Anastrozole in Pulmonary Arterial Hypertension

A Randomized, Double-Blind, Placebo-controlled Trial

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Abstract

Rationale: The aromatase inhibitor anastrozole blocks the conversion of androgens to estrogen and blunts pulmonary hypertension in animals, but its efficacy in treating patients with pulmonary arterial hypertension (PAH) is unknown.

Objectives: We aimed to determine the safety and efficacy of anastrozole in PAH.

Methods: We performed a randomized, double-blind, placebo-controlled trial of anastrozole in patients with PAH who received background therapy at two centers.

Measurements and Main Results: A total of 18 patients with PAH were randomized to anastrozole 1 mg or matching placebo in a 2:1 ratio. The two co-primary outcomes were percent change from baseline in 17 β -estradiol levels (E2) and tricuspid annular plane systolic excursion (TAPSE) at 3 months. Anastrozole significantly reduced E2 levels compared with placebo (percent change: -40%;

interquartile range [IQR], -61 to -26% vs. -4%; IQR, -14 to +4%; $P = 0.003$), but there was no difference in TAPSE. Anastrozole significantly increased the 6-minute-walk distance (median change = +26 m) compared with placebo (median change = -12 m) (median percent change: anastrozole group, 8%; IQR, 2 to 17% vs. placebo -2%; IQR, -7 to +1%; $P = 0.042$). Anastrozole had no effect on circulating biomarkers, functional class, or health-related quality of life. There was no difference in adverse events.

Conclusions: Anastrozole significantly reduced E2 levels in patients with PAH but had no effect on TAPSE. Anastrozole was safe, well tolerated, and improved 6-minute-walk distance in this small “proof-of-principle” study. Larger and longer phase II clinical trials of anastrozole may be warranted in patients with PAH.

Clinical trial registered with www.clinicaltrials.gov (NCT 1545336).

Key Words: pulmonary hypertension; clinical trial; anastrozole; sex hormones

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At a Glance Commentary

Scientific Knowledge on the

Subject: Experimental and observational evidence suggests that limiting estradiol exposure via aromatase inhibition may be a therapeutic strategy in pulmonary arterial hypertension (PAH), but the effects of hormonal manipulation with anastrozole have not been tested in human PAH.

What This Study Adds to the

Field: This is the first randomized clinical trial of a therapy targeting sex hormones in PAH. We have shown that anastrozole 1 mg daily lowers total, bioavailable, and free estradiol and estrone but did not affect tricuspid annular plane systolic excursion at 3 months. Anastrozole was safe and well-tolerated, and increased 6-minute-walk distance. Larger trials are necessary to determine if anastrozole is effective in treating PAH.

Pulmonary arterial hypertension (PAH) includes idiopathic and heritable forms, as well as PAH associated with a number of systemic conditions, such as connective tissue disease and portal hypertension. In PAH, the small muscular pulmonary arteries show endothelial proliferation and smooth muscle hypertrophy, *in situ* thrombosis, and plexiform lesions. Right ventricular (RV) failure ensues, leading to exercise limitation and death. Although a number of therapies have been approved for PAH, all treatments target the same three vascular pathways (prostacyclin, nitric oxide, and endothelin-1) and do not modify the disease.

The best established and strongest risk factor for idiopathic and heritable PAH is female sex. The first modern description from Dresdale and colleagues (1) in 1951 described three young women with idiopathic PAH, yet there were surprisingly few studies on the role of sex in patients with PAH until the past decade. Female sex is clearly a risk factor for the disease; however, women with PAH have better outcomes than men (2–11). Manipulation of estrogen (administration and deprivation) has shown inconsistent responses in animal models of pulmonary hypertension (PH). These contradictory observations in humans and

animals have been termed the “estrogen paradox” of pulmonary vascular disease [recently reviewed by Lahm and colleagues (12)]. A single-nucleotide polymorphism in the promoter region of aromatase (which converts androgens to estrogen and accounts for most of the estrogen production in postmenopausal women and men) is not only associated with a higher level of circulating 17β -estradiol (E2) but also increases the risk of PAH in patients with cirrhosis (13). We have recently shown that every 1-U increase in circulating E2 levels in men is associated with a more than 50-fold increase in the risk of PAH (14). In men with PAH, higher E2 levels have also been associated with a shorter 6-minute-walk distance (6MWD). Finally, recent studies have shown that aromatase is produced in the smooth muscle cells of the small muscular pulmonary arteries in both female animal models of PH and in women with PAH (15, 16). Administration of the aromatase inhibitor anastrozole (AN) reduced pulmonary arterial pressures, pulmonary vascular changes, and indexes of

RV hypertrophy in experimental PH (15, 16); metformin had similar effects via aromatase inhibition (17). Together, experimental and observational evidence suggests that limiting E2 exposure via aromatase inhibition may be a therapeutic strategy in PAH, but the effects of hormonal manipulation have not been tested in patients with pulmonary vascular disease.

AN has been approved by the U.S. Food and Drug Administration for patients with breast cancer for 20 years, and has minimal side effects in women without breast cancer (18, 19). In this pilot trial, we aimed to determine whether short-term AN would reduce relative levels of E2 and improve tricuspid annular plane systolic excursion (TAPSE) (percent change from baseline) compared with placebo in patients with PAH.

Methods

Study Design

Anastrozole in Pulmonary Arterial Hypertension (AIPH) was a two-center,

Figure 1. Flow diagram. PAH = pulmonary arterial hypertension; 6MWT = 6-minute-walk test.

randomized, double-blind, placebo-controlled “proof-of-principle” study of AN in patients with PAH. The protocol called for the recruitment of 18 patients with PAH; the first patient was randomized in January 2013 and the last in January 2015. Details of the methods are provided in the online supplement.

The trial protocol was approved by the institutional review boards at both participating centers and the Data Safety and Monitoring Board. The trial was registered at clinicaltrials.gov before initiating recruitment (NCT 1545336).

Study Participants

We included postmenopausal women and men older than 18 years of age with PAH who were able to perform 6-minute-walk testing. We excluded patients who were being treated with estrogen or antihormone therapy, those with a history of breast cancer, or World Health Organization (WHO) class IV functional status. Patients were allowed to receive concurrent treatment with any PAH specific medications as long as they were on a steady dose of medication for at least 3 months before entering the study. Complete inclusion and exclusion criteria are provided in Table E1 in the online supplement. Participants were recruited from two pulmonary vascular disease clinics (University of Pennsylvania and Rhode Island Hospital/Brown University). All participants provided written informed consent.

Study Procedures

Potentially eligible patients were identified by medical staff. After obtaining informed consent and screening, subjects were randomly assigned in a 2:1 ratio by a web-based computerized system to 1 mg of AN (Zydu Pharmaceuticals, Pennington, NJ) once daily or an identically encapsulated placebo. The randomization scheme was blocked and stratified by type of PAH (portopulmonary hypertension vs. other). All subjects and study personnel other than the research pharmacist were masked to treatment assignment and were not unmasked until the study was completed. Subjects were evaluated at the study centers at baseline, 6 weeks, and 3 months, with telephone calls at 1 and 3.5 months.

The two primary outcomes were the percent change from baseline in E2 levels

Table 1. Baseline Characteristics of Subjects Randomized to Anastrozole and Placebo

	Placebo (n = 6)	AN (n = 12)
Age, yr	59 ± 10	61 ± 13
Sex, female	2 (33)	7 (58)
Race/ethnicity		
White (non-Hispanic)	6 (100)	7 (59)
Hispanic or Latino	0 (0)	1 (8)
Black	0 (0)	4 (33)
Body mass index, kg/m ²	33 ± 9	29 ± 6
PAH diagnosis		
Idiopathic	2 (33)	5 (42)
Portopulmonary hypertension	1 (17)	2 (17)
Congenital systemic-to-pulmonary shunt	0 (0)	2 (17)
Systemic sclerosis	2 (33)	2 (17)
Other connective tissue disease	0 (0)	1 (8)
HIV	1 (17)	0 (0)
Right ventricular systolic pressure,* mm Hg	54 (21–59)	46 (39–60)
Concomitant PAH medications		
PDE5i	3 (50)	9 (75)
ERA	3 (50)	8 (67)
Epoprostenol or treprostinil, IV/SQ	0 (0)	4 (33)
Inhaled prostacyclin analog	1 (17)	2 (16)
Combination therapy	2 (33)	3 (25)
Warfarin	2 (33)	5 (42)
WHO functional class		
I	1 (17)	0 (0)
II	3 (50)	10 (83)
III	2 (33)	2 (17)
Six-minute-walk distance, m	378 ± 161	336 ± 119

Definition of abbreviations: AN = anastrozole; ERA = endothelin receptor antagonist; IV = intravenous; PAH = pulmonary arterial hypertension; PDE5i = phosphodiesterase type 5 inhibitor; SQ = subcutaneous; WHO = World Health Organization.

Data are shown as mean ± SD, median (interquartile range), or number (%).

*Placebo, n = 5; anastrozole, n = 9.

and TAPSE at 3 months. Secondary outcomes included differences from baseline in other sex hormone levels, 6MWD, tricuspid regurgitant jet peak velocity and other echocardiographic parameters,

N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) and other biomarker levels, WHO functional class, and Short Form-36 (SF-36) scores at 3 months after randomization. Details of

Figure 2. Percent change from baseline in serum estradiol levels at 6 weeks and 3 months in subjects receiving anastrozole (red) versus placebo (blue).

endpoint assessments and study monitoring are provided in the online supplement.

Statistical Analysis

All analyses proceeded according to the intent-to-treat principle. There was no imputation for missing data. Continuous variables are presented as mean $\pm$ SD or median (interquartile range [IQR]), and categorical variables are presented as number (%). Analyses of continuous outcomes compared the changes from baseline to 3 months between the AN and placebo groups using Wilcoxon rank sum tests. Secondary analyses incorporated the 6-week assessments. Fisher's exact tests were used to compare categorical outcomes at 3 months between the groups. There were no interim analyses or stopping rules

planned *a priori* for the trial. We set $\alpha = 0.05$ for each analysis and anticipated a 10% nonadherence and/or dropout rate. Previous studies have shown as great as a 90% reduction in E2 at 6 weeks with AN; we had projected having 99% power to detect this effect with 12 patients assigned to AN and 6 assigned to placebo. We estimated having 80% power to detect a difference in the change in TAPSE of 1.7 SDs compared with placebo.

Results

We screened 93 PAH patients during the enrollment period (see Table E2). Eighteen patients were randomized, and all completed the study period (Figure 1).

Twelve subjects were randomized to AN and six were randomized to placebo (Table 1). The two groups were similar in terms of age and diagnosis. There was some imbalance in sex between the groups secondary to the randomization of one male to the placebo group. Self-reported adherence with the study drug was $>95\%$.

AN significantly reduced E2 levels at 6 weeks and 3 months (Figure 2). AN reduced E2 levels by a median of 40% (IQR, -61 to -26%), whereas there was no effect of placebo (median reduction of 4%; IQR -14 to $+4\%$; $P = 0.003$) at 3 months. AN also decreased bioavailable and free E2 and estrone levels (Table 2 and see Figures E1–E3 in the online supplement). There was no difference between AN and placebo

Table 2. Baseline Hormone Levels at Six Weeks and Three Months

	n	Baseline	Change from Baseline at 6 wk (%)	P Value	Change from Baseline at 3 mo (%)	P Value
E2, pg/ml						
AN	12	12.3 (7.3 to 47.3)	−42.5 (−63.9 to −31.4)	0.010	−40.4 (−60.9 to −25.9)	0.003
Placebo	6	41.4 (22.1 to 53.6)	−5.0 (−27.9 to 30.9)		−3.9 (−14.3 to 3.6)	
Bioavailable E2, pg/ml						
AN	12	8.0 (3.8 to 26.5)	−43.8 (−63.9 to −33.0)	0.007	−41.6 (−60.1 to −26.8)	0.003
Placebo	6	18.9 (6.5 to 30.8)	−2.0 (−29.1 to 28.2)		−9.6 (−16.4 to −5.2)	
Free E2, pg/ml						
AN	12	0.3 (0.1 to 1.0)	−43.8 (−63.9 to −33.0)	0.007	−41.6 (−60.1 to −26.8)	0.003
Placebo	6	0.7 (0.3 to 1.2)	−2.0 (−29.1 to 28.2)		−9.6 (−16.4 to −5.2)	
Estrone, pg/ml						
AN	12	31.1 (18.4 to 73.2)	−76.2 (−82.1 to −68.4)	<0.001	−70.3 (−81.8 to −66.1)	<0.001
Placebo	6	49.6 (28.6 to 77.9)	−4.1 (−9.3 to 26.9)		−1.9 (−10.2 to 25.4)	
Progesterone, ng/ml						
AN	12	130.8 (82.1 to 337.1)	1.7 (−50.8 to 26.3)	0.75	−6.4 (−53.1 to 27.2)	0.49
Placebo	6	278.7 (134.5 to 303.6)	12.0 (−70.1 to 22.2)		18.1 (−52.0 to 36.7)	
Testosterone, ng/dl						
AN	12	34.0 (17.8 to 206.1)	3.3 (−9.6 to 60.7)	0.29	8.3 (−5.1 to 25.1)	0.89
Placebo	6	473.7 (9.8 to 791.6)	−6.7 (−18.7 to 43.1)		2.8 (−28.2 to 41.1)	
Bioavailable T, ng/dl						
AN	12	13.4 (6.0 to 104.4)	0.5 (−12.5 to 67.1)	0.55	8.2 (−12.5 to 27.2)	0.89
Placebo	6	243.3 (6.1 to 367.3)	−1.8 (−22.3 to 38.6)		10.7 (−32.8 to 29.6)	
Free T, pg/ml						
AN	12	5.5 (2.5 to 42.6)	0.5 (−12.5 to 67.1)	0.55	8.2 (−12.5 to 27.2)	0.89
Placebo	6	99.2 (2.5 to 149.7)	−1.8 (−22.3 to 38.6)		10.7 (−32.8 to 29.6)	
Androstenedione, ng/ml						
AN	12	0.5 (0.3 to 0.9)	3.2 (−6.1 to 42.0)	0.34	23.0 (−15.5 to 52.8)	0.39
Placebo	6	0.7 (0.4 to 1.2)	−9.8 (−18.7 to 11.6)		−9.9 (−16.3 to 0.4)	
DHEA-S, μ g/dl						
AN	12	18.9 (15.0 to 39.4)	1.8 (0.0 to 12.9)	0.42	0.0 (−2.5 to 9.6)	0.24
Placebo	6	38.8 (15.0 to 47.9)	0.0 (−15.8 to 4.4)		−3.8 (−14.2 to 0.0)	
SHBG, nmol/L						
AN	12	64.9 (36.5 to 90.6)	5.9 (−3.9 to 10.8)	0.21	2.6 (−4.9 to 14.3)	0.96
Placebo	6	53.3 (45.2 to 62.2)	−2.6 (−11.4 to 3.8)		9.9 (−8.8 to 15.3)	
Cortisol, μ g/dl						
AN	12	10.1 (9.0 to 12.2)	0.5 (−15.4 to 20.9)	0.68	16.8 (−30.9 to 37.3)	0.82
Placebo	6	9.1 (7.5 to 13.2)	12.3 (−5.0 to 23.6)		9.8 (8.0 to 18.8)	

Definition of abbreviations: AN = anastrozole; DHEA-S = dehydroepiandrosterone-sulfate; E2 = estradiol; SHBG = sex hormone-binding globulin; T = testosterone.

Data shown as median (interquartile range).

Figure 3. Percent change from baseline in tricuspid annular plane systolic excursion (TAPSE) at 3 months in subjects receiving anastrozole (red) versus placebo (blue).

in terms of the percent change in progesterone, testosterone (total, bioavailable, or free), androstenedione, dehydroepiandrosterone-sulfate, sex hormone-binding globulin, or cortisol levels. There were no differences in the change in TAPSE between the AN and

placebo groups (Figure 3; Table 3). AN had no effect on pulmonary artery systolic pressure, RV area, RV fractional area change, left ventricular (LV) diastolic sphericity index, or the RV:LV diameter ratio. Absolute changes are shown in Tables E3 and E4 and Figures E4 to E11.

Although there was no difference in the echocardiographic RV parameters, AN increased the 6MWD compared with placebo (Figure 4). The median change from baseline was +26 m (IQR, 6.0 to 48.5 m) in those allocated to AN, whereas it was −12 m (IQR, −27.0 to 3.0 m) in those who received placebo ($P = 0.023$) (median percent change from baseline was +8% vs. −2%, respectively; $P = 0.042$). We observed no differences between the AN and placebo arms in biomarkers of RV function, inflammation, or thrombosis, including NT-proBNP (Figure 5), monocyte chemoattractant protein-1, insulin, the homeostatic model assessment of insulin resistance index, IL-6, vascular endothelial growth factor, and von Willebrand factor levels, although variability was high in some of these markers (Table 4).

There were no differences in WHO functional class after treatment with AN or placebo and no differences in the occurrence of any specific side effect (Table 5) or in serious adverse events between the groups. AN did not affect health-related quality of life (HRQoL) as assessed by the SF-36 at 6 weeks or 3 months (see Table E5). There were no differences in safety laboratory results (such as cholesterol) or systemic blood pressure between the groups (data not shown).

Table 3. Echocardiographic Parameters at Baseline and Three Months

	n	Baseline	Change from Baseline at 3 mo (%)	P Value
TAPSE, cm				
AN	12	1.7 (1.4 to 1.9)	6.9 (0.0 to 27.8)	0.98
Placebo	6	2.0 (1.7 to 2.1)	10.0 (−5.9 to 33.3)	
RV systolic pressure, mm Hg				
AN	9	46.0 (38.9 to 59.9)	10.7 (−15.3 to 37.0)	0.15
Placebo	4	56.6 (37.3 to 71.8)	−21.5 (−36.2 to −8.2)	
RV FAC, %				
AN	11	36.7 (29.9 to 43.7)	18.9 (−20.6 to 37.0)	0.59
Placebo	6	40.0 (28.8 to 41.0)	−0.6 (−34.0 to 39.7)	
TR jet peak velocity, cm/s				
AN	10	337 (291 to 370)	1.8 (−9.9 to 17.1)	0.25
Placebo	5	385 (350 to 392)	−5.6 (−20.0 to −3.1)	
LV diastolic sphericity index				
AN	8	0.3 (0.3 to 0.3)	15.7 (10.4 to 24.0)	0.57
Placebo	6	0.4 (0.3 to 0.4)	13.3 (−1.3 to 18.7)	
Diastolic RV 4-chamber area, cm ²				
AN	11	22.9 (21.1 to 30.8)	−2.2 (−9.2 to 16.2)	0.66
Placebo	6	29.9 (26.7 to 32.4)	−2.1 (−16.6 to 7.0)	
RV:LV diameter ratio				
AN	10	0.9 (0.8 to 1.0)	6.6 (−9.0 to 10.6)	0.22
Placebo	6	1.0 (0.8 to 1.1)	−10.3 (−17.8 to 8.4)	

Definition of abbreviations: AN = anastrozole; FAC = fractional area change; LV = left ventricular; RV = right ventricle; TAPSE = tricuspid annular plane systolic excursion; TR = tricuspid regurgitant. Data are shown as median (interquartile range).

Figure 4. Absolute change from baseline in 6-minute-walk distance (6MWD) at 6 weeks and 3 months in subjects receiving anastrozole (red) versus placebo (blue).

Discussion

This is the first randomized clinical trial of a therapy targeting sex hormones in PAH. AN reduced circulating levels of E2, bioavailable and free E2, and estrone without changing other sex hormone levels. There was no effect of AN on TAPSE or other measures of RV function (including NT-proBNP levels). AN significantly increased 6MWD at 3 months. There were no differences in HRQoL or side effects in this small, proof-

of-principle study. AN was well-tolerated by postmenopausal women and men with PAH, with good self-reported adherence.

Female sex is consistently associated with pulmonary vascular disease in epidemiologic studies (2, 6–9). Idiopathic, heritable, connective tissue disease–associated PAH and portopulmonary hypertension are characterized by a female predominance (6, 10, 11, 20). Animal models of PH have increased female penetrance under some, but not all, experimental conditions

(21–24). Despite the increased risk of PAH conferred by female sex, estrogen has potentially beneficial proangiogenic, antiapoptotic, and cardioprotective effects on the RV, hence the “estrogen paradox.” Studies have shown that women without cardiovascular disease and women with PAH have better RV systolic function at baseline than men and that the RV ejection fraction increases more in women than men after PAH treatment, explaining the longer survival in women compared with men with PAH (3, 4, 25–27). Blocking E2 production in patients with PAH could lead to the loss of these adaptive or compensatory cardiac effects and the potential worsening of outcomes. Several animal studies of increased RV afterload have suggested that E2 improves RV function (whereas testosterone or ovariectomy has adverse effects) (28–30), with additional support from observational human studies (27). However, some animal models have suggested that ovariectomy reverses PH (31).

In this small feasibility study, AN did reduce circulating E2 levels (the primary endpoint) by 40%, but to a lesser effect than normally seen in postmenopausal women with breast cancer treated with AN (>60–90%) (32, 33). This suggests either reduced absorption of the study drug, reduced efficacy in this population (e.g., due to altered metabolism or inadequate dosing), imperfect self-reported adherence, or alternative sources of E2 production that do not respond to AN. However, 11 of 12 patients in the active treatment arm had a reduction in E2 levels at 3 months, and self-reported adherence was excellent. Although the effect estimate seen here could indicate reduced efficacy of the intervention, the effects of aromatase inhibition in PAH may actually be local rather than systemic, making the impact on circulating E2 levels less important. Mair and colleagues (15) have recently shown expression of aromatase in the media of the small pulmonary arteries from female Sugen-hypoxia animals and women with PAH. Administration of AN after Sugen-hypoxia administration reduced RV systolic pressure, pulmonary vascular remodeling, and RV mass in the female animals (in whom it also reduced E2 levels). This group also showed that metformin had a similar effect via aromatase inhibition (17). Small muscular pulmonary arterioles also expressed estrogen receptor (ER)- α in the

Figure 5. Percent change from baseline in N-terminal of the prohormone brain natriuretic peptide (NT-proBNP) at 6 weeks and 3 months in subjects receiving anastrozole (red) versus placebo (blue).

Table 4. Biomarker Levels at Baseline, Six Weeks, and Three Months

	N	Baseline	Change from Baseline at 6 wk (%)	P Value	Change from Baseline at 3 mo (%)	P Value
NT-proBNP, pg/ml						
AN	12	106.0 (46.7 to 673.9)	0.1 (−14.4 to 39.9)	0.80	19.2 (−18.1 to 55.3)	0.80
Placebo	5	98.6 (8.8 to 431.1)	−4.0 (−15.0 to 28.3)		22.5 (−5.4 to 73.0)	
MCP-1, pg/ml						
AN	12	486.8 (320.0 to 888.8)	6.1 (−22.8 to 32.4)	0.44	13.4 (−0.2 to 54.9)	0.33
Placebo	5	527.8 (410.9 to 763.2)	0.9 (−11.4 to 4.4)		5.0 (−4.3 to 5.2)	
Insulin, μ U/ml						
AN	12	15.6 (7.1 to 27.2)	17.0 (−25.0 to 75.0)	0.38	17.3 (−8.6 to 40.5)	0.23
Placebo	5	34.4 (7.3 to 61.5)	−12.4 (−52.5 to 17.1)		−16.6 (−33.7 to −5.7)	
HOMA index						
AN	10	4.3 (1.1 to 8.0)	—	—	17.5 (−41.5 to 55.6)	0.30
Placebo	4	14.3 (4.7 to 28.8)	—	—	−18.9 (−32.3 to −6.6)	
IL-6, pg/ml						
AN	12	1.9 (1.1 to 5.0)	−9.5 (−22.2 to 33.1)	0.34	20.2 (−13.6 to 40.0)	0.39
Placebo	6	4.6 (1.3 to 11.5)	37.0 (−3.9 to 42.1)		−2.1 (−12.0 to 13.6)	
VEGF, pg/ml						
AN	12	273.9 (146.3 to 834.1)	18.1 (−1.2 to 124.9)	0.20	35.8 (−11.7 to 154.2)	0.44
Placebo	5	374.4 (339.0 to 387.9)	−3.8 (−4.2 to 9.9)		0.8 (−9.0 to 3.4)	
von Willebrand factor, %						
AN	12	164.0 (110.0 to 228.0)	6.3 (−8.9 to 10.3)	0.75	3.0 (−14.3 to 22.2)	0.68
Placebo	6	201.5 (153.0 to 233.0)	3.9 (−19.7 to 10.3)		4.5 (0.0 to 12.5)	

Definition of abbreviations: AN = anastrozole; HOMA = homeostatic model assessment of insulin resistance; MCP-1 = monocyte chemoattractant protein-1; NT-proBNP = N-terminal prohormone of brain natriuretic peptide; VEGF = vascular endothelial growth factor. Data are shown as median (interquartile range).

serotonin transporter overexpressing animal model, the blockade of which reduced RV systolic pressure (16). Pulmonary artery smooth muscle cells from

women with PAH showed increased ER- α expression, whereas cells from men with PAH showed increased ER- β expression. Genetic variants in ESR-1 (which encodes

ER- α) were also associated with the presence of portopulmonary hypertension in a previous study of patients with advanced liver disease (13). If local production of E2 is mechanistically important in PAH, systemic E2 levels may not adequately reflect the main site of action, and measurement of E2 across the pulmonary circulation may provide an even greater signal. Although inflammation has been proposed to mediate the links between E2 and PAH (34–39), we did not see changes in IL-6 or other biomarkers despite reductions in E2.

AN did not significantly affect TAPSE or other measures of RV function at rest in this small study of 3 months. It is possible that (1) AN did not affect RV function, (2) AN only improved RV function in the setting of exercise, or (3) AN had a beneficial effect on the pulmonary vasculature and RV afterload, which was counterbalanced by adverse effects on RV function. It is also possible that we did not have sufficient power to detect a difference (either improvement or worsening) when there was one present (Type II error) or that measurement error biased to the null. We were powered to detect a relatively large effect on TAPSE (1.7 SDs in change compared with placebo), which may require a longer term study.

Table 5. Frequency of Side Effects and Adverse Events

	AN	Placebo	P Value
Myalgias	4 (33.3)	2 (33.3)	>0.99
Diarrhea	2 (16.7)	0	0.53
Fever	2 (16.7)	0	0.53
Abdominal distention	1 (8.3)	0	>0.99
Anorexia	1 (8.3)	1 (16.7)	>0.99
Confusion	1 (8.3)	0	>0.99
Cough	1 (8.3)	0	>0.99
Fatigue	1 (8.3)	2 (33.3)	0.25
Gastroenteritis	1 (8.3)	0	>0.99
Headache	1 (8.3)	2 (33.3)	0.25
Hemorrhoid	1 (8.3)	0	>0.99
Insomnia	1 (8.3)	0	>0.99
Jaw pain	1 (8.3)	1 (16.7)	>0.99
Oral thrush	1 (8.3)	0	>0.99
Pneumonia	1 (8.3)	0	>0.99
Side cramps	1 (8.3)	0	>0.99
Sinus congestion	1 (8.3)	0	>0.99
Upper respiratory infection	1 (8.3)	0	>0.99
Weight loss	1 (8.3)	0	>0.99
Hot flashes	0	1 (16.7)	0.33
Lightheadedness	0	1 (16.7)	0.33
Night sweats	0	1 (16.7)	0.33
Palpitations	0	1 (16.7)	0.33
Presyncope	0	1 (16.7)	0.33
Worsening of Raynaud's disease	0	1 (16.7)	0.33

Definition of abbreviation: AN = anastrozole. Data shown as n (%).

AN significantly increased the 6MWD, which remained stable (or decreased slightly) while the subjects received placebo. The median change from baseline in the AN arm (+26 m) approached the threshold of clinical relevance in PAH (40, 41). This finding persisted whether expressed as a percent change from baseline or as an absolute change, and it was consistent, in that 10 of 12 patients randomized to AN had an increase in walking distance compared with those randomized to placebo, who had minimal changes. There are several explanations for the improvement in exercise capacity seen with AN versus placebo without apparent changes in RV function or morphology. It is possible that AN may have improved the hemodynamic response to exercise; however, the indicators (such as echocardiography at rest and NT-proBNP) may not have been sufficiently sensitive (or precise) to reflect these changes. Alternatively, AN may have improved exercise tolerance via a mechanism beyond changes in cardiac morphology or systolic function, such as improved peripheral blood flow, systemic muscle function, or oxygen use. The improvement in 6MWD provides reassurance about the safety of AN in terms of cardiopulmonary function and suggests possible clinical efficacy; however, larger randomized studies are necessary to confirm this.

Our study had several limitations. This was a small phase II randomized clinical trial that demonstrated the feasibility of studies of estrogen reduction in PAH and provided an initial estimate of the biologic

effect of AN. However, the study was not powered to see small effects, some of which might only appear over a longer period of time, so that we could not draw inferences about the long-term efficacy, safety, and tolerability of AN in PAH. Although there was no early safety signal that would preclude larger studies, the small number of participants could have limited this determination. No conclusions could be drawn about the efficacy and safety of AN in premenopausal women with PAH, because aromatase inhibition in this setting is challenging and poses other risks. We did not include patients with severe PAH (e.g., WHO functional class IV patients), which limited generalizability to this group. Because of the “proof-of-concept” nature of the trial and its small sample size, we were also not powered to detect differences in response across PAH subgroups, and there was expected variation in the endpoints in both the treatment and placebo arms.

There were some imbalances between the arms that were not unexpected considering the sample size. Although there were more men in the placebo group, baseline E2 levels were actually higher in this group. As a randomized interventional study, these imbalances were likely by chance, so that we might still invoke causal inference, unlike in an observational study. The hemodynamic response to AN would have been of interest; however, the requirement for multiple right heart catheterizations on otherwise stable, treated patients would have rendered the trial

infeasible. In addition, we have shown that short-term hemodynamics are not adequate surrogate endpoints in PAH trials, so that conclusions based on early hemodynamic findings (or lack thereof) might be in error (42). We also did not measure E2 metabolites, which may have been of interest based on previous studies (25, 43, 44). Only larger and longer studies with clinically important endpoints can elucidate whether AN is a safe and effective treatment for PAH. A longer phase trial would require evaluation for bone loss based on the use of AN for its approved indications (45). There was a small amount of missing data. It is unlikely that the conclusions would have changed based on the single missing data points and effect estimates.

In summary, we have shown that 1 mg daily of AN lowered total, bioavailable, and free E2 and estrone, but it did not affect TAPSE or other RV measures. AN increased 6MWD but did not have a significant effect on HRQoL, functional class, or inflammatory biomarkers. Larger randomized clinical trials are necessary to determine if AN is effective in treating PAH. ■

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