Pitfalls of Drug Development: Lessons Learned from Trials of Denufosol in Cystic Fibrosis

Richard B. Moss, MD

The life-shortening genetic disease cystic fibrosis (CF) is caused by mutations in the *CF* gene on both alleles, resulting in failure of a defective CF transmembrane conductance regulator (CFTR) glycoprotein to normally regulate chloride and bicarbonate flux at the airway surface.[1] According to a leading theory of CF pathogenesis, the "volume depletion" hypothesis, this abnormal anion transport leads to reduced airway surface liquid, airway dehydration, and impaired mucociliary clearance, resulting in vulnerability to airway obstruction, microbial infection, and inflammation.[2]

In 1991, investigators at the University of North Carolina discovered that triphosphate nucleotides, such as adenosine-5'-triphosphate and uridine-5'-triphosphate, stimulated chloride secretion in both normal and CF respiratory epithelia,[3] offering a potential bypass mechanism for defective CFTR. Triphosphate nucleotides transduce a signal by binding to purinergic receptors on respiratory epithelial cells specifically responding to purine and pyrimidine nucleotides, termed P2Y2 receptors. This leads to release of intracellular calcium and activates calcium-dependent chloride channels (CaCCs) distinct from CFTR.[4] In vitro tissue culture studies have confirmed that adenosine-5'-triphosphate and uridine-5'-triphosphate can restore liquid transport in CF cultures within a few minutes, enhance tracheal mucus velocity in sheep models, and acutely increase sputum volume and mucociliary clearance in smokers.[5] Additional actions of P2Y2 agonists also have been reported from cell culture experiments, including stimulation of ciliary beat frequency, increased mucin secretion from goblet cells and surfactant from type II alveolar cells, and inhibition of epithelial sodium absorption,[5,6] the net sum of these plausibly resulting in improved airway hydration and increased mucociliary transport that should be beneficial in treating CF.

Native triphosphate nucleotides are rapidly metabolized by ectonucleotidases in an autocrine/paracrine manner, however.[7] This has led to the development of dinucleotides, INS365 and INS37217, that are more resistant to enzymatic degradation as assessed in 2 preclinical models: ex vivo exposure of the nucelotides to CF sputum samples and in vitro addition to human nasal ciliated epithelial cell cultures.[8] In these models, these dinucleotides had sufficient stability to merit consideration for drug development, with INS37217 (later named denufosol) outperforming INS365. Denufosol's half-life is 25 hours in ex vivo CF sputum and 3 hours when added in vitro to human respiratory epithelial cultures.[5,8] Moreover, local enzymatic degradation implies that systemic absorption and activity were negligible, minimizing the risk of systemic adverse effects or toxicity. Indeed, denufosol is metabolized within minutes after intravenous infusion, presumably by endogenous ectonucleotidases, and systemic levels are usually undetectable after nebulization.[9] Based on these data, denufosol was carried into clinical trials beginning in 2001 using a wet nebulization direct airway delivery approach.

Instead of success, however, the story of denufosol in CF is one of a great reversal. The results of a pivotal 6-month placebo-controlled Phase 3 trial of denufosol (named TIGER-1, an acronym for transport of ions to generate epithelial rehydration) and a 6-month open-label extension were published at the end of 2010.[10] These results suggested the possible discovery of a new type of treatment for CF, suitable for early intervention in patients with only mild lung function impairment, independent of CF genotype, and of potentially disease-modifying character.[11] The corporate developers of denufosol, Inspire Pharmaceuticals, and the entire CF community, from bench researchers to clinicians to patients, looked forward to confirmation of these results in the Food and Drug Administration-required replication Phase 3 trial (TIGER-2) that was already completed.

Instead, in an unexpected and disappointing development, less than 3 weeks after publication of the TIGER-1 data, Inspire Pharmaceuticals announced in a press release that the 466-patient, 48-week placebo-controlled international Phase 3 TIGER-2 trial had failed to demonstrate any benefit (unpublished data, January 2011). The complete results of TIGER-2 have now been published.[12] Inspire Pharmaceuticals' equity evaporated; the company lost $400 000 000 in a single day.[13] Within a few months, after the CF program was shut down, Inspire's remaining assets were acquired by Merck.[14]

In this article, I provide a personal review of the elements leading to this outcome. As always, in accordance with the aphorism "life is lived forward but understood backwards" aspects contributing to the failure of this drug development program are seen far more clearly in retrospect than

CaCC	Calcium-dependent chloride channel
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
FEV_1	Forced expiratory volume in 1 second

From the Department of Pediatrics, Stanford University School of Medicine, Stanford, CA

R.M. was a Principal Investigator in several denufosol clinical trials and has served on the Inspire Pharmaceuticals Medical Advisory Board.

Portions of this article were presented at the North Cystic Fibrosis Conference in Anaheim, CA, November 5, 2011.

 http://dx.doi.org/10.1016/j.jpeds.2012.11.034

contemporaneously, and as a former Advisory Board member to Inspire, the unsuccessful outcome is a source of abiding personal humility. Nonetheless, I believe that there are useful lessons to be learned in sharing one view of the denufosol story. When assessing potential harbingers of therapeutic failure, it may be helpful to consider these in a sequence of basic science and preclinical understanding, followed by aspects of the drug development program in the clinic through Phase 1, 2, and 3 trials in patients.

Basic and Preclinical Understanding

The absence of a meaningful impact of denufosol in Phase 3 studies, described in detail below, suggests that the preclinical assessment of denufosol's effect on CF respiratory epithelium was possibly flawed in several key respects. Denufosol's stability in CF sputum ex vivo and its 3-hour half-life when applied to human nasal epithelium in vitro seemed to offer a viable path to clinical treatment based on a mininum of thrice-daily administration. However, accelerated metabolism of P2Y2 agonists in CF respiratory epithelial cultures in vitro, based on up-regulation of ectonucleotidase activity, was discovered when the program was already well into a Phase 2 clinical trial, and the mechanism is still under investigation.[15,16] This finding may underlie the disappointing "real world" compartmental pharmacokinetics of denufosol in vivo in CF patients' airways that was not to be explored for a decade until TIGER-2, as discussed below. Meanwhile, the drug development program progressed through clinical trials that ultimately involved more than 1000 patients (many individually enrolled for 1 year or longer), dozens of CF centers, and hundreds of millions of dollars. This turned out to be a crucial omission. Clinical development spurred by unmet needs ran too fast and far ahead of scientific understanding of target organ drug metabolism.

Another important basic science discovery postdated the introduction of denufosol into clinical trials. The physiological role of CaCCs, the putative target of denufosol, and the effect of abolished or reduced CFTR function in CF on CaCC activity, were poorly understood. Well after denufusol was being tried in CF patients, researchers at the University of North Carolina discovered that shear stress on respiratory epithelium (dependent on the rate of change) releases endogenous luminal adenosine-5'-triphosphate, thereby activating CaCCs to secrete chloride.[17] This aspect of CaCC activation opens the possibility that CaCCs may be more physiologically active in CF than anticipated, and thus potentially less augmented by pharmacologic stimulation than hoped. Airway clearance regimens, such as high-frequency chest wall oscillation vests, cause shear stress, as does positive expiratory pressure, cough, vigorous exercise, and even routine activities of daily living and the very motion of breathing.[18] Might CaCCs be refractory targets in CF?

Related to this physiological question is the more fundamental one of identifying a CaCC's target for purinergic agonists in concrete molecular terms. The identity of the CaCC was discovered only after denufosol was already entering Phase 3 trials. Now that the molecular identity of at least 1 CaCC has been confirmed as the anoctamin protein TMEM16A, it may be possible to evaluate this with more precision.[19] However, controversy exists regarding the activity of various anoctamins as respiratory epithelial CaCCS,[20] and so the action of denufosol or other purinergic agonists on CaCCs still has not been explored sufficiently.[21] The clinical effect of inhaled osmotic agents (eg, hypertonic saline, mannitol) likely points to the viability of strategies to improve airway surface hydration in CF,[22,23] although other mechanisms for the clinical efficacy of these agents may be relevant as well.[24] In general, it seems fairly certain that the fates of investigational drugs activating CaCCs and/or inhibiting epithelial sodium absorption, involving other mechanisms such as osmotic hydration, stimulating cough, activating neural reflexes, or affecting host defense and inflammation, will be affected by further basic research even while under evaluation in clinical trials.

Finally, another mechanistic factor potentially affecting respiratory epithelial responses to P2Y2 receptor agonists could be any variation in receptor activity in the population. In fact, such polymorphisms have been described during the course of the denufosol clinical trials,[25] but their distribution in the general population and effect on clinical responses in patients with CF remain unknown.

Early Clinical Trials

Phase 1 trials are used to screen for tolerance and adverse effects of investigational agents, to determine potential clinical doses based on safety signals and extrapolation from in vitro or preclinical levels needed for a potentially beneficial pharmacodynamic effect, and to explore dosage intervals based on at least single-dose pharmacokinetics. Each of these assessments is crucial in clinical drug development. The first trials of denufosol established a good safety profile, with single-dose tolerability at up to 200 mg in healthy adult male nonsmokers and 80 mg in smokers. Cough was noted in a majority of nonsmokers at the highest test dose of 320 mg and in smokers at 100 mg, along with a transient decline in forced expiratory volume in 1 second (FEV_1).[5] However, in a Phase 1 trial in adult and pediatric CF patients, the highest tested dose was 60 mg, given initially as a single ascending dose on consecutive days and then in a second part of the study twice daily for 5 days.[26] The maximal dose of 60 mg apparently was chosen based on concerns that higher doses could cause excessive cough or other respiratory intolerance, although these concerns were not empirically tested with denufosol but rather were suggested by a previous trial with the earlier-generation dinucleotide INS365.[27] Because the 60 mg dose was well tolerated, this maximum dose was carried forward in the subsequent trials. Although somewhat more wheezing was evident at 60 mg than at lower doses, could higher doses have been more effective with acceptable levels of intolerability? If acute increases in airway hydration were feared as a potential cause of adverse events, strategies to deal with this problem (eg, via dose titration) could have been tried.