Correction of Nonsense *BMPR2* and *SMAD9* Mutations by Ataluren in Pulmonary Arterial Hypertension

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Heritable pulmonary arterial hypertension (HPAH) is a serious lung vascular disease caused by heterozygous mutations in the bone morphogenetic protein (BMP) pathway genes, *BMPR2* and *SMAD9*. One noncanonical function of BMP signaling regulates biogenesis of a subset of microRNAs. We have previously shown that this function is abrogated in patients with HPAH, making it a highly sensitive readout of BMP pathway integrity. Ataluren (PTC124) is an investigational drug that permits ribosomal readthrough of premature stop codons, resulting in a full-length protein. It exhibits oral bioavailability and limited toxicity in human trials. Here, we tested ataluren in lung- or blood-derived cells from patients with HPAH with nonsense mutations in *BMPR2* ($n = 6$) or *SMAD9* ($n = 1$). Ataluren significantly increased BMP-mediated microRNA processing in six of the seven cases. Moreover, rescue was achieved even for mutations exhibiting significant nonsense-mediated mRNA decay. Response to ataluren was dose dependent, and complete correction was achieved at therapeutic doses currently used in clinical trials for cystic fibrosis. BMP receptor (BMPR)-II protein levels were normalized and ligand-dependent phosphorylation of downstream target Smads was increased. Furthermore, the usually hyperproliferative phenotype of pulmonary artery endothelial and smooth muscle cells was reversed by ataluren. These results indicate that ataluren can effectively suppress a high proportion of *BMPR2* and *SMAD9* nonsense mutations and correct BMP signaling *in vitro*. Approximately 29% of all HPAH mutations are nonsense point mutations. In light of this, we propose ataluren as a potential new personalized therapy for this significant subgroup of patients with PAH.

Keywords: pulmonary arterial hypertension; nonsense mutation; ataluren; bone morphogenetic protein signaling

Pulmonary arterial hypertension (PAH) is a potentially fatal disorder of the lung vasculature in which proliferation of endothelial and smooth muscle cells progressively obliterates the pulmonary arterioles. This causes a sustained elevation in pulmonary artery pressure and can lead to right heart failure. Current treatments slow disease progression and alleviate symptoms, but do not cure the disease. PAH may be idiopathic or associated

CLINICAL RELEVANCE

Pulmonary arterial hypertension (PAH) is a life-threatening lung disease. Approximately 40% of cases are idiopathic or familial, and, of these, about 25% have a mutation in the bone morphogenetic protein (BMP) pathway. This study shows that ataluren, a drug that induces ribosomal readthrough of nonsense mutations, can restore BMP signaling *in vitro*, and therefore opens up the possibility of future personalized therapy for the subset of patients with PAH with this type of mutation.

with an underlying condition. About 6% of patients have a positive family history, where the disease is inherited as an autosomal dominant trait with an estimated 27% penetrance (1). All patients with familial disease and/or an identifiable mutation are now grouped together as heritable PAH (HPAH) (2, 3).

The primary genetic predisposition to PAH is a heterozygous mutation of one of four genes in the bone morphogenetic protein (BMP) signaling pathway, most commonly *BMPR2*, which encodes a type II BMP receptor (BMPR-II). Mutations in the *BMPR2* gene have been found in up to 80% of affected families (3–6) and 10–40% of patients with apparently sporadic idiopathic PAH (5, 7, 8). Mutation-positive patients with idiopathic PAH may represent either *de novo* mutations or inheritance of a mutation that was nonpenetrant in the carrier parent. To date, over 200 distinct mutations have been identified in nearly 300 individuals, with nonsense point mutations accounting for approximately 29% overall (3, 6). Less commonly, mutations are found in other BMP pathway genes: activin receptor-like kinase 1 (gene symbol, *ACVRL1*), endoglin (*ENG*), or Smad8 (gene symbol, *SMAD9*) (9–16).

BMP signaling regulates many processes related to growth, differentiation, and development. Receptor–ligand binding stimulates phosphorylation of Smad1, -5, and -8, which, in canonical signaling, then associate with Smad4. There are also several Smad4-independent functions, including post-transcriptional up-regulation of a subset of microRNAs (miRs) (17, 18). Recently, we showed that, in cells from the explant lungs of patients with HPAH, mutations in *BMPR2* or *SMAD9* abrogate this BMP-mediated miR induction (16). Two of the affected miRs, miR-21 and miR-27a, are growth suppressive in pulmonary vascular cells. The inability to up-regulate these miRs in HPAH cells may therefore contribute to their hyperproliferative phenotype (16). In contrast, canonical signaling remained relatively intact, indicating that the miR pathway is an especially sensitive measure of the integrity of BMP signaling. Moreover, we demonstrated that overexpression of the wild-type gene can correct miR processing and proliferation rate in HPAH cells (16). We therefore considered that restoration of normal gene function could be a novel therapeutic approach in PAH.

Ataluren is a small molecule that potentially promotes readthrough of premature stop codons without affecting normal translational stop signals (19, 20). In clinical trials for cystic

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fibrosis, it appears to be well tolerated with few adverse effects (21–23). In this study, we investigated its utility as a therapeutic intervention for PAH by examining the ability of ataluren to restore BMP signaling and reverse the hyperproliferative phenotype in HPAH cells. Our results suggest that ataluren is highly effective in correcting BMP signaling, and may be applicable to a broad range of HPAH nonsense mutations.

MATERIALS AND METHODS

Study Population

Seven different HPAH nonsense mutations were studied (Table 1). Pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs) were available from explant lung tissue for two cases; the remaining five were studied in blood-derived cells, either lymphoblastoid cell lines (LCLs) or late-outgrowth endothelial progenitor cells (L-EPCs). PAH PAECs and PASMCs were isolated as previously described (24, 25). Control cells were purchased commercially (Lonza, Allendale, NJ). Cells were maintained in EGM-2 (PAEC) or SmGM-2 (PASMC) medium (Lonza) and used at passages 4–9. L-EPCs were isolated as previously described (26), and maintained in EGM-2MV (Lonza) supplemented with 10% FBS. LCLs were generated from peripheral blood mononuclear cells and maintained in RPMI-1640 supplemented with 20% FBS. Analysis for mutations in *BMPR2* and *SMAD9* was performed as previously described (27, 28).

Quantitative PCR

PAECs were plated at 8.8×10^4 cells per well and PASMCs at 4.2×10^4 cells per well in 12-well plates. At 24 hours after seeding, cells were treated with ataluren (Selleck Chemicals, Houston, TX) dissolved in cell-culture grade DMSO at various concentrations for 24 hours, then 3 nM BMP4 (PASMC, LCL) or BMP9 (PAEC, L-EPC) was added for 4 hours. This choice of ligands reflects known tissue-specific responsiveness (29). Each experimental condition was performed in triplicate. Total RNA was extracted using miRNeasy kits (Qiagen, Valencia, CA). For mRNA quantitation, 250 ng of RNA was reverse transcribed using random primers and SuperScript III (Invitrogen, Carlsbad, CA), and analyzed using QuantiTect SYBR Green mastermix (Qiagen). Results were normalized to *GAPDH*. Mature miRs were quantified using TaqMan microRNA kits (Applied Biosystems, Foster City, CA) and normalized to *RNU48*. All assays were performed in triplicate for each of the three independent RNA preparations.

Western Blotting

Cells were seeded at 1×10^6 cells per plate in 10-cm plates and treated as detailed in the RESULTS section. Cells were lysed in 500 μ l Laemmli buffer, boiled for 5 minutes, and 50–150 μ l was resolved on 10% SDS

polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA) using Trans-blot Turbo apparatus (Bio-Rad). Blots were probed with the relevant antibodies: mouse monoclonal BMPR-II (BD Biosciences, San Jose, CA); α -tubulin mouse monoclonal (Sigma-Aldrich, St. Louis, MO); phospho-Smad1/5 rabbit monoclonal (Cell Signaling, Danvers, MA); and total-Smad1/5/8 rabbit monoclonal (Santa Cruz, Dallas, TX). After chemiluminescent detection, blots were quantitated with Quantity One software (Bio-Rad).

Proliferation Assays

Cell proliferation assays were performed over a 72-hour time course using direct live cell counts or XTT (2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carbox-anilide) assay (R&D Systems, Minneapolis, MN). For live cell counts, cells were seeded at 7.35×10^4 per well in 12-well plates, then harvested at 12, 24, 36, 48, or 72 hours, stained with trypan blue, and counted using a hemocytometer. For XTT assays, cells were seeded in 96-well plates at 4,000 (PAECs) or 800 (PASMCs) cells per well. Assays were performed in triplicate and normalized to a standard curve of relevant control cells. Data were tested for statistical significance at the 72-hour time point using one-way ANOVA, followed by pair-wise multiple comparison procedures (Holm-Sidak method).

RESULTS

We first tested ataluren at a concentration of 3 μ M, the dose that maximized nonsense readthrough in the initial luciferase assays (20). We used BMP-mediated induction of miR processing as our reporter, because this is the most sensitive readout of BMP signaling at our disposal (16), focusing specifically on miR-27a because it is expressed across all the cell types that we studied. Results were validated with miR-21 in endothelial and smooth muscle cells (data not shown). Endothelial cells with three different mutations all showed a partial recovery of miR-27a induction after treatment with 3 μ M ataluren (Figure 1A). We also tested four additional mutations in transformed LCL. These cells respond poorly to BMP4, and neither patient nor control LCL showed significant induction of miR-27a in the absence of ataluren. Despite this, three mutations (R213X, Q433X, and E845X) did show significant induction by BMP4 after ataluren treatment (Figure 1B). R332X was the only mutation that did not respond. Notably, with the exception of W9X, all mutations showed evidence of significant nonsense-mediated mRNA degradation (NMD) upon cDNA sequencing (Figure E1 in the online supplement). These results suggest that the majority of *BMPR2* and *SMAD9* nonsense mutations

TABLE 1. SUMMARY OF MUTATIONS AND CELL TYPES ANALYZED IN THIS STUDY

				Response to Ataluren (miR-27a Fold Change)
Mutation	Nonsense Codon*	Cell Type	% Mutant Transcript Present in cDNA	P Value
<i>BMPR2</i>				
W9X	UGAc	L-EPC	100 (no NMD)	<0.001
R213X	UGAt	LCL	~50	<0.05
R321X	UGAg	PAEC, PASMC	<10 (complete NMD)	<0.001
R332X	UGAg	LCL	~25	NS
Q433X	UAGa	LCL	~20	<0.001
E845X	UAAa	LCL	~25	<0.001
Exon 1-8 deletion (Δ ex1-8)	N/A	PAEC	0 (promoter is deleted)	NS
<i>SMAD9</i>				
R294X	UGAa	PAEC, PASMC	~10	<0.001

Definition of abbreviations: LCL, lymphoblastoid cell line; L-EPC, late-outgrowth endothelial progenitor cell; miR, microRNA; N/A, not applicable; NMD, nonsense-mediated mRNA degradation; NS, no significant change; PAEC, pulmonary artery endothelial cell; PASMC, pulmonary artery smooth muscle cell.

*The sequence of the nonsense codon is indicated in upper case, followed by the succeeding base in lower case. Previous studies have indicated that ataluren is most effective in UGAc codons (20), but no clear correlation was seen in heritable pulmonary arterial hypertension cells.

Figure 1. Ataluren significantly increases microRNA (miR) processing across a range of nonsense pulmonary arterial hypertension (PAH) mutations. Cells were treated with 3 μ M ataluren for 24 hours, then the appropriate bone morphogenetic protein (BMP) ligand for 4 hours. RNA was analyzed for miR-27a, normalized to RNU48. Results are shown as fold change of miR-27a with BMP treatment compared with vehicle. (A) Pulmonary artery endothelial cells (PAECs; R321X and R294X) or late-outgrowth endothelial progenitor cells (L-EPs; W9X) treated with BMP9. (B) Lymphoblastoid cell line (LCL) treated with BMP4. Means ($\pm$ SEM) of nine replicates are shown. * $P < 0.05$; ** $P < 0.001$.

may be amenable to readthrough mediated by ataluren, irrespective of their NMD status.

The first studies of ataluren efficacy in primary human cells established that the maximal effect was achieved at 17 μ M, more than 5-fold higher than the luciferase assays (20). Dosing in subsequent mouse studies and human clinical trials has aimed to maintain a serum level in this range (19, 21). We therefore performed a dose-response analysis in PAH PAECs to determine the optimal concentration for this cell type. In the absence of drug treatment, R321X PAECs showed no BMP9-mediated induction of miR-27a (Figure 2A). When treated with ataluren, there was a dose-dependent increase in miR-27a induction up to 20 μ M, after which it plateaued. A dose of 15 μ M was sufficient to achieve complete correction of the fold change in comparison to control cells (Figure 2A). Control PAECs consistently showed a threefold induction that did not change with drug treatment. As a measure of canonical (Smad4-mediated) BMP signaling, we also analyzed induction of *ID1* mRNA. However, there was no appreciable deficit in PAH cells in comparison to controls, and no consistent change with ataluren treatment (Figure 2B, Figure E2), supporting our previous finding that the Smad4-independent miR pathway is a more sensitive readout for the integrity of BMP signaling than the canonical pathway (16). Consistent with data published for other genes, ataluren did not alter the level of *BMPR2* or *SMAD9* transcript (Figure E3), nor the amount of NMD (data not shown).

In view of these promising results, we reanalyzed the three endothelial mutations at a concentration of 15 μ M ataluren. BMP-mediated induction of miR-27a was completely normalized for both *BMPR2* mutations at this dose (Figure 3). *SMAD9*:R294X

showed a significant recovery of miR induction, but the fold change remained below that seen in controls, possibly a reflection of the lower basal level of miR-27a expression in these cells. Similar results were obtained at 20 μ M ataluren and with miR-21, a second reporter for this pathway (data not shown). As an additional control, we included a *BMPR2* deletion mutation (Δ ex1–8), which should not be correctable with ataluren treatment. Indeed, this mutation showed no induction of miR-27a at 15 μ M (Figure 3) or 20 μ M (data not shown), confirming that restoration of miR processing was specific to nonsense mutations. PSMCs were also available from the two explant cases, and here the fold change for *SMAD9*:R294X was completely corrected (Figure 3). We also retested the R332X mutation in LCL isolated from two different members of the same family. However, even at a concentration of 20 μ M ataluren, the results were inconsistent and, in view of the apparently low level of BMP signaling in LCL, we did not reanalyze the other LCL mutations at higher doses of ataluren.

To determine whether ataluren-mediated readthrough resulted in an increase in BMPR-II protein levels, we performed Western blot analysis of endothelial cell lysates. As expected for a heterozygous nonsense mutation, the basal level of wild-type protein was reduced by at least 50% in PAH cells compared with control (Figure 4A). After treatment with 20 μ M ataluren for 24 hours, we observed a 1.9- and 3.7-fold increase in BMPR-II in R321X and W9X mutant cells, respectively (Figure 4A). To determine whether this increase leads to an up-regulation of BMP signaling, we then measured receptor-Smad phosphorylation. Cells were treated with 20 μ M ataluren for 24 hours in serum-free media and then induced with BMP9 for 4 hours.

Figure 2. Response of PAH PAECs to ataluren is dose dependent. PAECs with the *BMPR2* R321X mutation were treated for 24 hours with a range of ataluren concentrations as indicated, followed by the addition of BMP9 for 4 hours. (A) miR-27a normalized to RNU48. Induction of miR-27a is dose dependent to 20 μ M, then plateaus. (B) *ID1* mRNA normalized to *GAPDH*, as a readout of canonical BMP signaling. R321X shows no deficit in *ID1* induction and no change with ataluren treatment. Means ($\pm$ SEM) of nine replicates are shown.

Figure 3. Ataluren corrects miR processing in endothelial cells and pulmonary artery smooth muscle cells (PASMCs). Cells were treated with 15 μ M ataluren for 24 hours, then BMP9 (PAEC, L-EPC) or BMP4 (PASMC) for 4 hours. RNA was analyzed for miR-27a, normalized to RNU48. Results are shown as fold change of miR-27a with BMP treatment compared with vehicle. Means ($\pm$ SEM) of nine replicates are shown.

This led to a 1.4-fold increase in receptor-Smad phosphorylation, restoring the fold change in mutant cells to the range seen in controls (Figure 4B).

We also observed an additional band of approximately 112 kD in W9X cells that was not present in controls or R321X (Figure E4). Given the very early termination of the W9X transcript and the absence of NMD, we postulated that this represents a reinitiation product from a downstream Kozak sequence. Interestingly, when the cells were treated with ataluren, the abundance of the truncated protein decreased, consistent with the reinitiation being competitively inhibited by ataluren-mediated readthrough and the increase in full-length protein.

Pulmonary artery endothelial and smooth muscle cells from patients with HPAH are hyperproliferative compared with controls, a phenotype that we have previously corrected by overexpressing the wild-type gene (16). To investigate the potential physiologic effects of ataluren treatment in the pulmonary artery, we performed a growth curve analysis of control and R321X cells treated with 20 μ M ataluren, and counted live cells at each time point. Treatment with ataluren significantly reduced R321X cell growth, but had no effect on control cells (Figure 5A). One-way ANOVA of cell counts at 72 hours was statistically significant ($P < 0.001$), with highly significant pair-wise differences between ataluren-treated and untreated R321X cells. This decrease in proliferation occurred in the absence of significant cell death or

senescence (data not shown). Figure 5B shows the effect of BMP9 on the cells, and suggests that treatment with ataluren is also able to restore BMP-mediated growth suppression.

We next analyzed the growth parameters of patient PAECs using XTT assays. All HPAH cells proliferated significantly faster than controls at baseline (Figure 5C). Treatment with ataluren corrected baseline proliferation in cells with truncating mutations, restoring their growth to a rate comparable with unstimulated control cells (Figure 5E). Importantly, this suppression was not observed in PAECs with the *BMPR2* deletion (Δ ex1-8), confirming that the effect was specific to readthrough of nonsense mutations (Figure 5E). As previously reported, BMP stimulation decreased the overall proliferation rate, but HPAH cells still grew faster than unstimulated controls (Figure 5D). Figure 5F shows that BMP responsiveness was also restored after ataluren treatment. Results in PASMCs from the same patients directly mirrored those in PAECs (Figures E5A–E5D). Thus, overall, ataluren was able to normalize both basal proliferation rates and BMP-mediated growth suppression in PAECs and PASMCs from patients with nonsense mutations, with no evidence of adverse effect on the growth of control cells.

DISCUSSION

In the current treatment era, the average life expectancy of patients with PAH is estimated at 5–7 years after diagnosis, with significant

Figure 4. Ataluren restores bone morphogenetic protein receptor (BMPR)-II protein levels and increases Smad phosphorylation. (A) Human endothelial cells were treated with 20 μ M ataluren for 24 hours. Total BMPR-II protein levels were assessed using immunoblotting. Blots were reprobed for α -tubulin and normalized to account for loading differences. Quantification was performed across two replicate blots, and is indicated by the values and plots below the blot. (B) W9X L-EPCs were treated with 20 μ M ataluren for 24 hours and then induced with BMP9 for 4 hours. Immunoblotting was performed with antibodies against phospho-Smad1/5 or total-Smad1/5/8. Blots were quantitated and the phospho-Smad signal was normalized to total-Smad levels. Bars indicate the fold change of phospho-Smad in BMP9-treated cells compared with vehicle.

Figure 5. Effects of ataluren readthrough on the proliferation of PAECs. Live cell counts show that ataluren treatment leads to growth suppression of R321X (A) and restores BMP-mediated growth repression in response to BMP9 (B) (*post hoc* *P* values versus control at 72-h: R321X + ataluren, $P < 0.001$; R294X + ataluren + BMP9 versus control + BMP9, $P = 0.42$). In cell proliferation assays using XTT: (C) untreated PAECs from heritable PAH (HPAH) proliferate significantly faster than controls (*post hoc* pairwise *P* values at 72 h; all $P < 0.001$); (D) BMP9 treatment slows proliferation in all cells, but HPAH cases still proliferate faster than untreated control cells ($P < 0.001$); (E) Ataluren treatment significantly represses the growth of R321X and R294X cells, but has no effect on Δex1–8 cells ($P < 0.001$); (F) BMP9 induction shows restoration of BMP growth repression in R321X and R294X, but not Δex1–8 cells. Means ($\pm$ SD) of triplicate wells are shown.

morbidity (30, 31). Current therapies for PAH target the prostacyclin, endothelin, or nitric oxide pathways, and are believed to be effective by diminishing vasoconstriction and decreasing endothelial and smooth muscle cell proliferation (32–34). However, these therapies have only demonstrated modest clinical improvements in patients. In particular, patients with *BMPR2* mutations are less likely to respond to acute vasodilator challenge and calcium channel blocker treatment (35–37). The ideal therapy for PAH would not only ameliorate current symptoms, but also drive the reversal of pathological features, such as vascular remodeling and vasoconstriction in patient lungs. A recent study using an adenoviral *BMPR2* vector to reverse established PAH in rodents (38), as well as our own data showing the effectiveness of overexpressing the wild-type gene *in vitro* (16), establishes restoration of *BMPR2* levels as a potential new mode of therapeutic action in PAH.

One approach to correct a subset of HPAH mutations is to promote readthrough of premature termination codons. High concentrations of aminoglycosides, such as gentamicin, allow translational readthrough of truncating mutations, and have been shown to functionally eliminate premature stop codons in a number of diseases, including cystic fibrosis (39, 40) and the muscular dystrophies (41, 42). Gentamicin has also been tested on two HPAH mutations (43, 44). However, the potential renal and otic toxicities of aminoglycosides prohibit long-term use (45, 46). Ataluren was identified in a high-throughput screen for compounds that induce readthrough of nonsense mutations by altering ribosomal proofreading activity on premature termination codons, while remaining ineffective on *bona fide* termination codons (20). Trials in adults and children with cystic

fibrosis have shown promising initial results (21–23). Here, we have demonstrated that ataluren is also effective in increasing *BMPR2* protein levels and *Smad* phosphorylation, correcting BMP-regulated miR processing and restoring normal proliferation rates in PAECs and PSMCs from patients with PAH with nonsense mutations. These effects were achieved at the concentrations currently used in clinical trials.

One striking observation of our study is that we saw no correlation between the extent of NMD and the ability of ataluren to correct miR processing. This is in contrast to cystic fibrosis mutations where the capacity of ataluren to correct *CFTR* mutations is inversely related to the amount of NMD (47). Induction of the BMP pathway led to a temporary increase in *BMPR2* and *SMAD9* expression (Figure E3), and we considered that this *de novo* transcription might lead to transiently higher levels of nonsense-containing transcripts that could be available for ataluren readthrough. However, we found no evidence for a relative increase in mutant transcripts over a 4-hour time course (data not shown), and so the basis of this difference between cystic fibrosis and PAH mutations remains unclear. In addition, although ataluren shows maximal activity with UGA codons (20), we did not see any correlation between the sequence context of individual PAH mutations and their response to the drug (Table 1). Therefore, our data suggest that ataluren may be amenable to treating a high proportion of HPAH nonsense mutations—six of seven in this study.

To date, 40 distinct nonsense mutations have been reported in *BMPR2* (3, 6), and two in *SMAD9* (14, 16). Due to the rarity of lung transplant tissue from patients with HPAH, we were only able to test ataluren on two mutations in explant lung cells.

However, blood-derived L-EPCs proved to be a good surrogate for PAECs, demonstrating robust miR up-regulation in response to BMP stimulation and similar levels of correction by ataluren. With the advent of personalized medicine, future clinical trials will require a relevant tissue in which to determine the efficacy of drugs against specific mutations. To this end, the performance of L-EPCs in our study was superior to LCLs, and offers an excellent substrate for studying ataluren across a broad spectrum of mutations, including *ACVRL1* and *ENG*. We also propose that restoration of miR processing is a robust readout that could be used to test alternative approaches for restoring BMPR-II levels in other classes of mutation, such as reducing lysosomal degradation (48) or chemical chaperones to improve trafficking of misfolded mutant BMPR-II protein to the cell surface (49, 50).

In summary, our study suggests that ataluren is capable of correcting multiple aspects of BMP signaling at physiologically relevant doses, even in the presence of significant nonsense-mediated mRNA decay, and therefore warrants further study as a potential therapy specific to patients with HPAH harboring nonsense mutations. One limitation is that we currently do not know whether correcting BMPR-II levels would be successful in treating a patient with advanced disease. Established PAH can be reversed by adenoviral expression of *BMPR2* in both the monocrotaline and hypoxia rat models (38), but the extent to which advanced disease in humans is *BMPR2* driven is unknown. Promisingly, however, in this study, ataluren corrected the hyperproliferative phenotype of PAECs and PSMCs as efficiently as gene overexpression (16), and may therefore be able to reverse established disease. Although few patients have a family history of PAH, systematic genetic testing identifies *BMPR2* mutations in approximately 25% of all patients with familial or idiopathic disease (51). Of these, around 29% will have nonsense mutations (3). The potential use of ataluren as a therapeutic molecule for this sizeable subset of patients with PAH heralds an exciting new era in which PAH treatment may be personalized to specific genetic causes.

Author disclosures are available with the text of this article at www.atsjournals.org.

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