)es

CLINICAL

Novel therapeutics for cystic fibrosis: what does the future hold?

Keywords: CFTR • cystic fibrosis • disease modifying agents • high-throughput screening • livacaftor

Cystic fibrosis (CF) is a life-shortening autosomal recessive disease caused by mutations in the gene encoding CFTR, a protein with anion channeling properties that is critical for electrolyte homeostasis at many mucosa [1]. Although the earliest descriptions of signs associated with CF as a lethal condition in early childhood can be traced back to folk tales of the middle ages [2], the first formal descriptions of the disease occurred independently in the late 1930's by Fanconi [3] and Andersen [4]. Both investigators described the presence of pancreatic, pulmonary and intestinal lesions with accumulation of mucoid material and glandular ducts blocked by inspissation of mucus. It was not until the early 1950s through the seminal work of DiSant-Agnese describing the high concentrations of sodium and chloride in sweat [5] that electrolyte transport abnormalities were recognized as key in the pathophysiology of the disease. This was later demonstrated by Quinton to be the product of impermeability of the sweat duct to chloride transport [6]. The cloning of the gene in 1989 [7] and later localization of the wild-type CFTR protein to the apical surface of mucosal epithelium [8] was followed by an explosion of incremental knowledge that identified important aspects of the pathophysiology of the disease as well as pinpointing potential targets for intervention in the cascade of events that, particularly in the lung, lead to progressive end organ damage.

Although a multisystemic defect, it is well recognized that the morbidity associated with CF primarily centers on its pulmonary manifestations. In the airway surface, a striking defect in mucociliary clearance is the hallmark and results in an environment prone to chronic bacterial infection and severe inflammation. The airways of CF patients are typically overburdened with microorganisms, oxidants and proteases, as well as by large concentrations of inflammatory mediators. A chronic, progressive process slowly destroys the airways and often results in severe lung dysfunction and death from respiratory failure. Remarkable progress has been accomplished in the last decade in our understanding of the basic pathophysiologic mechanisms produced by CFTR dysfunction, and this has been aided greatly by the development of animal models of CF [9]. In parallel, therapeutic developments informed by this knowledge have steadily been advanced and focused primarily on curtailing the progression of the lung disease. Despite this, to date, most of the morbidity and mortality associated with CF continues to be a result of its pulmonary manifestations. Not surprisingly then, most therapeutic development efforts historically focused on therapies to clear the airways from obstruction and targeted antibiotic therapies. These efforts led to the development and approval of three drugs with a specific CF indication: rhDNAse (Pulmozyme®), Tobramycin for Inhalation (TOBI®) and Aztreonam Lysine for Inhalation (Cayston[®]). However, the observed clinical response to these therapies can be variable from patient to patient. This has been attributed in part to the fact that these interventions are targeting steps downstream from the basic defect, and thus with limited disease modifying potential.



Carlos E Milla^{*,1} 'Center for Excellence in Pulmonary Biology, Stanford University, 770 Welch Road, Suite 350, Palo Alto, CA 94304, USA Tel.: +1 650 723 8325 Fax: +1 650 723 5201 cmilla@stanford.edu



Certainly, true disease modification can in theory only be accomplished by restoring normal CFTR function at the cellular level through either correction of the gene defect or the defective protein. A clear recognition of this fact led to the understanding that the application of molecular tools at the cellular level was required for a better chance at success in identifying the most effective therapies. As a result we have witnessed remarkable advances in CF fueled by the application of high-throughput screening methodology that permitted to test ion channeling activity in cell cultures for a large numbers of compounds [10]. The availability of a platform that permitted testing large libraries of compounds allowed in a more efficient way to test the concept proposed two decades ago of restoring CFTR function by rescuing from degradation and potentiating misfolded CFTR protein so that its activity in the apical cell membrane could be restored [11]. The approval in 2012 of the first drug aimed at the basic defect in patients carrying the G551D CFTR mutation (Ivacaftor, N-[2,4-di-tert-butyl-5-hydroxyphenyl]-1,4dihydro-40x0quinoline-3-carboxamide) demonstrated the value of this approach for efficient drug discovery in CF, as the in vitro findings accurately predicted the clinical response [12,13]. Besides the benefits realized by a selected small group of patients, this can also be taken as a successful proof of concept. It is now established that targeting the CF basic defect can lead to effective restoration of CFTR function and this translates in detectable clinical benefits. Following this paradigm, a growing number of compounds and chemical classes with potential CFTR activity are being identified and the number is likely to increase in the next few years. As a result, additional compounds with promising in vitro effects targeting the basic CFTR defect are increasingly entering clinical trials. As importantly, through application of *in vitro* cell culture assays, it has been possible to identify other CFTR mutations amenable to targeting with Ivacaftor as a therapeutic agent [14]. Given the very low frequency in this patient population of a large number of mutations known to be associated with CF, this has introduced the concept of in vitro cell-based platforms as tools to screen for patients who could potentially benefit from drugs targeting CFTR. This certainly presents the opportunity of a real innovation in CF clinical drug development, as it will permit a targeted and more efficient approach instead of attempting to conduct large clinical trials for very limited eligible-patient populations. However, two important challenges lie ahead. First, from a trials-design perspective the need for more accurate and practical outcome measures has become critical. As none of these therapies will effectively be a cure as the gene defect is not targeted, the main goal is still pri-

marily to identify therapies that, by targeting CFTR, will modify the trajectory of the pulmonary disease. Given all the advances made in the care of the pulmonary disease in CF, it has already become apparent that traditional measures applied to monitor the lung disease progression, such as the forced expiratory volume in 1 s, could miss an important signal in the short term for an effective therapy [15]. A recent example is the reported top line results from a study of Ivacaftor in patients with a mild CFTR mutation predicted in vitro to have strong responses [16]. Certainly better tools will have to be identified and moved forward to address this important issue. The recently reported results on the application of an alternative sensitive measure of airway function, the lung clearance index, point to the feasibility of applying innovative measures to detect important effects in future trials [17].

Second, it has become increasingly apparent that perhaps a combination of drugs targeting CFTR and the mechanisms involved in its proteostasis will be required to achieve the strongest responses. It was initially proposed that to address the most common defect seen in patients, the F508del mutation, a combination-therapy approach with the use of drugs aimed at correcting the folding defect complemented with drugs such as Ivacaftor to potentiate the activity of the rescued protein could give the highest level of efficacy. Preliminary results of ongoing clinical trials testing the effects of potential correctors for CF patients homozygous for the F508del mutation in combination with Ivacaftor have already shown some promising results [18,19]. However, although it is already well demonstrated that the F508del mutation disrupts CFTR folding and this prevents normal trafficking to the cell membrane, there is now accumulating evidence that even if the defective protein is rescued from degradation, it will have decreased activity and potentially a low residence time in the membrane. Perhaps as importantly, there is a recognized need to better identify those key mechanisms involved in misfolded protein processing that are involved in the pathophysiology of CF [20]. Thus, it may be necessary to develop combination regimens with drugs that address many of these mechanisms to restore the highest levels of CFTR activity back. Although this is a feasible approach, it will impose a challenge in the drug development process as it will be difficult to test multiple drugs in combination until their effects are fully proven independently.

So, what does the future hold for the field? Specifically for CF, a new era has dawned where, although a true cure is not yet in sight, novel therapeutics targeting the basic defect will effect true disease modification. However, there is now a clear recognition that clinical trials will have to be designed to accommodate for the innovative aspects is these potential disease modifying agents, including the development and validation of outcome measures capable of demonstrating these effects. In addition, this has provided a nice demonstration of the importance of genomicdriven therapeutics, where the specific genotype of the patient is a key determinant of the therapeutic regimen. Given the remarkable and unprecedented results seen in patients treated with Ivacaftor, this is also a demonstration of the power of the concept of individualized medicine. As an extension for other rare genetically based conditions, CF has paved the way for the development of drug discovery programs that

References

- Welsh MJ, Ramsey BW, Accurso F, Cutting GR. Cystic Fibrosis. In: *The Metabolic and Molecular Basis of Inherited Disease*. 8th Edition. Scriver CR, Beaudet AL, Sly WS, Valle D (Eds). McGraw-Hill Professional, NY, USA (2001).
- 2 Busch R. On the history of cystic fibrosis. Acta Univ. Carol. Med. 36, 13–15 (1990).
- 3 Fanconi G, Uehlinger E, Knauer C. Das coeliakiesyndrom bei angeborener zysticher pankreasfibromatose und bronchiectasien. *Wiener Klin Wochen* 86, 753 (1936).
- 4 Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease. *Am. J. Dis. Child.* 56, 344–399 (1938).
- 5 Di Sant'Agnese PA, Darling RC, Perera GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. *Pediatrics* 12, 549–563 (1953).
- 6 Quinton PM. Chloride impermeability in cystic fibrosis. *Nature* 301, 421–422 (1983).
- 7 Riordan JR, Rommens JM, Kerem B *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245, 1066–1072 (1989).
- 8 Crawford I, Maloney PC, Zeitlin PL *et al.* Immunocytochemical localization of the cystic fibrosis gene product CFTR. *Proc. Natl Acad. Sci. USA* 88, 9262–9266 (1991).
- 9 Fisher JT, Zhang Y, Engelhardt JF. Comparative biology of cystic fibrosis animal models. *Methods Mol. Biol.* 742, 311–334 (2011).
- 10 González JE, Oades K, Leychkis Y, Harootunian A, Nequlescu PA. Cell-based assays and instrumentation for screening ion-channel targets. *Drug Discov. Today.* 4(9), 431–439 (1999).
- 11 Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 73(7), 1251–1254 (1993).
- 12 Van Goor F, Hadida S, Grootenhuis PD *et al.* Rescue of CF airway epithelial cell function *in vitro* by a CFTR

are firmly grounded on the basic scientific knowledge and tightly linked to a strong clinical base. It certainly provides a perfect demonstration of the value of translational research.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert t-estimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

potentiator, VX-770. *Proc. Natl Acad. Sci. USA* 106(44), 18825–18830 (2009).

- 13 Ramsey BW, Davies J, McElvaney NG et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. N. Engl. J. Med. 365(18), 1663–1672 (2011).
- 14 Van Goor F, Yu H, Burton B, Hoffman BJ. Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J. Cyst. Fibros.* 13(1), 29–36 (2014).
- 15 Ramsey BW. Outcome measures for development of new therapies in cystic fibrosis: are we making progress and what are the next steps? *Proc. Am. Thor. Soc.* 4(4), 367–369 (2007).
- 16 Vertex announces results of Phase 3 study of Ivacaftor in people with CF who have the R117H mutation (2013). http://investors.vrtx.com/releasedetail. cfm?ReleaseID=814799
- 17 Davies J, Sheridan H, Bell N *et al.* Assessment of clinical response to ivacaftor with lung clearance index in cystic fibrosis patients with a G551D-CFTR mutation and preserved spirometry: a randomised controlled trial. *Lancet Respir. Med.* 1(8), 630–638 (2013).
- 18 Boyle MP, Bell S, Konstan M, McColley SA, Kang L, Patel N. The investigational CFTR corrector, VX-809 (Lumacaftor) co-administered with the oral potentiator ivacaftor improved CFTR and lung function in F508del homozygous patients: phase II study results. *Pediatr. Pulmonol. Suppl.* 47(S35), 315 (2012).
- 19 Donaldson SH, Pilewski J, Griese M, Dong Q, Rodman D. VX-661, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the F508Del-CFTR mutation: interim analysis. J. Cyst. Fibros. 12(Suppl. 1), S14 (2013).
- 20 Balch WE, Sznajder JI, Budinger S *et al.* Malfolded protein structure and proteostasis in lung diseases. *Am. J. Respir. Crit. Care Med.* 189(1), 96–103 (2013).