4. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, *et al*.; VX17-445-103 Trial Group. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *Lancet* 2019;394:1940–1948.
5. Middleton PG, Mall MA, Dřevínek P, Lands LC, McKone EF, Polineni D, *et al*.; VX17-445-102 Study Group. Elexacaftor-tezacaftor-ivacaftor for cystic fibrosis with a single Phe508del allele. *N Engl J Med* 2019;381:1809–1819.
6. Mall MA, Mayer-Hamblett N, Rowe SM. Cystic fibrosis: emergence of highly effective targeted therapeutics and potential clinical implications. *Am J Respir Crit Care Med* 2020;201:1193–1208.
7. Accurso FJ, Rowe SM, Clancy JP, Boyle MP, Dunitz JM, Durie PR, *et al*. Effect of VX-770 in persons with cystic fibrosis and the G551D-CFTR mutation. *N Engl J Med* 2010;363:1991–2003.
8. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Cao D, Neuberger T, *et al*. Rescue of CF airway epithelial cell function *in vitro* by a CFTR potentiator, VX-770. *Proc Natl Acad Sci USA* 2009;106:18825–18830.
9. Sun X, Yi Y, Yan Z, Rosen BH, Liang B, Winter MC, *et al*. *In utero* and postnatal VX-770 administration rescues multiorgan disease in a ferret model of cystic fibrosis. *Sci Transl Med* 2019;11:eaau7531.
10. Ernst SE, Stoltz DA, Samuel M, Karp P, Tan P, Stroik M, *et al*. Poster Session, poster #447: development of a G551D porcine model of cystic fibrosis. *Pediatr Pulmonol* 2019;54(Suppl 2):S155–S480.
11. Bose SJ, Krainer G, Ng DRS, Schenkel M, Shishido H, Yoon JS, *et al*. Towards next generation therapies for cystic fibrosis: folding, function and pharmacology of CFTR. *J Cyst Fibros* 2020;19(Suppl 1):S25–S32.
12. Birket SE, Davis JM, Fernandez-Petty CM, Henderson AG, Oden AM, Tang L, *et al*. Ivacaftor reverses airway mucus abnormalities in a rat model harboring a humanized G551D-CFTR. *Am J Respir Crit Care Med* 2020;202:1271–1282.
13. Tuggle KL, Birket SE, Cui X, Hong J, Warren J, Reid L, *et al*. Characterization of defects in ion transport and tissue development in cystic fibrosis transmembrane conductance regulator (CFTR)-knockout rats. *PLoS One* 2014;9:e91253.
14. Birket SE, Davis JM, Fernandez CM, Tuggle KL, Oden AM, Chu KK, *et al*. Development of an airway mucus defect in the cystic fibrosis rat. *JCI Insight* 2018;3:e97199.
15. Rosen BH, Evans TIA, Moll SR, Gray JS, Liang B, Sun X, *et al*. Infection is not required for mucoinflammatory lung disease in CFTR-knockout ferrets. *Am J Respir Crit Care Med* 2018;197:1308–1318.
16. Bruscia EM, Zhang PX, Ferreira E, Caputo C, Emerson JW, Tuck D, *et al*. Macrophages directly contribute to the exaggerated inflammatory response in cystic fibrosis transmembrane conductance regulator-/- mice. *Am J Respir Cell Mol Biol* 2009;40:295–304.
17. Hisert KB, Heltshe SL, Pope C, Jorth P, Wu X, Edwards RM, *et al*. Restoring cystic fibrosis transmembrane conductance regulator function reduces airway bacteria and inflammation in people with cystic fibrosis and chronic lung infections. *Am J Respir Crit Care Med* 2017;195:1617–1628.
18. Barnaby R, Koeppen K, Nymon A, Hampton TH, Berwin B, Ashare A, *et al*. Lumacaftor (VX-809) restores the ability of CF macrophages to phagocytose and kill *Pseudomonas aeruginosa*. *Am J Physiol Lung Cell Mol Physiol* 2018;314:L432–L438.
19. Zhang S, Shrestha CL, Kopp BT. Cystic fibrosis transmembrane conductance regulator (CFTR) modulators have differential effects on cystic fibrosis macrophage function. *Sci Rep* 2018;8:17066.
20. Jarosz-Griffiths HH, Scambler T, Wong CH, Lara-Reyna S, Holbrook J, Martinon F, *et al*. Different CFTR modulator combinations downregulate inflammation differently in cystic fibrosis. *Elife* 2020;9:e54556.
21. Dreano E, Bacchetta M, Simonin J, Galmiche L, Usal C, Slimani L, *et al*. Characterization of two rat models of cystic fibrosis-KO and F508del CFTR-generated by Crispr-Cas9. *Animal Model Exp Med* 2019;2:297–311.
22. McCarron A, Cmielewski P, Reyne N, McIntyre C, Finnie J, Craig F, *et al*. Phenotypic characterization and comparison of cystic fibrosis rat models generated using CRISPR/Cas9 gene editing. *Am J Pathol* 2020;190:977–993.

A Plot TWIST in Pulmonary Arterial Hypertension

The treatment of pulmonary arterial hypertension (PAH) has been a success story in pulmonary medicine. Major advances in our understanding of the mechanisms driving PAH have suggested a complicated interplay of many processes, including endothelial cell dysfunction, perivascular inflammation, smooth muscle cell hyperproliferation, and vasoconstriction (1). There are three classes of drugs that have led to improvements in symptoms and survival. Despite these advances, median survival is only 6 years (2), with death typically occurring as a result of cor pulmonale. Existing therapies for PAH primarily target sustained pulmonary vasoconstriction (3) despite the presence of several other pathophysiologic pathways that may be amenable to intervention.

One attractive approach to PAH therapy could be to target the proproliferative/prosurvival phenotype of pulmonary artery smooth muscle cells (4). Uncovering the role of a potential "oncogene" in PAH would certainly fit the bill. In this issue of the *Journal*, Fan and colleagues (pp. 1283–1296) report their exciting findings that argue for the role of the transcription factor TWIST1 in the pathogenesis of PAH (5). How is TWIST1 relevant to PAH? *TWIST1* is a well-known oncogene implicated in metastasis and resistance to chemotherapy (6). In idiopathic pulmonary fibrosis, *Twist1* transcription has been shown to be highly upregulated in idiopathic pulmonary fibrosis lungs and to promote lung fibroblast accumulation by inhibiting apoptosis (7). Similarly, in PAH, *Twist1* has already been shown to be overexpressed in the lungs and to contribute to so-called endothelial-to-mesenchymal transition through TGFβ–Smad2 signaling (8). Therefore, TWIST1 may drive this quasineoplastic pulmonary artery smooth muscle cell (PASMC) phenotype in PAH.

In contrast to data reported in a previous study (9), Fan and colleagues have shown that TWIST1 expression is increased in PASMCs from patients with familial PAH. Furthermore, in rodent models, PASMC-specific loss of *twist1* resulted in the attenuation of pulmonary hypertension. Overexpression of *Twist1* drove PASMC proliferation and migration and overcame the effects of harmine, a small molecule that is reported to promote TWIST1 degradation (10).

To understand the mechanism behind these findings, the team turned to familiar targets, including BMPR2, the so-called PAH

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gene (11). Silencing of *TWIST1* increased BMPR2 expression, and, inversely, TWIST1 overexpression decreased *Bmpr2* transcription. Although this finding might suggest that TWIST1 interacts with the *Bmpr2* promoter, this was not observed. Through mass spectrometry analysis the team identified a physical interaction of TWIST1 with GATA-6, a transcription factor associated with PASMC growth, and they confirmed this finding by coimmunoprecipitation. *TWIST1* overexpression decreased GATA-6 protein levels despite having no effect on the level of *GATA6* mRNA. This indeed was a surprising finding. TWIST1 is a transcriptional inhibitor (12). If the effect of TWIST1 on GATA-6 is not mediated by changes in mRNA levels, then does it regulate protein stability? Indeed, the authors found that reduction of GATA-6 levels driven by TWIST1 was mediated by the ubiquitin E3 ligase activity of MDM2. This reduction in GATA-6 protein levels led to decreased engagement of the BMPR2 promoter, completing the link between TWIST1 overexpression and decreased BMPR2 signaling.

This is a plot twist in our understanding of TWIST1. Instead of showing binding to the promoter regions to reduce transcription of *GATA6* or *Bmpr2* as might be expected of a transcription factor, the authors instead demonstrated a direct interaction between TWIST1 and GATA-6 proteins, and that this interaction led to increased proteasomal degradation of GATA-6. Although TWIST1 expression appeared to increase GATA-6–MDM2 interaction leading to GATA-6 ubiquitination, the exact mechanism by which TWIST1 promotes this interaction is not entirely clear. TWIST1 does not increase MDM2 expression, but through its interaction with GATA-6, it might induce a conformational change and increase the capacity for MDM2 binding and the destabilization of GATA-6. Further exploration of this relationship could identify a new druggable target in PAH.

Although transcription factors are notoriously difficult drug targets, the β-carboline alkaloid compound harmine has been shown to be a potent TWIST1 inhibitor (10). However, previous attempts at using harmine as a cancer therapy have been hampered by significant neurotoxicity, so the ability to create harmine derivatives with anti-TWIST1 activity and acceptable safety is an open question (13). In addition, the complete inhibition of TWIST1 may be inadvisable, as data from our group suggest that the loss of *twist1* in the mesenchymal compartment may increase inflammation and worsen fibrosis (12). The effect of TWIST1 activity on the ubiquitination and degradation of GATA-6 does perhaps unveil a more promising opportunity for therapy. The ubiquitin–proteasome system has been associated with many lung diseases (14) and has been proposed as a potential therapeutic target. Ubiquitin E3 ligases and subunits, each with highly specific substrate–ligase binding pockets, are potentially amenable to small molecule inhibitors (15). The development of drugs targeting the ubiquitin system is an active area of research (16), particularly within cancer therapeutics. Notably, there are multiple ongoing cancer clinical trials examining the effects of compounds blocking the E3-ligase MDM2 (17), which the authors implicate here as being integral for TWIST1-mediated GATA-6 degradation. Perhaps a similar approach could be employed to inhibit TWIST1-driven loss of GATA-6 and reduce PASMC hypertrophy and proliferation? These exciting findings might identify a new class of therapies that may synergize with existing success stories in PAH. ■

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References

1. Bourgeois A, Omura J, Habbout K, Bonnet S, Boucherat O. Pulmonary arterial hypertension: new pathophysiological insights and emerging therapeutic targets. *Int J Biochem Cell Biol* 2018;104: 9–13.
2. Thenappan T, Ormiston ML, Ryan JJ, Archer SL. Pulmonary arterial hypertension: pathogenesis and clinical management. *BMJ* 2018;360: j5492.
3. Spiekerkoetter E, Kawut SM, de Jesus Perez VA. New and emerging therapies for pulmonary arterial hypertension. *Annu Rev Med* 2019;70: 45–59.
4. Pullamsetti SS, Savai R, Seeger W, Goncharova EA. Translational advances in the field of pulmonary hypertension: from cancer biology to new pulmonary arterial hypertension therapeutics. targeting cell growth and proliferation signaling hubs. *Am J Respir Crit Care Med* 2017;195:425–437.
5. Fan Y, Gu X, Zhang J, Sinn K, Klepetko W, Wu N, *et al*. TWIST1 drives smooth muscle cell proliferation in pulmonary hypertension via loss of GATA-6 and BMPR2. *Am J Respir Crit Care Med* 2020;202:1283–1296.
6. Yochum ZA, Cades J, Wang H, Chatterjee S, Simons BW, O'Brien JP, *et al*. Targeting the EMT transcription factor TWIST1 overcomes resistance to EGFR inhibitors in EGFR-mutant non-small-cell lung cancer. *Oncogene* 2019;38:656–670.
7. Bridges RS, Kass D, Loh K, Glackin C, Borczuk AC, Greenberg S. Gene expression profiling of pulmonary fibrosis identifies Twist1 as an antiapoptotic molecular "rectifier" of growth factor signaling. *Am J Pathol* 2009;175:2351–2361.
8. Mammoto T, Muyleart M, Konduri GG, Mammoto A. Twist1 in hypoxia-induced pulmonary hypertension through transforming growth factor-β-Smad signaling. *Am J Respir Cell Mol Biol* 2018;58:194–207.
9. Wang C-C, Ying L, Barnes EA, Adams ES, Kim FY, Engel KW, *et al*. Pulmonary artery smooth muscle cell HIF-1α regulates endothelin expression via microRNA-543. *Am J Physiol Lung Cell Mol Physiol* 2018;315:L422–L431.
10. Yochum ZA, Cades J, Mazzacurati L, Neumann NM, Khetarpal SK, Chatterjee S, *et al*. A first-in-class TWIST1 inhibitor with activity in oncogene-driven lung cancer. *Mol Cancer Res* 2017;15:1764–1776.
11. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, *et al*. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000;67:737–744.
12. Tan J, Tedrow JR, Nouraie M, Dutta JA, Miller DT, Li X, *et al*. Loss of twist1 in the mesenchymal compartment promotes increased

fibrosis in experimental lung injury by enhanced expression of CXCL12. *J Immunol* 2017;198:2269–2285.
13. Li S, Wang A, Gu F, Wang Z, Tian C, Qian Z, *et al*. Novel harmine derivatives for tumor targeted therapy. *Oncotarget* 2015;6:8988–9001.
14. Weathington NM, Sznajder JI, Mallampalli RK. The emerging role of the ubiquitin proteasome in pulmonary biology and disease. *Am J Respir Crit Care Med* 2013;188:530–537.
15. Meiners S, Evankovich J, Mallampalli RK. The ubiquitin proteasome system as a potential therapeutic target for systemic sclerosis. *Transl Res* 2018;198:17–28.
16. Huang X, Dixit VM. Drugging the undruggables: exploring the ubiquitin system for drug development. *Cell Res* 2016;26: 484–498.
17. Tisato V, Voltan R, Gonelli A, Secchiero P, Zauli G. MDM2/X inhibitors under clinical evaluation: perspectives for the management of hematological malignancies and pediatric cancer. *J Hematol Oncol* 2017;10:133.