# Synergy between growth hormone and bisphosphonate in a mouse model of osteogenesis imperfecta

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## Overview

Osteogenesis Imperfecta (OI) or **brittle bone disease** is a genetic disorder with a prevalence of **1 in 15,000** newborns worldwide.

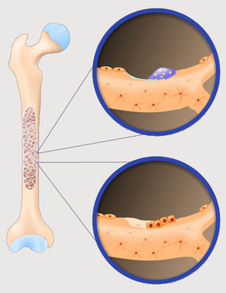
Characterised by bone fragility and frequent fractures, OI affected children can experience **10 times more fractures** than their healthy peers throughout childhood.


An example of a moderate form of OI - OI Type IV

Bisphosphonates are a class of **bone anti-resorptive drugs** widely used in OI management. However, there is increasing interest in the **off-label use** of human growth hormone (GH) to **promote bone growth** and counter short stature.

Here, we examined the effects of GH in the context of bisphosphonate dosing using a relevant **OI mouse model**. Detailed **bone phenotyping analysis** was conducted via microCT, bone histomorphometry and biomechanical testing.

## Aim & Hypothesis



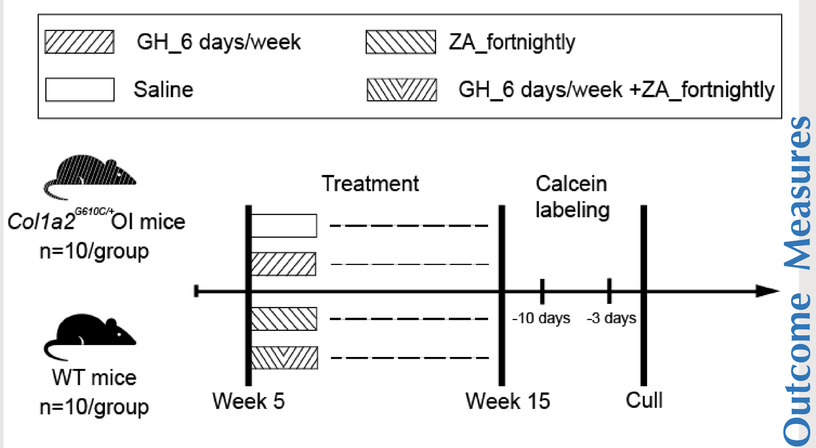
Osteoclasts mediated bone resorption ↓ inhibited by ZA

Osteoblasts driven bone formation ↑ promoted by anabolic GH

→ Synergistic effects on OI bone strength ?

We hypothesized that the **combined therapy** of GH and a bisphosphonate (zoledronic acid; ZA) would have **synergistic benefits** on the bone strength in a mouse model of **mild to moderate** OI (*Col1a2*$^{+/G610C}$).

## Research plan

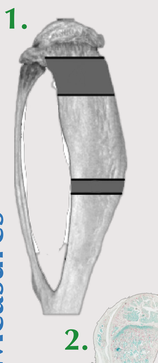


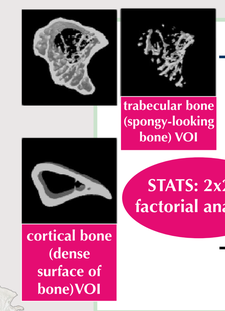

### I. Animal Experiments

We tested **ZA and GH, alone or in combination**, in a total of 80 OI mice and their wildtype (WT) littermates. Drugs were administrated to mice post-weaning for **10 weeks**.

### II. Outcome Measures

1.

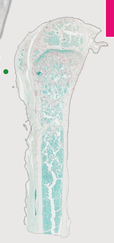





- MicroCT analysis for volumetric parameters of trabecular and cortical bone
- X-ray to measure femur length

STATS: 2x2x2 factorial analysis

2.

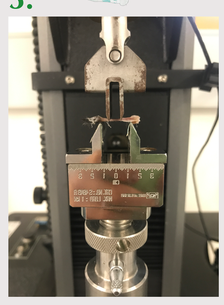


Dynamic histomorphometry

H&E staining

TRAP osteoclasts staining

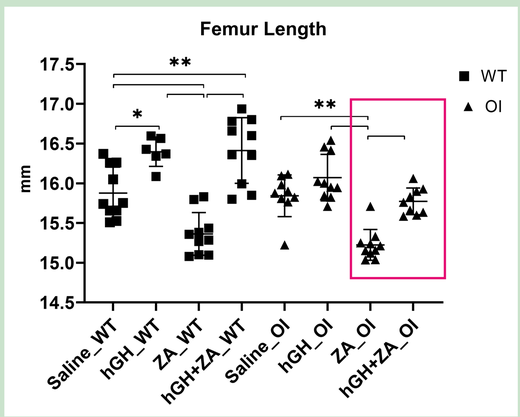
3.



Tibial 4-point bending test &.
L4 vertebral compression test to examine the actual strength of the bone

## Results

### 1. GH improved cortical bone volumetric parameters

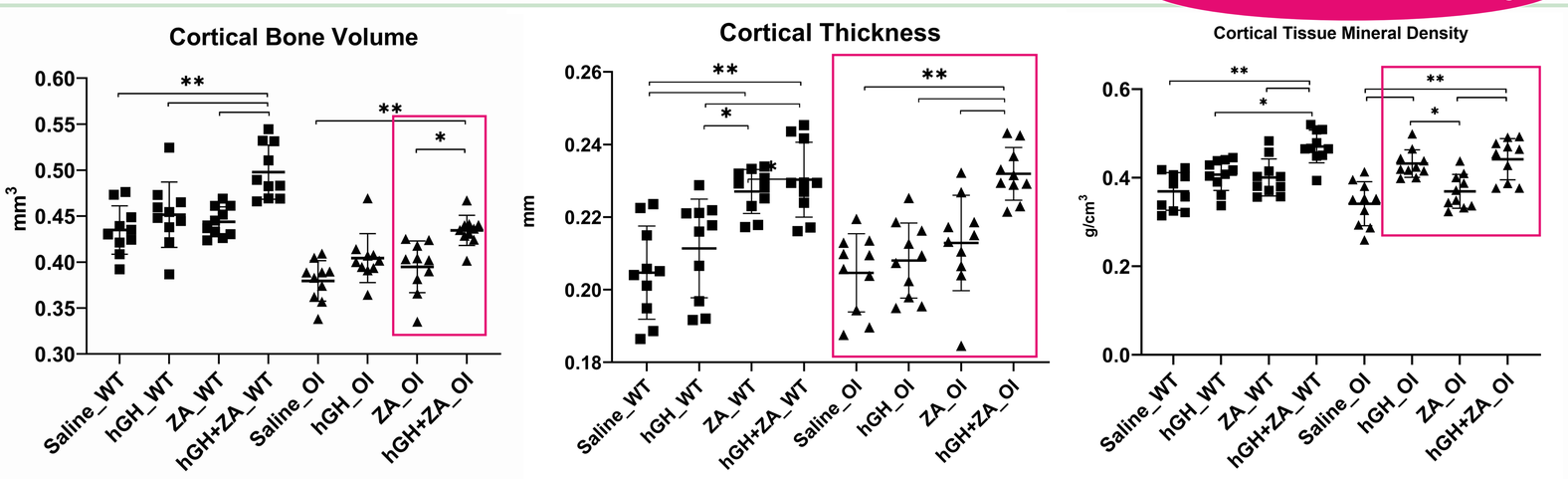



GH/ZA co-treatment reversed the worsened short stature in ZA treated OI mice. Their femur length was elongated by a significant 0.55mm on average ($p<0.05$).

MicroCT scanning separately examined the trabecular and cortical regions of tibial samples. While the trabecular bone showed minimal responses to GH treatment, the drug yielded considerable increases on cortical bone parameters.

GH/ZA treatment outperformed ZA alone in OI mice, which significantly improved the cortical bone volume, thickness and tissue mineral density (TMD) by +10%, +9% and +30%, respectively ($p<0.05$).

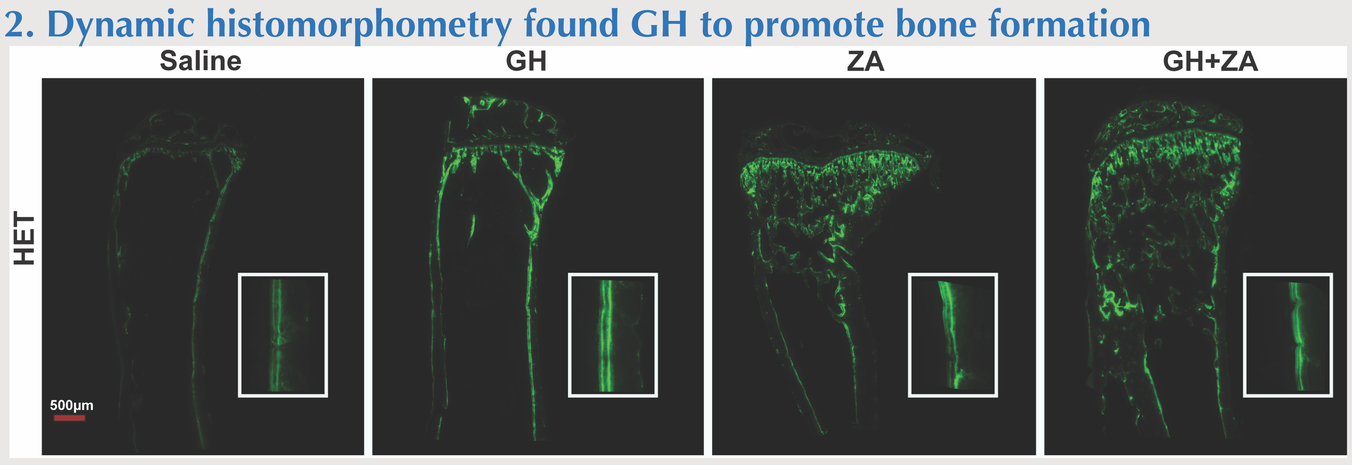
→ Reflects the bone mass & strength




The improvement of cortical thickness demonstrated a synergistic 3-way interaction between the genotype, GH and ZA. Indicated by the 2x2x2 factorial statistical analysis, GH was associated with increased cortical thickness in OI mice only at the presence of ZA.

GH treatment alone also significantly increased cortical TMD compared with saline control (+27%, $P<0.01$), confirming the anabolic efficacy of the therapy.

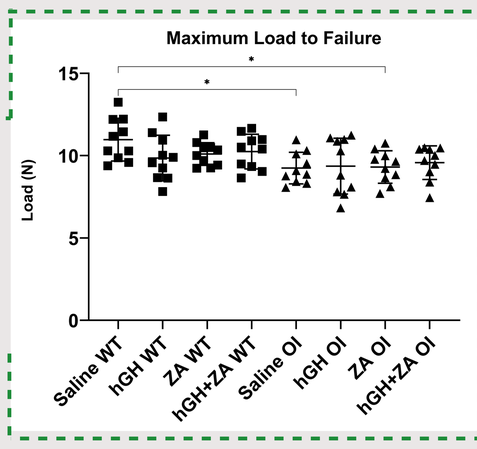
### 2. Dynamic histomorphometry found GH to promote bone formation



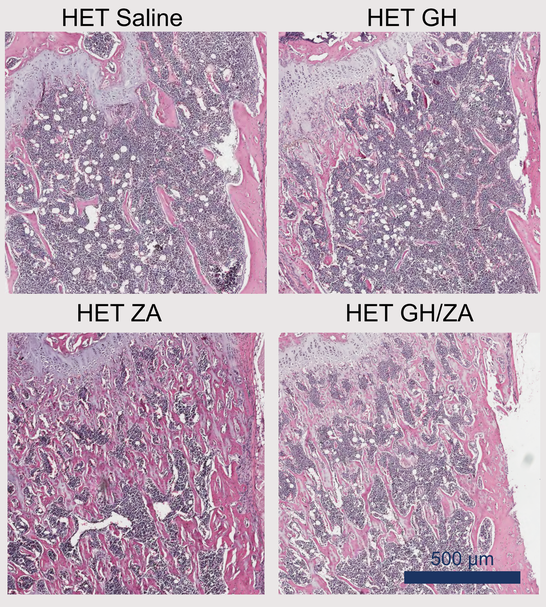

The effects of GH treatment on bone formation and mineral apposition rate (MAR) were analysed by using dynamic labelling of newly formed bone surfaces.

$$MAR = \frac{\text{Distance between the labelled surfaces}}{\text{\#of days in between the two calcein doses}}$$

Preliminary data suggested GH alone significantly increased the MAR on the endosteal bone surface by +60% ($p<0.05$) compared with ZA treatment alone in OI mice.




Representative images of hematoxylin and eosin staining demonstrated the dramatically increased trabecular bone in ZA and ZA/GH treated OI femurs.




### 3. Four-point bending test on tibia confirmed the diminished bone strength in OI

The maximum load on the tibia before its catastrophic failure revealed the bone's resistance to breaking. This test confirmed the reduced bone strength in OI compared with WT mice. Although no significant impact was found between treatment groups within each genotype, this could be attributed to the variance within each group.

## Conclusion & Future directions

This study found GH was effective in improving the bone phenotype in a mild-moderate OI mouse model. The anabolic effect of GH was particularly pronounced in the cortical region of the bone rather than the trabecular region.

The data raise no concerns regarding the use of GH in OI individuals receiving or having received bisphosphonates therapy. However, the clinical benefits of combination therapy will need to be further assessed in randomized control trials relative to the standard of care.