Porcupine inhibition as a novel therapeutic paradigm for sclerosteosis

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INTRODUCTION: Sclerosteosis (OMIM accession number 269500) is an ultra-rare, severe autosomal recessive high bone mass (HBM) disorder that has no available pharmacological treatment. The disease is characterized by increased bone mineral density and generalized skeletal overgrowth. Acute clinical symptoms, which include facial paralysis, hearing loss and elevated intracranial pressure, are managed through difficult and high-risk surgical interventions. Sclerosteosis is caused by *SOST* mutations that result in loss of functional sclerostin, a secreted glycoprotein which plays a pivotal role in controlling bone formation by antagonizing the Wnt/β-catenin signaling pathway. LGK974, a small molecule porcupine (PORCN) inhibitor, blocks palmitoylation of Wnt ligands and subsequent transport to the extracellular membrane, thus suppressing Wnt signaling. Herein we assessed the potential effectiveness of LGK974 both *in vitro* and in an *in vivo* mouse model as a novel therapeutic for sclerosteosis.

METHODS: *In vitro* cell assays explored the effect of LGK974 on osteoclast and osteoblast biology by assessing osteoclast number, bone resorption, osteoblast mineralization, alkaline phosphatase (ALP) activity and expression of Wnt pathway/osteogenic markers. To assess the potential of LGK974 to reduce HBM, 6-week-old male and female *Sost* deficient (*Sost*$^{-/-}$) mice, a well-studied model of sclerosteosis, were treated intermittently with 6 mg/kg LGK974 or DMSO vehicle for 4 weeks (n = 20; both sexes). Right tibiae were loaded (peak load of 20N) during the first two weeks of treatment to simulate loading events experienced in early childhood, with left tibiae serving as a contralateral non-loaded control. All animal procedures were approved by the Royal Veterinary College Ethics Committee and were performed in accordance with UK Animals (Scientific Procedures) Act 1986. Tibiae, vertebrae and skulls were collected and analyzed by microcomputed tomography (μCT).

RESULTS: A phenotypic screen of 5 small molecule Wnt signaling inhibitors revealed LGK974 as a promising drug candidate. LGK974 (100 nM) was non-toxic and significantly reduced osteoblast ALP activity and mineralization ($p < 0.0001$). Treatment with LGK974 decreased Axin2 (Wnt marker) expression ($p < 0.0001$) during early osteoblastic differentiation whilst Runx2 and OCN (osteoblast markers) expression were reduced ($p < 0.0001$ and $p < 0.01$, respectively) in mature osteoblasts. Osteoclast number and resorption were unaffected by LGK974 treatment *in vitro*. In *Sost*$^{-/-}$ mice, LGK974 treatment significantly reduced cortical bone volume in male and female loaded (19.2% and 16.6%, respectively; $p < 0.0001$) and non-loaded (18.2% and 18.1%, respectively; $p < 0.0001$) tibiae. Notably, the effect on tibial geometry was limited. In the axial skeleton, a significant reduction in male and female trabecular number (19.9% and 18.7%, respectively; $p < 0.0001$) was recorded in the L4 vertebral trabecular bone of LGK974 treated mice. The average bone volume of the skull was decreased significantly by 11.6% ($p < 0.05$) in males and a trend to reduced parietal bone thickness observed ($p = 0.13$). The foramen magnum diameters remained unaffected. Upon analysis of middle and inner ear parameters, LGK974 treatment significantly reduced the bone volume and surface area of the auditory ossicles (10.6%; $p < 0.01$ and 14.3%; $p < 0.0001$) and bone area of the otic capsule (7.8%; $p < 0.05$) in male mice, with a trend towards diminished otic capsule thickness noted (5.6%; $p = 0.28$). Interestingly, although reduced bone parameters were observed in females following LGK974 treatment, changes were generally significant in treated males.

DISCUSSION: In this study the Wnt pathway inhibitor LGK974 affected osteoblast biology in a manner analogous to sclerostin. Moreover, we showed, for the first time, that PORCN inhibitor treatment results in greater levels of protection against bone gains in loaded tibiae compared to non-loaded, suggesting that treatment may also be effective in highly active young sclerosteosis patients. LGK974 treatment reduced hyperostosis in the male otic cavity and ossicles, indicating that patient hearing loss could potentially also be alleviated. A decrease in skull bone formation observed in our mouse model of sclerosteosis indicates that treatment with LGK974 may also result in protection against the most dangerous and life-threatening pathology in sclerosteosis - intracranial pressure. The more pronounced LGK974-related protection against HBM phenotypes observed in males indicates potential sexual dimorphism in response to treatment and aligns with data from human sclerosteosis patients and heterozygous carriers when assessing hearing loss and patient Z-scores.

SIGNIFICANCE/CLINICAL RELEVANCE: This study discloses that PORCN inhibitor therapy reduces bone mass in multiple sites associated with the most severe pathology in sclerosteosis i.e. skull and ear, as well as the axial and appendicular skeleton. This provides the first evidence of a disease modifying treatment strategy to alleviate symptoms of sclerosteosis and other *Wnt* related HBM conditions.

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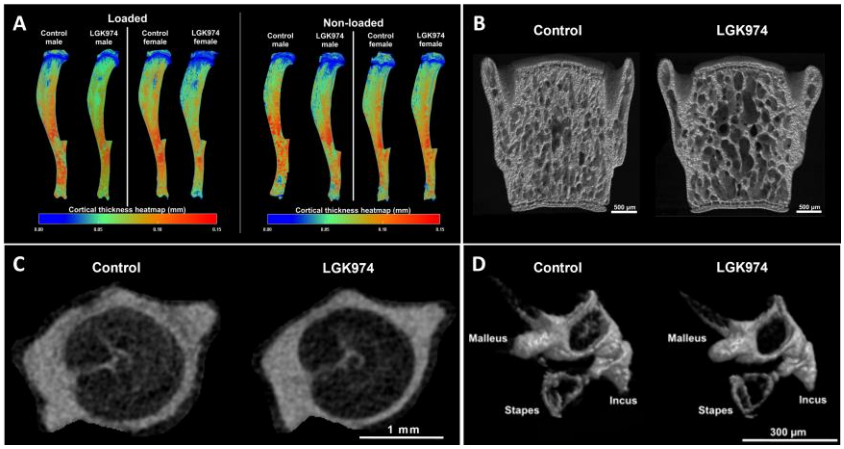

Figure 1: Effect of LGK974 treatment on *Sost*$^{-/-}$ mouse skeleton A) Representative 3D μCT images of loaded and non-loaded tibiae (heat map of cortical bone thickness; male and female) treated with vehicle (control) and LGK974. B) Changes in trabecular number are visible in 3D μCT images of the L4 vertebral bodies of treated male mice. C) Representative 2D μCT images of the otic capsule (inner ear) of vehicle control and LGK974 treated mice. D) Representative 3D μCT images of the middle ear ossicles, with distinguishable malleus, incus and stapes.