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# Learning more about Palovarotene
## An editorial for the FOP community

A commentary on:

Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with the human *ACVR1*$^{R206H}$ Fibrodysplasia Ossificans Progressiva (FOP) mutation

(Journal of Bone and Mineral Research, online March 2016)

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Heterotopic ossification (HO) in fibrodysplasia ossificans progressiva (FOP) is a multi-step process that includes an intermediate stage of cartilage formation and concludes with the formation of mature mineralized bone in soft tissues such as skeletal muscles. This cartilage-to-bone process is known as endochondral ossification. The primary goal of research for FOP is to identify effective treatments to prevent the extra-skeletal endochondral bone formation that begins early in childhood and progresses throughout life. A milestone was reached in July 2014 with the beginning of a multicenter phase 2 clinical trial for FOP. The drug that is being evaluated in the phase 2 trials is palovarotene, a retinoid agonist that activates a specific component of the retinoic acid signaling pathway (RARγ) in cells and tissues.

The clinical trial for FOP was initiated based on the strength of previous studies by two of us (Iwamoto and Pacifici) and their coworkers demonstrating that RARγ agonists are particularly powerful to prevent HO at the cartilage stage and were effective in blocking it in injury and genetic mouse models (Shimono et al 2011). Palovarotene is a drug that was previously tested for emphysema. Although the drug was not developed as a treatment for emphysema, it was shown to produce few side effects in phase 2 clinical trials in adults.

However, many questions remained unanswered about palovarotene and its use for treating FOP, including whether the drug would be effective in preventing HO triggered by the most common FOP *ACVR1*$^{R206H}$ human mutation, whether it would also improve skeletal function and joint movement, and whether it may actually impair the normal process of endochondral ossification required for skeletal growth and elongation during childhood.

These and related questions were investigated in a study that was recently published online in the *Journal of Bone and Mineral Research*: **Palovarotene inhibits heterotopic ossification and maintains limb mobility and growth in mice with the human *ACVR1*$^{R206H}$ Fibrodysplasia Ossificans Progressiva (FOP) mutation**. This study was a joint effort and the result of a highly productive collaboration between researchers at the Children's Hospital of Philadelphia (Kenta Uchibe, Maurizio Pacifici, Masahiro Iwamoto) and those at the Center for Research in FOP and Related Disorders at the University of Pennsylvania School of Medicine (Salin Chakkalakal, Michael Convente, Deyu Zhang, Frederick Kaplan, Eileen Shore).

The results of this study provide strong and clear support for palovarotene as an effective potential treatment to not only prevent HO in FOP, but also protect and sustain skeletal growth and joint function.

***Palovarotene inhibits HO in a mouse model with the human FOP mutation***

We previously created and described transgenic mice in which we inserted the R206H mutation into the ACVR1 gene (Chakkalakal et al 2012). The mice mimicked human FOP and developed HO and the characteristic toe malformations, but they did not allow us to initiate the HO process at specific times as is often the case in FOP patients following a flare-up. In the current study, we used a new 'conditional' mouse model that permits us to activate the mutation at will, allowing us to adjust timing and location as well as the cell types expressing the mutation depending on the question to answer. The conditional FOP mouse model was created by Aris Economides and his colleagues at Regeneron Pharmaceuticals in collaboration with researchers at Penn. Dr. Economides is also a co-author on the current study.

In a first set of experiments, we activated the FOP mutation in all cells of young mice and demonstrated that injury to skeletal muscles induced local formation of HO, just as in children with FOP. When we treated these mice with palovarotene, however, very little HO formed and the HO process had been halted at the cartilage formation stage as we expected. Importantly, we also found that palovarotene had elicited additional beneficial effects at the early stages of HO formation, specifically a reduced immune cell response.

A severe functional consequence of HO during FOP is that it may eventually impair or completely block skeletal movement and joint function. Very promisingly, we found that palovarotene-treated mice retained good skeletal function and could move and function well.

***Prenatal expression of ACVR1$^{R206H}$ causes limb skeletal malformations and HO***

The *ACVR1*$^{R206H}$ mutation is likely to affect cells during embryonic development since most FOP patients display the great toe malformation at birth. In order to determine whether the new conditional mouse line was able to mimic this characteristic feature of FOP, we activated the mutation in the cells of the developing embryonic limbs by using a genetic approach (*Prxx1*$^{+}$ cells). Note that we had to restrict the mutation to those cells because we had learned from our previous studies that general activation of the mutation throughout the whole body in developing mouse embryos was too aggressive and resulted in premature death.

When the Prrx1-*ACVR1*$^{R206H}$ mice (Prrx1-R206H mice) were examined right after birth, the first digits of the hind feet (the mouse equivalent of human great toes) were in fact malformed. In a

manner analogous to FOP children, the newborn mice did not display HO, but began to develop HO spontaneously starting around 2 to 4 weeks of age. The extraskeletal bone formed consistently and in the absence of injury. Because the mutation was active only in limb cells, the mice did not develop HO in other parts of their body that are often affected in FOP patients such as the back and neck.

We also noted that the Prrx1-R206H mice were slightly shorter than siblings lacking the mutation. We measured lengths of several limb bones and determined that, indeed, the bones were on average shorter in FOP mice. Bones, such as the femur in the leg and the humerus in the arm, normally grow in length through the expansion and development of cells in 'growth plates' located at each end near the joints. This expansion, like HO in FOP, occurs through the process of endochondral ossification. When we examined the growth plates of Prrx1-R206H mice, we found that there was a significant reduction in the region of the growth plate that contains the more mature cartilage cells and is responsible for long bone elongation.

***Palovarotene reduces spontaneous HO in Prrx1-R206H mice***

HO in the Prrx1-R206H mice forms in the absence of injury, and this feature provided us with the opportunity to test the efficacy of palovarotene for spontaneous HO. Since HO in these mice does not begin until after birth, we began treatment with palovarotene at birth. Palovarotene was administered to nursing mouse mothers for the first 2 weeks, with the expectation that the drug would be passed to the pups through breastfeeding. It was then given directly to the 2 week-old mice for another two weeks until about 1 month of age. At the end of this period, Prrx1-R206H

mice that had not been treated with palovarotene had extensive HO, but those treated with the drug had substantially reduced amounts of it.

***Palovarotene rescues skeletal malformations and growth in Prrx1-R206H mice***

We also examined the effects of palovarotene on long bone elongation and the growth plates. When mice without the FOP mutation were treated with palovarotene, skeletal growth was slightly reduced and there was some impairment of growth plate cartilage function, consistent with known effects of other retinoid agonists on skeletal growth. However, when Prrx1-R206H mice were treated with palovarotene, long bone growth and elongation were significantly improved and were similar to those in untreated mice without the FOP mutation. In addition, and very importantly, the growth plates of palovarotene-treated Prrx1-R206H mice were restored to a near normal appearance, and the mice remained mobile and functioned well over time.

Individually, both the treatment with palovarotene and the increased basal BMP signaling activity by the $ACVR1^{R206H}$ mutation can impair normal skeletal bone growth. Because palovarotene treatment blocked HO and reversed the skeletal defects in Prrx1-R206H mice, our data indicate that there is a fine balance between BMP and retinoid signaling - and in particular between ACVR1 and RARγ - that is critical for growth plate function and skeletal growth. Further characterization of ACVR1 and RARγ interactions will not only increase our understanding the action and mechanisms of such key regulators, but may also identify additional druggable targets and even more effective potential treatment regimens.

***Summary and Conclusions***

The goals of the present study were to determine whether palovarotene can block HO caused by the human ACVR1$^{R206H}$ mutation and whether the drug could also correct other skeletal defects caused by the mutation. The results clearly demonstrate that palovarotene can do so and, quite significantly, appears to be able to act on neonates via the mother's milk. While the cellular mechanisms through which palovarotene and ACVR1$^{R206H}$ interact remain to be elucidated, our findings are a major step in establishing whether palovarotene is an effective and preventative treatment for FOP, possibly administrable from infancy.

HO is highly damaging to the well being of FOP patients because it progressively interferes with, and limits, multiple body functions including walking, bending, breathing, mastication, and swallowing. Since palovarotene inhibits the cartilage stage of heterotopic endochondral ossification, palovarotene treatment would be needed at each flare-up to reduce or possibly prevent each new round of HO, possibly starting from a young age. Once formed, HO is permanent in FOP patients; the ectopic bone cannot be removed by surgery because the resulting tissue damage triggers additional episodes of HO. Given the potency of palovarotene to prevent spontaneous as well as injury-induced HO, it has the potential to suppress HO initiation following surgery in FOP patients, a therapeutic feat if indeed possible. Notably, the near complete recovery of growth plate structure and function that we observe in palovarotene-treated FOP mice supports the possibility that drug treatment of skeletally-immature patients might enable suppression of HO during childhood while restoring skeletal growth, an attainment that originally seemed counter-intuitive with a retinoid agonist.

For the first time we show that palovarotene:

- prevents HO that is caused by a mutation that occurs in human patients. The ACVR1$^{R206H}$ mutation examined in our study is the most common mutation in patients with FOP;
- rescues defects in mutant bone growth and preserves joint function;
- can be given to lactating mothers to inhibit HO in nursing pups, raising the possibility of safe and early, or even pre-natal, intervention for HO in children;
- restores growth plate function in mutants, in contrast to the predicted detrimental effects on wild-type growth plates - a well-known side-effect of retinoids. This remarkable observation indicates that mutant and wild-type cells and tissues respond differentially to this drug, an observation with major implications for clinical trials and drug testing, and drug safety and use.

The data in this study provide strong support for palovarotene to not only prevent HO, but also to restore long bone growth and maintain joint function and mobility. Our data demonstrate that palovarotene is not only highly effective, but could also be safe for use in children with FOP, offering the first possibility of a chronic preventive treatment for FOP.