individual patient's response to a 6-hour resuscitation bundle in septic shock is quite variable. This begs the question of whether participants in the −/+ or +/+ subgroups may actually benefit from an alternative resuscitation strategy. Several RCTs are specifically seeking to clarify the optimal resuscitation strategy in participants with septic shock (15–17). The present study supports that the trajectory of biomarker measurements may inform prognostic enrichment strategies for clinical trial enrollment (18). The authors should be commended for their continuous advances in moving [TIMP-2] × [IGFBP7] from risk assessment toward clinical management. In sepsis, we are constantly searching for better tools to risk stratify patients. Perhaps the trajectory of kidney function is the canary in the coal mine that can inform clinical management and guide development of effective therapeutics for patients with septic shock. ∎

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🔓 Rats Race to Keep Pace in the Growing Cystic Fibrosis Model Space

In cystic fibrosis (CF), which occurs in people with two mutant copies of the CFTR (cystic fibrosis transmembrane conductance regulator) gene, chronic airway infection and inflammation are the major causes of morbidity and mortality. Although the mechanisms underlying

disease can be elegantly dissected using in vitro systems, a clear understanding of disease pathophysiology relies on effective animal models (1). A number of CF animal models have been developed through disruption of CFTR loci, with advantages and disadvantages to each (2). Mice and rats are less expensive to purchase and house, have faster reproductive cycles, and can be studied with commercially available reagents for immunologic evaluations. However, small mammals are more anatomically divergent from humans than larger mammals, and these models fail to develop all manifestations of CF pathophysiology (3). The ferret and pig CF models develop lung pathology more closely resembling human CF

lung disease, and manifest nonpulmonary disease manifestations of CF (pancreatic and hepatobiliary disease). However, intensive husbandry requirements and increased expense limit the availability of the pig and ferret models.

With the approval of the newest combination of CFTR modulators (elexacaftor/tezacaftor/ivacaftor), approximately 90% of people with CF in the United States are now eligible for highly effective CFTR modulator therapy (4, 5). The enthusiasm that CFTR modulators will lead to sustained improvements in quality and quantity of life for people with CF is coupled with an awareness that we have incomplete knowledge of the long-term effects of highly effective CFTR modulator therapy medications (6). Animal models that incorporate CFTR genotypes that respond to CFTR modulator therapy will help fill this knowledge gap. Although relatively uncommon in the population compared with the CFTR-ΔF508 mutation, the CFTR-G551D mutation was targeted first, because this mutation causes severe deficiency in CFTR activity and is highly responsive to ivacaftor alone (7, 8). Ivacaftor-sensitive models of both CF ferrets (9) and pigs (10) engineered to express the CFTR-G551D mutations exist. However, because ivacaftor does not potentiate rodent CFTR under most conditions tested (8, 11), development of smaller animal models in which to study modulator-induced restoration of CFTR activity has been more challenging.

In this issue of the *Journal*, Birket and colleagues (pp. 1271–1282) describe the first modulator-responsive small animal model for studying restoration of CFTR activity *in vivo* (12). The authors generated an ivacaftor-responsive CF rat by inserting most of the human CFTR-G551D variant into the rat genome under control of the endogenous rat promoter. Rats homozygous for the humanized CFTR-G551D (hG551D) construct demonstrated the same gross phenotypes as the authors' previously developed CFTR-knockout rat (13, 14), including gastrointestinal obstruction, decreased pup size, poor weight gain, white incisor phenotype, and development of airway submucosal gland hypertrophy and mucous hyperviscosity. The authors verified expression and correct orientation of the hG551D construct in the rat lungs and confirmed impaired CFTR channel function using short-circuit measurements on excised rat tracheae. The addition of ivacaftor increased short-circuit current measurements in hG551D rats to approximately 50% of wild-type measurements, consistent with the effects of similar concentrations of ivacaftor to potentiate CFTR-G551D channel activity in human cells (7, 8). Finally, and most importantly, the authors demonstrated that ivacaftor potentiates hG551D CFTR channels *in vivo* and reverses findings associated with deficient CFTR activity. In juvenile rats, ivacaftor treatment normalized nasal potential differences and restored growth rate. In mature rats that had reached the age at which insufficient CFTR activity produces airway mucous abnormalities, ivacaftor treatment increased airway surface liquid depth, decreased mucous viscosity, and enhanced mucociliary transport.

The hG115D rat adds to CF research capability by increasing the accessibility of animal models that respond to CFTR modulators and by complementing the CFTR-G551D pig and ferret. Shorter gestational periods and decreased costs make the hG115D rat more amenable to prolonged experiments, such as the evaluation of outcomes of modulator initiation in animals with more advanced pathology. Although the CF rat does not develop all the hallmarks of the human CF disease, notably pancreatic and hepatobiliary disease,

it can be used to study gastrointestinal disease and many features of CF lung disease. One significant limitation of the CF rat model, like the CF mouse, is that it does not develop spontaneous airway infection when kept in specific pathogen-free housing. However, data from ferrets (15) and mice (16) deficient in CFTR activity demonstrate that deleterious hyperinflammatory immune responses are present in the CF airway even in the absence of colonizing bacteria. As immunologic reagents are more readily available for rats than pigs and ferrets, investigation of the impact of CFTR modulators on chronic airway inflammation is an area of research for which the hG551D rat model may be particularly well suited.

Another potential use for the hG115D rat is the study of modulator therapy effects on CF airway infection. Unlike the mouse, the CF rat develops airway mucous abnormalities and impaired mucociliary transport, features that could predispose adult animals to develop airway colonization following direct inoculation with exogenous bacteria. Further studies are needed to determine if chronic infections can be achieved in CF rats. Because data indicate that adults with established infections who start modulators do not eradicate chronic bacterial infections (17), the ability to study chronic airway infections over time in a small animal model, and how CFTR modulator therapy affects these infections, will be a major advance.

As the majority of people with CF start treatment with CFTR modulators, many questions remain regarding long-term consequences of these medications; the hG511D rat model will be an excellent model to investigate some of these concerns. Long-term sequelae of modulators may reflect consequences of prolonged pharmacologic enhancement of CFTR channel activity, off-target effects of individual modulators, or CFTR mutation class–dependent effects on cellular physiology. Data are emerging that different modulators have differential effects on cellular functions, particularly immune cell functions (18–20). The hG551D rat model, like CFTR-G551D ferret and pig models, uses ivacaftor to restore CFTR channel activity; other models will be needed to assess long-term effects of tezacaftor and elexacaftor. Two groups recently published generation of ΔF508 homozygous rats, by genetically altering native rat CFTR (21, 22); however, it is unclear if either model exhibits *in vivo* responses to CFTR modulator therapy. Comparison of phenotypes of multiple animal models with different mutations, and how they change with individual and combinations of modulators, will help predict outcomes for our patients and will help determine the ways modulators may alter cellular function beyond CFTR activity restoration. ∎

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🔓 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