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Low Dose FK506 (Tacrolimus) in End-Stage Pulmonary Arterial Hypertension

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Contribution: ES, YS, MR & RTZ were responsible for the design, implementation, and analysis of the results. MAA was involved in genetic sequencing. RD, PY performed the cardiac MRIs. MVV, AVN, AJS, AL were responsible for blinded interpretation of the cardiac MRIs. JLB, ES, & RTZ were responsible for development of the dosing algorithm. ES & DS carried out

and interpreted serologic biomarker assays. MB was responsible for blood collection and biobanking. All authors contributed to the development of the manuscript.

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Disclosures: A patent application has been filed for low dose FK-506 as a treatment for PAH at Stanford University. ES and MR are listed as inventors on the patent. ES and RZ are advisors for a start-up company Selten Pharma, Inc.

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Despite recent advances in therapy, pulmonary arterial hypertension (PAH), characterized by occlusive vasculopathy of the pulmonary arteries, remains a progressive disease without a cure (1-3). While the currently approved PAH medications haven't demonstrated anti-remodeling properties in humans, novel anti-proliferative strategies have shown some benefits but also raised safety concerns (4-6), none target a genetic predisposition of PAH, the dysfunctional Bone Morphogenetic Protein Receptor 2 (BMPR2) signaling.

Loss-of-function mutations in BMPR2 in familial and idiopathic (I)PAH patients (7-9) are associated with increased pulmonary vasculopathy (10). Furthermore, reduced BMPR2 expression is observed even in patients without a mutation, reinforcing the importance of decreased BMPR2 in PAH (11). In a high throughput screen of 3,600 FDA approved drugs we identified low-dose FK506 (tacrolimus) as a potent BMPR2 activator that reversed experimental PAH (12). We therefore hypothesized that low dose FK506 would be beneficial in PAH patients by increasing BMPR2 signaling.

Based on these findings, we initiated a randomized, double-blind, placebo-controlled phase IIa trial (TraNsFORM, NCT#01647945) to evaluate the safety and tolerability of FK506 in stable PAH patients. Here, we report our clinical experience with compassionate use of low-dose FK506 in three end-stage PAH patients who did not qualify for TraNsFORM due to severity of illness (patient details in supplement). We assessed traditional clinical parameters, New York Heart Association (NYHA) functional class, six-minute walk distance (6MWD), serologic biomarkers, hospital admissions as well as protocolized cardiac magnetic resonance imaging (cMRI) assessed by blinded readers (13, 14). All patients remained on stable doses of PAH medication and diuretics throughout the 12-months period.

Patient #1: 36-year-old historically athletic female, NYHA-IV with rapidly progressive IPAH requiring rapid up-titration of epoprostenol, and addition of sildenafil and ambrisentan for recurrent hospitalizations for RV failure (**Table 1**). Despite aggressive treatment, she still reported NYHA-III/IV symptoms, an elevated N-terminal-pro-B type natriuretic peptide (NT-proBNP) and a REVEAL risk score of 11, stratifying her as high risk with a potential 1-year mortality of 15-30% (3, 15), she was listed for lung transplantation. At that time she was offered compassionate treatment with FK506 (goal trough blood level 1.5-2.5 ng/mL).

Within 1 month of FK506 initiation, she reported substantial improvement in symptoms (**Figure 1**). Within 2 months she was placed on hold for transplantation by the lung transplant team. After 3 months, her 6MWD distance improved by 100 meters, her NT-proBNP decreased >50% and she reported NYHA-I symptoms (**Figure S1A, Table 1**). Over the 12-month period, cMRI showed a stable RV ejection fraction (RVEF), RV end-diastolic volume index (RVEDVi), and cardiac index (CI) (**Figure S1B**). Her REVEAL risk score decreased to 3 (range 3-6), placing her in the low risk category (**Table 1**). While the 12 months prior to FK506 were characterized by 3 hospitalizations for RV failure, the subsequent 12 months were free of any PAH associated hospitalizations (**Table 1**). At the time of this submission, the patient is 27 months from the initiation of FK506, continues to report NYHA-II symptoms, and has been free from hospitalization or clinical deterioration.

Patient #2: 50-year-old female with end-stage systemic sclerosis associated PAH on intravenous treprostinil, sildenafil, ambrisentan, as well as an intravenous dopamine infusion for end-stage RV failure and hypotension. Patient continued to report NYHA-III/IV symptoms, had an elevated NT-pro BNP (range 4,926–15,161 pg/mL) and 4 hospitalizations for progressive RV

failure and palliative paracenteses (**Table 1**). Given the lack of further therapeutic options, she was offered FK506.

cMRI at baseline, 3 and 6 months showed substantial improvement in RVEF, stable RVEDVi and improvement in RVSVi and CI (**Figure S1B**), with a reduction back to baseline at 12 months. Her REVEAL risk score decreased from 12 to 11. At 12-month follow-up, she had stable NYHA-III symptoms, a 94-meter increase in 6MWD, 30% reduction in her NT-proBNP and no PAH related hospitalizations since being on FK506 (**Table 1**). Patient is currently 26 months post FK506 initiation and has not experienced further clinical deterioration.

Patient #3: 55-year-old female with severe end-stage drugs-and-toxins-associated PAH, NYHA-III/IV, on high dose IV treprostinil, sildenafil, intolerance to ERAs, listed for lung transplantation was offered FK506. Despite initial symptomatic improvement (**Table 1**), patient voluntarily discontinued FK506 after 4.5 months. Unfortunately, over the ensuing 7 months, she showed progressive clinical worsening, culminating in a ICU admission for RV failure and large pericardial effusion. Upon the patient's wish, she was restarted on FK506 and is currently 12 months post her second FK506 initiation, feeling much better with compensated NYHA-II symptoms and without any further hospital admission for RV failure.

Serologic Biomarkers:

None of the three patients had mutations in BMPR2, SMAD9 or caveolin-1. We measured BMPR2 expression and specific BMPR2 associated genes and molecules (Id1, Smurf-1, IL-6, LIMK1, Cofilin-1, miRNAs 21 and 27a) at baseline, 3, 6 and 12 months FK506 treatment in patients versus healthy controls (n=12) (see supplement). Patients had significantly lower BMPR2 mRNA expression at baseline (**Figure 1**) with near normalization of BMPR2 and

associated genes after 12 months of FK506 treatment. Strikingly, Patient #3 who stopped FK506 after 4.5 months and who worsened clinically over the following 7 months, showed a 12-months BMPR2 profile that was opposite to that of patients still on FK506 therapy.

Discussion:

Our results suggest potential clinical benefit of low dose FK506 in end-stage PAH, judged by the marked clinical response, stabilization in cardiac function and freedom from hospitalization for RV failure. Despite the overall positive experience, we caution that these findings are highly preliminary. The efficacy of this therapy must be validated in appropriate, well-designed, prospective clinical trials. Our choice of low-dose FK506 was based on data from pre-clinical studies (12) and the desire to avoid major immunosuppressive side effects in patients with indwelling lines. We did not observe an increase in line-sepsis or opportunistic infections. We also did not observe serious adverse effects of posterior reversible encephalopathy syndrome, acute kidney injury or worsening of creatinine, an elevated systemic blood pressure, hyperglycemia, hyperkalemia, anemia, or a change in white blood cell count, The currently underway-clinical phase IIa trial will address safety and tolerability in greater detail, as even low-dose immunosuppression over time can lead to complications.

This is the first study in PAH patients that repurposes FK506 to increase BMPR2 signaling. The changes in serologic biomarkers are encouraging and show that we have indeed targeted BMPR2 in patients with reduced levels of BMPR2. It will be of interest to determine whether the same effect can be achieved in patients with documented mutations and whether a subset of patients are particularly sensitive to the beneficial effects of this strategy and could therefore be identified up-front as potential “responders” – based on BMPR2 levels.

Figure Legends:

Table 1: Timeline of symptoms, clinical parameters, events, and therapies for patients #1-3 before and after initiation of FK506. REVEAL Risk Score %1-year survival: Score 1-7 = 95-100% (low risk), 8 = 90-95% (average risk), 9 = 85-90% (moderately high risk), 10-11 = 70-85% (high risk), 12 or above = <70% (very high risk)(3). Events reported as those related to PAH: RVF= right ventricular failure, HepF = hepatic failure, Sync = syncope, Tx List = listed for heart and/or lung transplantation, Tx Hold = placed on hold for transplantation due to clinical improvement. Prost = prostacyclin, PDE-5I = phosphodiesterase-5 inhibitor, ERA = endothelin receptor antagonist, Dop = dopamine, NYHA = New York Heart Association functional class.

Figure 1: Biomarkers for patients #1-3 before and after initiation of FK-506. (A) BMPR2 mRNA/GAPDH in PBMCs of healthy controls at baseline (B) (n=12) and 3 patients (n=3) at baseline (B), 3 months (3) and 6 months (6) and 12 months (12). The red dotted line indicates the time FK506 was stopped in Patient #3. (B) Id1 /GAPDH mRNA in PBMCs. (C) SMURF-1/GAPDH mRNA in PBMCs. (D) IL-6 plasma levels via ELISA. (E) LIMK1/GAPDH mRNA in PBMCs. (F) Cofilin-1/GAPDH mRNA in PBMCs. (G) and (H) respectively miR21/RNU48 and mir27a/RNU48 RNA expression in PBMCs, respectively.

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