MEDICINE

Making precision medicine personal for cystic fibrosis

Molecular defects in the cystic fibrosis gene prompt creative approaches to treatment

By **Candela Manfredi[1], Janice M. Tindall[2], Jeong S. Hong[1], Eric J. Sorscher[1]**

Cystic fibrosis (CF) is an inherited, life-threatening disease that primarily involves exocrine tissues (such as lungs, pancreas, and liver) for which highly active pharmacotherapies have recently emerged. More than 1700 disease-associated variants are described in the CF transmembrane conductance regulator (*CFTR*) gene, which encodes an epithelial cell ion channel that is defective in patients with CF. On the basis of classifying CFTR mutant proteins according to pathogenic mechanisms, the disease has been viewed as a model for personalized therapeutics. However, *CFTR* variants may have pleiotropic effects, which complicates assignment of specifically tailored drugs to discrete mechanistic subcategories. In addition, the cost of new CFTR modulators constrains third-party reimbursement and has delayed drug availability for certain patient groups, including individuals with ultrarare *CFTR* variants for which the treatments are not formally approved but may still be effective. Issues such as these are being addressed by innovative and powerful approaches to promote CF precision medicine.

Failure of the CFTR ion channel causes altered composition and volume of exocrine secretion, giving rise to thick, hyperviscous mucus that obstructs secretory organs including the lungs, pancreas, and liver, and diagnostic findings such as increased amount of chloride in sweat. The resulting inflammation, chronic infection, and fibrotic scarring of respiratory parenchyma represent the major causes of morbidity and mortality. For many years, a conceptual approach to CF intervention has pursued tailored small molecules (modulators) designed to rescue specific CFTR defects (*1*–*3*). These are grouped according to errors in CFTR protein synthesis (class I), maturation processing of the protein (class II), ion channel opening or gating (class III), conductance through the ion-selective pore (class IV), or steady-state protein concentrations (class V) (see the figure). A separate group (class VI) is sometimes used to specify class V *CFTR* variants that disrupt plasma membrane stability of the encoded protein (*4*).

Personalized treatment strategies based on this early annotation have led to impressive therapeutic progress. Ivacaftor, for example, is a "potentiator"-type modulator (a drug that helps open the CFTR ion channel gate). The compound is suitable for overcoming certain CFTR class III (gating) defects and improves lung function (*5*). The drug has gained U.S. Food and Drug Administration (FDA) approval for 38 *CFTR* variants (it is approved for fewer variants elsewhere), comprising ~15% of the patient population. In addition, ivacaftor in combination with lumacaftor (a "corrector" of decreased CFTR biogenesis) is marketed for individuals with two copies of the class II Phe508del CFTR protein maturation abnormality (~45% of patients; this is the most common CFTR mutation) (*2*). Tezacaftor, a corrector that functions similarly to lumacaftor, was approved in 2018 by the FDA in combination with ivacaftor to treat individuals with two copies of Phe508del, as well as those who carry one of 26 other ivacaftor-responsive CFTR mutations (*3*). These modulators, developed from studies of molecular pathogenesis and a personalized therapeutic strategy, can markedly improve respiratory manifestations of CF and have conferred new optimism worldwide among patients, families, and caregivers.

It was originally anticipated that well-established disease subclasses would serve as an organizing principle for precision CF treatments and help identify specific compounds for targeting CFTR mutants in a mechanism-directed manner. However, it has become increasingly clear that most *CFTR* variants result in not just one, but numerous subclasses of molecular defects in the CFTR protein, which makes personalized approaches complex. For example, the Phe508del mutant exhibits not only inadequate biogenesis (the traditional class II grouping), but also improper gating (class III) and increased plasma membrane turnover (class V) (*6*). Class I mutants, such as Glu831X (where X indicates premature stop), might be predicted to show no response to currently available modulators owing to *CFTR* messenger RNA (mRNA) instability and protein truncation. However, a fraction of Glu831X *CFTR* mRNA can produce full-length protein lacking only amino acid 831, which maintains residual activity (*7*). Accordingly, individuals with the Glu831X mutation are approved by the FDA to be treated with ivacaftor with or without tezacaftor. Numerous *CFTR* mRNA splicing defects (class V) generate proteins with large deletions or insertions and might otherwise be expected to exhibit negligible response to drugs such as ivacaftor. However, certain of these class V CFTR mutants produce alternatively spliced mRNA (e.g., 2789+5G→A, 3272-26A→G, 3849+10kbC→T) and reduced CFTR protein with residual function, and patients with these mutations are approved for ivacaftor treatment with or without tezacaftor. Thus, there is a need to recast some original assumptions that underlie CFTR mutation–tailored therapies for CF.

In agreement with the observation that *CFTR* variants are mechanistically pleiotropic, modulator drugs developed for a specific CFTR mutation or *CFTR* variant subcategory typically exhibit a broad spectrum of activity (*8*). For example, ivacaftor as single agent or in combination with tezacaftor leads to clinical benefit across all five mechanistic categories (see the figure). Similarly, emerging triple drug combination therapies (TCTs, e.g., ivacaftor in combination with tezacaftor and elexacaftor) have undergone extensive clinical testing (*9*). Elexacaftor appears to work through a Phe508del corrector mechanism independent from tezacaftor and enhances overall clinical effectiveness. TCTs have the potential to benefit a sizable majority of individuals with CF worldwide because the agents are directed toward patients with at least one allele encoding Phe508del CFTR.

Even if the promise of TCT is fully realized, thousands of patients will continue without effective modulators owing to refractoriness of the underlying mutant protein (e.g., those with untreatable mechanistic defects such as premature truncation, mRNA splicing defects, abnormal ion conductance, or aberrant protein folding). These patients highlight the continuing need to better understand intransigent CFTR pathophysiology and advance treatment of disease sequelae such as glandular obstruction by mucus (mucostasis), or respiratory infection and inflammation. Other individuals with CF have been unable to obtain modulator treatment because the mutations they possess are exceedingly rare.

[1]Emory University School of Medicine, Atlanta, GA, USA.
[2]University of Alabama at Birmingham, Birmingham, AL USA.
Email: esorscher@emory.edu

Recent estimates describe over 1000 *CFTR* variants represented by fewer than five patients each (*10*). Establishing processes so that individuals with poorly characterized or ultrarare *CFTR* variants can access effective modulator treatment remains one of the most vexing challenges in the field.

Many drugs approved by government regulatory agencies for one condition also show robust activity in other clinical settings. Despite lack of formal regulatory approval for these other indications, physicians are generally allowed flexibility to prescribe the treatments (with third-party insurance reimbursement) if patient benefit is anticipated. A difficulty arises in the case of CFTR modulators, however, because of high treatment cost (over $300,000 annually for certain regimens). Barriers to reimbursement have blocked modulator access in the United Kingdom and other countries, and drug expense can be a limiting economic burden for U.S. third-party payers, where insurance coverage for modulators has generally been restricted to patients with genotypes for which the drugs are formally approved. For example, variants with FDA approval comprise 39 CFTR mutations from among more than 1700 associated with the disease. Although many patients with ultrarare mutations would likely benefit from modulators already approved for other genotypes, access is constrained. Moreover, an attempt to broaden approval and include additional *CFTR* genotypes presents a formidable challenge. The number of patients with a particular ultrarare mutation is typically so small that a phase 3, double-blind placebo-controlled trial (often required by regulatory agencies) is not possible.

Innovative strategies have been devised to address the complex issue of drug authorization for ultrarare *CFTR* variants. For example, in part because pronounced clinical benefit from modulator treatment can often be obtained within a matter of weeks, N-of-1 trials have been evaluated. Such studies are designed so that individual patients with ultrarare mutations are monitored before, during, and after experimental treatment for evidence of improved disease manifestations. N-of-1 trials may be combined to encourage expanded drug approvals, but it can be difficult to show consistent modulator benefit in small cohorts, even those with identical *CFTR* genotypes. This is due to differences in, for example, age, past environmental exposures, chronic lung scarring, inflammation, severity of infection, and disease trajectory. As an alternative approach, patients with similar molecular phenotype (e.g., residual CFTR function determined by in vitro testing), or evidence of mild clinical disease (pancreatic sufficiency or sweat chloride value in an intermediate range), have been evaluated in a manner less bound by specific gene defect or mutation subcategory (*3*). For a cohort of patients with rare variants that can be meaningfully classified according to strong mechanistic rationale, a pronounced clinical response to CFTR modulators may be useful for broadening the regulatory indication for the entire group (*11*).

In recent, path-breaking decisions from the FDA, ivacaftor approvals were extended to comprise rare variants by applying in vitro data (*10*). The idea that compelling in vitro findings could contribute to modulator approval represents an innovative shift in CF personalized medicine. In vitro systems capable of predicting CF clinical improvement provide a compelling means to rationally expand drug access to patients with ultrarare genotypes. Well-validated cell models expressing recombinant CFTR mutants, CF intestinal organoids (cell cultures that form three-dimensional systems), and other cell-based strategies have been advanced for this purpose (*10*, *12*).

A single modulator or combination treatment can markedly improve disease phenotype for a large number of divergent CFTR molecular defects. A global potentiator of CFTR gating, for example, might favorably enhance ion transport across numerous *CFTR* variant mechanistic subcategories. Corrector agents that augment CFTR biogenesis typically confer improvement across multiple classes of variants. Based on the experience from CF, it is reasonable to imagine that similar drug versatility might be expected for other inherited conditions, including those with a considerable array of genetic abnormalities, such as adrenoleukodystrophy, certain muscular dystrophies, Pompe disease, etc. Specialized tools and leading-edge patient protocols are already being applied in the clinical setting toward the objective of making precision medicine more personal, and less strictly focused on subclasses of disease. Thus, emerging tailored therapies will be refined (for CF, cancer, inflammatory disorders, neurodegenerative conditions, and others) with increasingly informative data directed toward knowing what works best rather than relying on genotype alone. ■

Disease-causing mutation subclasses in cystic fibrosis

There are over 1700 cystic fibrosis (CF) transmembrane conductance regulator (CFTR) mutations, which are classically divided into five categories based on pathogenic mechanism. Thirty-nine CFTR mutations are approved by the U.S. Food and Drug Administration for modulator treatment. The proportion of patients with CF and at least one mutation in a subclass and the proportion of those patients who can receive modulators are indicated. Figures are based on publicly available data (*13*).

Class	I	II	III	IV	V
Type of mutation	Protein synthesis	Maturation processing	Ion channel gating	Ion channel conductance	Reduced protein
Example of modulator-approved mutation	Glu831X	Phe508del/ Phe508del	Gly551Asp	Arg117His	3849+ 10kbC→T
Representative cellular compartment where defect occurs	Ribosome	Golgi/ER	Plasma membrane CFTR		Plasma membrane, spliceosome
Patients with mutation class*	22%	88%	6%	6%	5%
Patients with a modulator-approved genotype	<0.5%	39.2%	4.6%	2.6%	3.5%

*Patients with heterozygous *CFTR* variants in two classes are counted twice.

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ACKNOWLEDGMENTS

We thank M. Konomos for help with the figure and H. Evans and B. Sorscher for useful suggestions. Funding was obtained through grants from NIH (R01HL139876, R01HL136414) and CFF (SORSCH13XX0). E.J.S. serves as a board member, medical advisory council chair, and consultant for the CF Foundation.

10.1126/science.aaw0553

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Science **365** (6450), 220-221.
DOI: 10.1126/science.aaw0553