovery from the initial injury. Pyrophosphate (PPi) is a potent inhibitor of mineralization. Here we describe a cardiotoxin-muscle injury model in Abcc6$^{-/-}$ mutant mice, which have a 50% reduction of circulating PPi levels compared to WT mice and exhibit a susceptibility to ectopic mineralization (). ENPP1-Fc is currently being used in clinical trials to treat disorders of low pyrophosphate including ENPP1 and ABCC6 Deficiencies. In pre-clinical models a surrogate of ENPP1-Fc administration prevents mineralization of soft tissues and restores circulating PPi levels. Here we utilize a modified version of ENPP1-Fc termed IMA2a which exhibits a longer half-life.

## AIM

The aim of the study is to assess if treatment using a surrogate of ENPP1-Fc (IMA2a) is able to prevent ectopic mineralization of muscle in a cardiotoxin-induced muscle injury model. Mineralization was assessed by µCT analysis and muscle tissue responses by RNAseq analyses.

## METHODS

- Abcc6$^{-/-}$ male mice (10-12 weeks old) received an IP injection of immunosuppressant (anti CD4 antibody, GK1.5) on days 1, 2 and 8, Intramuscular cardiotoxin (CTX) on day 2, and subcutaneous injection of IMA2a on days 3 and 8




**References**

Li Q et al. Mouse models for pseudoxanthoma elasticum: genetic and dietary modulation of the ectopic mineralization phenotypes. PLoS One. 2014.

Moore-Lotridge SN et al. Trauma-Induced Nanohydroxyapatite Deposition in Skeletal Muscle is Sufficient to Drive Heterotopic Ossification. Calcif Tissue Int. 2019.

Jacob U et al. INZ-701, a recombinant ENPP1 enzyme, prevents ectopic calcification in an Abcc6-/- mouse model of Pseudoxanthoma Elasticum. Exp. Derm. 2022.

## 1 Mineralization peaks 14 days after injury

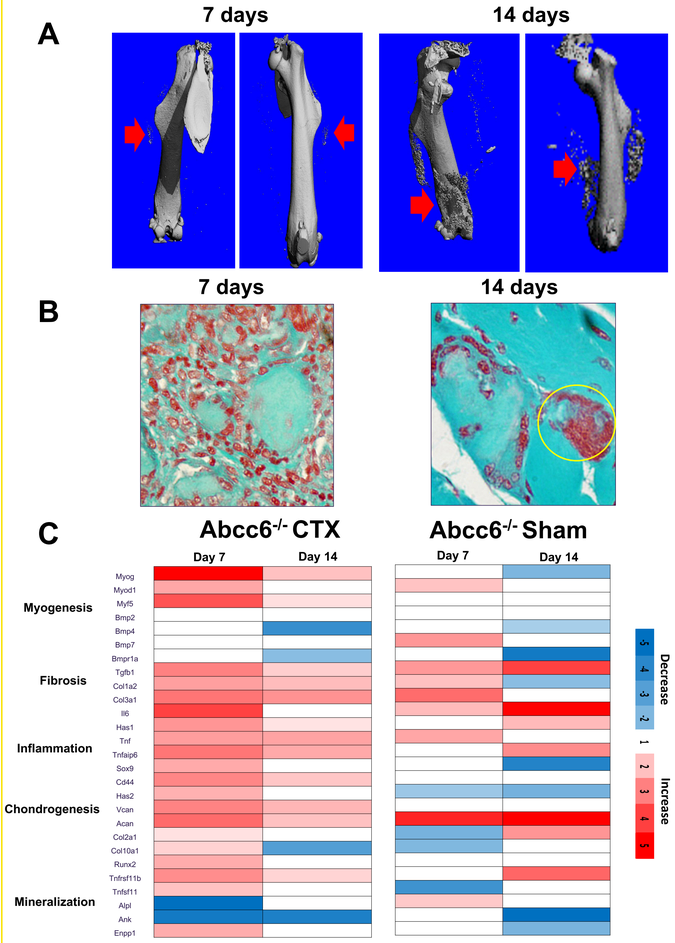



Figure 1: A) µCT of femurs of CTX injected limb at 7 and 14 days. Red arrows shows calcified area. B) Safranin-O staining of injured muscle at 7 and 14 days. Area of chondrogenic clustering of progenitor cells clustering is circled. C) Individual gene expression changes for ABCC6$^{-/-}$ mice on day 7 and 14 after CTX or sham (PBS) injury.

## 2 IMA2a prevents mineralization in this model

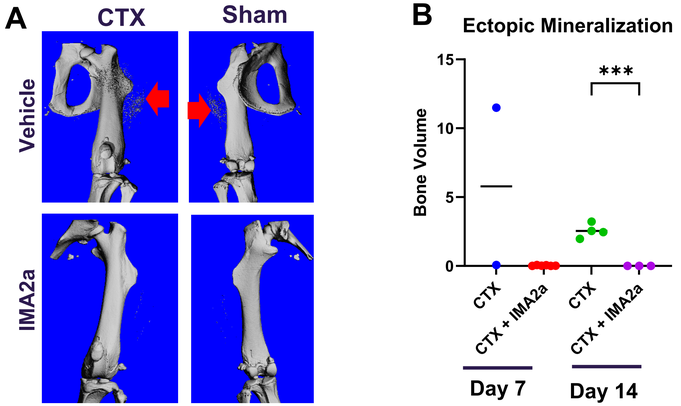



Figure 2: A) Representative µCT of limbs injected with Cardiotoxin (CTX) or contralateral limb (PBS) of animals treated with Vehicle or IMA2a. Areas of calcification are shown with the red arrows. B) Quantitation of calcification. Unpaired t-test (p = 0.0004)

## 3 IMA2a shows favorable PK

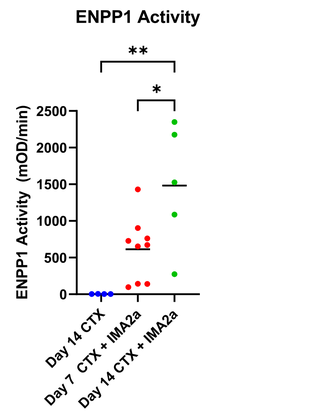
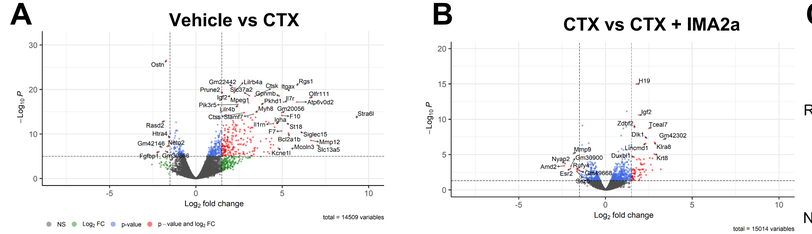



Figure 3: IMA2a concentration in the plasma of vehicle or IMA2a treated animals. One-Way ANOVA with Tukey's post-test. * P=0.0286, ** P=0.0026.

## 4 Differentially expressed genes




C

| | Gene ID | log FC vs No Drug | p value | Reference |
|---|---|---|---|---|
| Muscle Regeneration | *Ripply1* | **3.20** | 0.00122 | PMID: 25772056 |
| | *Rtl1 (Peg11)* | **2.56** | 0.00003 | PMID: 32878916 |
| | *Toeal7* | **2.51** | 0.00000 | PMID: 37047236 |
| | *Dlk1* | **2.36** | 0.00000 | PMID: 21124733 |
| | *Lincmd1* ** | **2.32** | 0.00000 | PMID: 31572510 |
| | *Myh3* ** | **2.21** | 0.00005 | PMID: 36253149 |
| | *Myh8* | **2.03** | 0.00016 | PMID: 19181095 |
| | *Mir329* | **1.99** | 0.00774 | PMID: 25086941 |
| | *Gjd4* | **1.98** | 0.00168 | PMID: 38936363 |
| | *Mymk* | **1.66** | 0.00158 | PMID: 31642939 |
| | *Duxbl1* ** | **1.58** | 0.00002 | PMID: 32010697 |
| | *Mmp9* | **-1.85** | 0.00001 | PMID: 25252612 |
| Neuromuscular Junctions | *Unc13a* | **1.89** | 0.00132 | |
| | *Chrng* | **1.57** | 0.01132 | |
| | *Sez6* | **-1.65** | 0.00234 | |
| | *Nyap2* | **-2.32** | 0.00019 | PMID: 21946561 |

Figure 4: A) Volcano plots showing DEGs in CDTX vs Naïve muscle tissues. B) Volcano plots showing DEGs in CDTX injured muscle tissues after IMA2a treatment. C) IMA2a treatment activated genes included those associated with muscle and neuromuscular junction regeneration.

## CONCLUSIONS AND FUTURE DIRECTIONS

- Treatment with IMA2a prevents ectopic mineralization of CTX-injured skeletal muscle tissue.
- Bulk RNAseq analyses of muscle tissues from additional IMA2a treatment times to delineate a therapeutic mechanism
- Evaluation of IMA2a efficacy to attenuate ectopic mineralization in other ectopic mineralization models such as in a post-burn injury murine model