Repurposing FK506 to Increase BMPR2 Signaling and Improve Pulmonary Arterial Hypertension: A Fast Track From Cells to People

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To improve long-term survival in pulmonary arterial hypertension (PAH), new treatment approaches that target occlusive vasculopathy instead of vasoconstriction of pulmonary arteries are necessary. Identifying genes or pathways that unify different pathologies and etiologies in PAH is a crucial first step for drug development. The bone morphogenetic protein receptor 2 (BMPR2) pathway, originally described as the cause of familial PAH, has gained significant interest over the past few years as a potential master switch in PAH, and therefore presents a promising treatment target in PAH. FK506 (tacrolimus) was found in a high-throughput screen of US Food and Drug Administration (FDA)-approved drugs to significantly increase BMPR2 signaling. Low-dose FK506 was able to restore normal BMPR2 signaling and function in patient pulmonary artery (PA) endothelial cells, inhibit proliferation and induce apoptosis in PA smooth muscle cells, and prevent and reverse pulmonary hypertension (PH) in 3 experimental rodent models of PH. As an FDA-approved drug, it was possible to relatively quickly (in approximately 2 years) translate the basic science discoveries from the bench to the clinic. A Phase 2 proof-of-concept safety and tolerability trial is underway (ClinicalTrials.gov Identifier: NCT01647945) to evaluate the use of low-dose FK506 in PAH and to identify patients who might respond best to FK506 depending on their impairment in BMPR2 signaling.

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BMPR2 expression in pulmonary artery smooth muscle cells (PASMCs): HIV-TAT by repressing the BMPR2 promoter and HIV-nef potentially by interfering with the intracellular trafficking of BMPR2. Macaques with infected chimeric simian/human immunodeficiency virus (SHIV) containing HIV-1-derived Nef protein develop severe pulmonary hypertension (PH) characterized by occlusive pulmonary vasculopathy mimicking the human disease. The effect of an HIV infection on BMPR2 expression is even more pronounced in the presence of drugs such as cocaine, commonly used by HIV-positive intravenous drug users. On the contrary, reconstitution of athymic rats with regulatory T cells prevents PH induced by the vascular endothelial growth factor (VEGF) receptor blocker SUGEN5416, and is associated with an increase in BMPR2 expression in pulmonary arteries.

With regard to the role of BMPR2 in metabolism, recent data suggest that enhanced estrogenic activity decreases BMP signaling in a susceptible host, whereas altered BMP signaling modifies estrogenic activity. BMPR2 is involved in the regulation of hepcidin gene expression and iron metabolism, and most recently has been shown to be associated with insulin resistance and impaired fatty acid oxidation in experimental PH. In addition, metabolic reprogramming in PH occurs downstream of BMPR2, which is supported by studies in mice with a disease-causing mutation in BMPR2 that are more susceptible to oxidant injury in mitochondrial membranes compared to wild-type animals. Furthermore, it was recently shown that the human neutrophil inhibitor elafin interacts with caveolin-1 to facilitate BMPR2 signaling and endothelial homeostasis, linking elevated elastase activity to a reduced BMPR2 signaling. In addition, Drake et al showed that mutations in BMPR2 impaired normal micro RNA processing of miR-21 and miR-27a, which likely contributes to vascular cell proliferation in PH. These examples illustrate that BMPR2 signaling presents a critical pathway in PAH and a potential master switch linking different PAH pathologies and etiologies. We hypothesize that there is a threshold of BMPR2 signaling below which PAH develops, either due to a BMPR2 mutation, environmental factors, or modifier genes. Therefore, targeting the BMPR2 pathway could be a promising treatment approach in PAH (Figure 1).

INCREASING BMPR2 SIGNALING WITH FK506

We performed a high throughput screen of >3000 US Food and Drug Administration (FDA)-approved drugs and bioactive compounds using a reporter cell line where the BMP response element (BRE) from the Id1 promoter was linked to luciferase to identify activators of Id1 expression and, respectively, BMPR2 signaling. FK506 (tacrolimus) was identified as the best BMPR2 activator. Even at low, sub-immunosuppressive doses (0.2-2 ng/mL), FK506 was able to bind to FKBP12, and by doing so removed the BMPR2 inhibitor FKBP12 from the type 1 receptors ALK1, ALK2, and ALK3, allowing for phosphorylation of the type 1 receptor FKBPI2 from the type 1 receptors ALK1, ALK2, and ALK3, allowing for phosphorylation of the type 1 receptor and subsequent downstream smad dependent and independent signaling. In addition, FK506 inhibited the phosphatase calcineurin, which also facilitated phosphorylation of the type 1 receptor. Previous studies had shown that FKBPI2 was necessary for SMURF-1 mediated degradation of type 1 and type 2 TGF-β receptors. By removing FKBPI2 from the type 1 receptor, FK506 is able to prevent SMURF-1 and smad7 mediated type 1 and type 2 receptor degradation, leading to an increase in BMPR2 protein.
expression (unpublished data). FK506 was superior to rapamycin and cyclosporin in increasing BMPR2 signaling, due to its dual function as a calcineurin inhibitor and binder of FKBP12, as well as its ability to remove FKBP12 from all 3 type 1 receptors, ALK1, ALK2, and ALK3, which are implicated in BMP signaling (Figure 2).

**FK506 PREVENTS AND REVERSES EXPERIMENTAL PH**

Increasing BMPR2 signaling by low-dose FK506 (blood level 0.2 ng/mL) prevented the development of hypoxia-induced PH in mice with a conditional endothelial deletion of BMPR2 by improving endothelial health and preventing loss of small vessels. Low-dose FK506 was also able to reverse established severe PH in rats exposed to the endothelial toxin monocrotaline by reducing medial hypertrophy. Furthermore, low-dose FK506 reversed severe PH and occlusive vasculopathy in rats where PH was induced by the VEGF receptor blocker SUGEN5416, followed by 3 weeks exposure to 10% hypoxia and 5 weeks normoxia. This animal model refined by Abe et al mimics the clinical disease very well, as it is characterized by severe PH accompanied by an occlusive vasculopathy of small pulmonary arteries.

**FK506 IN CLINICAL USE FOR PAH**

FK506 has been used for more than 20 years as a potent immunosuppressive agent in solid organ transplantation. Low-dose FK506 is used in certain autoimmune diseases such as psoriasis and rheumatoid arthritis, again for its anti-inflammatory properties. Furthermore, FK506 was found to increase endothelial expression of ALK1 and endoglin. As loss-of-function mutations in these genes are observed in PAH associated with hemorrhagic hereditary telangiectasia (HHT), FK506 could also be of potential benefit in patients with this disorder. In fact, FK506 was given following liver transplant in a patient with HHT who had multiple arteriovenous malformations, and it was noted that internal and external telangiectases, epistaxes, and anemia disappeared, suggesting that the mechanism of action of FK506 involved partial correction of endoglin and ALK1 haploinsufficiency.

We have treated 3 end-stage PAH patients with low-dose FK506 with very promising results (unpublished data). Subsequently, a Phase 2 proof-of-concept safety and tolerability trial was initiated at Stanford University to test whether low-dose FK506 treatment is feasible in PAH, and to identify patients who might benefit most from low-dose FK506 depending on their BMPR2 impairment (ClinicalTrials.gov Identifier: NCT01647945). One goal of this trial is to develop a “BMPR2 signature” in the blood of PAH patients at baseline and follow-up to first prove that the BMPR2 pathway is targeted by the study drug, and second to define and develop criteria that might reflect which patient subgroup potentially responds best to therapy—valuable information for the design of a subsequent efficacy trial.

**THE ADVANTAGES OF DRUG REPOSITIONING IN PAH—FROM CELLS TO PEOPLE**

Recycling is good for the environment and for drug development, as well. There are multiple advantages to repurposing existing drugs for the treatment of PAH. New drug development is a
Changing the paradigm of drug development from novel compounds for PAH to repurposing existing drugs offers the advantage of a known safety and toxicity profile, resulting in shorter dose-finding studies and accelerating the use of a potential disease-modifying drug in patients.

References
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