these outcome measures in parallel into interventional studies to understand not only their sensitivity and variability over time but also their responsiveness to treatment. Assessing treatment efficacy in infants and preschool children has been a difficult frontier to tackle, but because therapies such as cystic fibrosis transmembrane regulator–modulators likely have their most pronounced effect when implemented early, this is a challenge we need to address. ■

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Toward Harnessing Sex Steroid Signaling as a Therapeutic Target in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a sexually dimorphic disease that predominantly affects women. Studies investigating the most abundant female sex hormone, 17β-estradiol (E2), have identified E2 (or its 16α-hydroxyestrone metabolite) as either a disease mediator or a protective agent (1). This heterogeneity reflects the complex clinical observation that women, although more prone to developing PAH, exhibit better survival than their male counterparts (2–4).

Based on data implicating aromatase in PAH pathogenesis (5, 6), in this issue of the *Journal*, Kawut and colleagues (pp. 360–368) performed a phase 1 study of aromatase inhibition in postmenopausal women or men with PAH (7). Eighteen subjects were randomly assigned in a 2:1 ratio to 1 mg/day anastrozole or placebo. Primary outcomes were change from baseline in E2 levels and tricuspid annular plane systolic excursion (TAPSE) after 12 weeks. Anastrozole decreased serum E2 levels by 40% and increased 6-minute-walk distance (6MWD) while not exerting significant effects on TAPSE, N-terminal pro-brain natriuretic peptide (NT-pro-BNP) levels, proinflammatory cytokines, other sex hormones or circulating biomarkers, functional class, or quality of life. The authors conclude that anastrozole therapy is safe and warrants further evaluation in a phase 2 study.

The authors are to be commended on a well-designed, well-executed, and clinically relevant study that answers several important questions. In particular, the authors demonstrate that treatment with anastrozole is feasible in postmenopausal female and male patients with PAH. No patient discontinued the study drug, indicating it is well tolerated. Given recent data that E2 exerts right ventricle (RV)-protective effects in PAH (8, 9), the lack of negative effects on RV function in the study group as a whole is reassuring. The positive effect on 6MWD (+38 m vs. placebo) is striking.

However, the study also has several weaknesses. These include lack of measurement of 16α-hydroxyestrone levels (a mediator of E2's detrimental effects in PAH [6, 10]), lack of hemodynamic measurements before enrollment, enrollment of predominantly New York Heart Association functional class I or II patients, and reliance on TAPSE (a highly preload-dependent parameter [11]) as sole indicator of RV function. In addition, the study excluded premenopausal patients. Notably, 93 patients were screened to enroll 18 subjects, raising concerns about generalizability to a larger PAH population. Last, although the authors hypothesized

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they would decrease serum E2 levels by 90%, levels were only lowered by 40%.

Interestingly, inspection of patient-level data suggests heterogeneity in responses to anastrozole. Some patients decreased E2 levels by 60–80%, whereas others only decreased by 5% or less. Of note, E2 tended not to decrease in patients with low levels (<10 pg/ml), suggesting that in those patients, there simply was not a target for anastrozole. We do not know whether these patients improved their 6MWD despite unchanged E2 levels (which would suggest anastrozole effects that are independent of lowering E2 levels).

Even though individual patient data in small pilot trials need to be interpreted with caution, the variety in TAPSE, NT-pro-BNP, and/or 6MWD responses is striking. For example, TAPSE increased in six patients, did not change in four patients, and decreased in two patients. NT-pro-BNP remained unchanged in nine patients, but increased in three. 6MWD tended to increase in most subjects, but decreased in two patients. We unfortunately do not know anything about the characteristics of patients with more favorable responses versus those whose responses did not go in the right direction (e.g., sex, age, disease stage, PAH medication). It also remains unknown whether there were relationships between changes in E2 levels and changes in TAPSE, NT-pro-BNP, or 6MWD. The only clear pattern is that NT-pro-BNP levels tended to increase in those patients with higher baseline levels in this parameter. In light of data indicating E2 as a mediator of superior RV function in health and disease (8, 9, 12, 13), these findings raise the question of whether at least some study subjects are dependent on E2 signaling for maintaining RV adaptation. Do those patients who exhibited decreased TAPSE and 6MWD and/or increased NT-pro-BNP require a certain level of E2 in the RV to prevent transition to maladaptive remodeling? Although the study population as a whole did not experience harm from aromatase inhibition, a subset of patients may not tolerate such a strategy.

As with many studies, this study answers important questions but raises even more questions: Which patients with PAH benefit from aromatase inhibition? Who are the responders and nonresponders? Could a subset of patients do worse? Should we measure aromatase activity or E2 levels before inhibiting aromatase in everyone with PAH? Does dosing need to be varied according to baseline aromatase activity, E2 levels, or estrogen receptor abundance? What about premenopausal women? Where is the primary target of anastrozole in PAH? The latter is a particularly interesting question, as aromatase is widely expressed (e.g., gonads, adipose tissue, heart, systemic and pulmonary vasculature), and the more pronounced effect on 6MWD as compared with other endpoints could suggest prominent peripheral muscle or metabolic effects. Last, what are potential adverse effects of long-term aromatase inhibition? Decreases in bone density in postmenopausal women and negative effects on peripheral muscle health and/or mental health have been described (14). Patients would need to be monitored accordingly.

If we accept that E2 is detrimental for the pulmonary vasculature but beneficial for the RV, how could such a paradigm be harnessed therapeutically? One strategy would be to inhibit E2 only in patients with mild disease or in subjects at risk for disease development (e.g., women with *BMPR2* mutations and/or *CYP1B1* SNPs [15]). Conversely, inhibiting E2 may be detrimental in patients with or at risk for severe RV disease; they may benefit from enhancing E2-dependent pathways (e.g., as bridge to transplant or short-term treatment during RV failure crises). Local inhibition of E2 signaling in the pulmonary vasculature or local activation in the RV through organ-targeted delivery (e.g., aerosolized therapies, right coronary artery–selective infusion) or molecular approaches involving lung- or RV-specific pathways could harness compartment-specific effects while avoiding off-target effects.

Kawut and colleagues have taken an elegant and important step in the right direction. The time is ripe for further well-designed clinical trials and rigorous basic science studies focused on harnessing sex steroid signaling to improve outcomes for patients of either sex with PAH. ■

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