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Landmark papers in respiratory medicine

The era of CFTR modulators: improvements made and remaining challenges

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene [1]. The CFTR protein is an ion channel that mediates chloride and bicarbonate transport in epithelial cells of multiple organs including lungs, pancreas and intestine [2, 3]. A defective CFTR protein produces an impaired ion and fluid secretion in the epithelial cells affecting several organs and leading to severe lung disease. More than 2000 CF-causing mutations have been identified [4, 5]. The most common mutation, the deletion of phenylalanine at position 508 (F508del), induces misfolding of the protein that is retained in the endoplasmic reticulum and degraded by proteasomal pathways [6].

Until the last decade, the only available treatments for CF were focused on managing the symptoms of the disease. CFTR correctors are pharmacological compounds that rescue the CFTR protein to the cell surface. Thus, these treatments that target the underlying cause of CF have the potential to change the course of CF clinical disease [7, 8]. In the present study we reviewed the CFTR modulators currently in the clinic, the improvements made as well as the challenges that still need to be overcome in the field of CF treatments.

CFTR modulators currently in the clinic

The clinical introduction of CFTR modulators, which are able to restore some CFTR function, has significantly improved the disease course of CF patients over the past years. The first U.S. Food and Drug Administration (FDA) approved drug was ivacaftor (VX-770; Kalydeco, Vertex Pharmaceuticals) [9], a "potentiator" that affected the gating properties of mutant CFTR channel activity (*e.g.* G551D, S549N, R117H, R347P) [10]. The clinical trial of ivacaftor showed a reduction of sweat chloride concentration under the CF diagnostic range and an increase in lung function of 10% [9, 11]. Subsequently, a CFTR "corrector" drug, lumacaftor (VX-809), in combination with ivacaftor (lumacaftor/ivacaftor or Orkambi, Vertex Pharmaceuticals) [12] also showed a modest clinical improvement for patients bearing F508del mutation [13]. After 24 weeks of treatment with Orkambi, patients homozygous for F508del experienced a reduction in the rate of pulmonary exacerbations (30–39%), an absolute change in body mass index (BMI, 0.13–0.41) and an increment of percentage of predicted forced expiratory volume in 1 s (FEV_1 % pred) between 4.3% and 6.7% [13].

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The entry into the clinic of CFTR modulators such as TRIKAFTA has significantly improved life for ~90% CF patients carrying one or two F508del mutations but challenges remain for rare *CFTR* mutations and the management of lung infections @SaraOcana1 https://bit.ly/3aRafQF

It has been demonstrated that lumacaftor acts to stabilise the mutant CFTR in the early stages of biogenesis by interacting directly with the CFTR protein [14, 15]. However, long-term treatment (24–48 h) of lumacaftor in combination with ivacaftor *in vitro* diminishes the pharmacological correction of F508del-CFTR [16, 17].

Instead, tezacaftor/ivacaftor (Symdeko, Vertex Pharmaceuticals) appeared to have a more favourable adverse event and drug-drug interaction profile [7, 18, 19] than lumacaftor/ivacaftor. Symdeko was approved, alongside F508del, for a large number of "residual function" *CFTR* mutations based on *in vitro* culture responses, paving the way for the triple CFTR modulation [7]. Symdeko was associated with a significantly lower frequency of pulmonary exacerbations and improvement of FEV_1 % pred (3.4% mean) compared to baseline, although no significant differences were observed in BMI. This was followed by phase 2 clinical studies where a triple combination of CFTR modulators was tested in patients who were heterozygous for the F508del *CFTR* mutation and a minimal-function mutation (F508del–minimal function genotype), demonstrating improvements in CFTR function and clinical outcomes. These encouraging results led to the first phase 3, multicentre, randomised, double-blind, placebo-controlled clinical trial where the triple-combination, elexacaftor, tezacaftor, and ivacaftor, was randomised *versus* placebo for 403 patients with F508del–minimal function genotypes for 24 weeks [20]. A second phase 3 clinical trial was done for 107 patients homozygous for the F508del mutation, who were randomised for the triple combination *versus* tezacaftor/ivacaftor for 4 weeks [21]. Moreover, at trial completion, participants were given the option to enrol in a 96-week open-label extension trial.

The results of these phase 3 clinical studies were similar for the F508del–minimal function and F508 homozygous patients, showing an improvement of FEV_1 % pred (in the range of 10.4–13.8%) compared to the control [20] or actively-controlled patients [21], reduction of the sweat chloride concentration (–39.1 to –43.4) and higher patient-reported quality of life. Moreover, a reduction of exacerbations was observed for patients with F508del–minimal function genotypes compared to placebo [20], as well as improvements of BMI [20, 21], which in CF, usually correlates with better survival. The triple CFTR modulation therapy demonstrated good tolerability with only mild or moderate adverse effects and very low rate of discontinuation [20, 21]. However, there is a main and highly relevant difference between these two studies. While patients homozygous for F508del are usually prescribed either lumacaftor plus ivacaftor or tezacaftor plus ivacaftor, patients with only one copy of F508del are rarely prescribed these treatments given their reported inefficacy. Although the triple-combination therapy achieved an improvement in patients homozygous for F508del much higher than previous CFTR modulators, reaching levels comparable to the benefit of ivacaftor for G551D patients, the benefit observed for patients with only one F508del copy involved more profound significance for the CF treatment paradigm of these patients.

Overall the triple-combination, elexacaftor, tezacaftor, and ivacaftor (marketed as Trikafta, Vertex Pharmaceuticals), demonstrated statistically significant and/or clinically meaningful improvements in lung function (10–14% of FEV1 % pred) and respiratory-related quality of life for patients with one copy of the F508del mutation and a minimal-function mutation and patients with two F508del copies [20, 21]. While this discovery constituted an improvement for numerous CF patients F508del homozygous, it is a life changing treatment for those patients with F508del–minimal function genotypes, in whom previous CFTR modulators were ineffective. The triple-combination or Trikafta has recently received FDA approval for patients aged 12 years or older who have at least one copy of the F508del *CFTR* mutation [22, 23]. However, the effectiveness of the same CFTR modulator combination therapy to rescue the processing defect for other rare *CFTR* mutations remains to be determined.

Patient response to CFTR modulators

Among the approved CFTR modulators, Trikafta can be applicable to the largest number of CF patients [20, 22], as it aims to target those with at least one copy of the F508del *CFTR* mutation, accounting for up to 90% of people with CF [24]. However, accumulating evidence from previous CFTR modulators prescriptions suggest that not all the patients who are predicted to respond to these treatments might experience the expected benefit. This patient-to-patient variability has been represented *in vitro* using patient samples, where it was observed that patient responses to lumacaftor or Orkambi can largely vary even among people carrying the same *CFTR* mutation [25–27]. Driving causes proposed for this variability [28] as well as disease severity [29] have been the genetic variants within and outside the *CFTR* locus [30]. Differences have also been described between the response observed in females and males under treatment with ivacaftor [31].

Therefore, in order to better tailor personalised treatment choices, new research directions need to identify reliable *in vitro* systems to predict individual patient responses [32–35]. The identification of reliable individual response prediction tools is even more important for those patients carrying refractory CFTR variants not addressed by available modulators and for those who carry extremely rare

CFTR mutations [35–37]. It has been demonstrated that Orkambi showed a modest response to some of these rare mutations (A455E, M1101K, N1303K) in heterologous expression systems [38, 39]. However, this correction was not recapitulated in patient-derived tissues [40]. On the other hand, it has been recently demonstrated that a combination of CFTR modulators alongside a small molecule that inhibits the nonsense-mediated RNA decay can rescue the functional expression of W1282X-CFTR in heterologous systems and primary nasal epithelial cells [41–43].

Experts in the CF field still maintain that "*structural biology and functional studies are a powerful combination to elucidate fundamental knowledge about CFTR and are key for the development of better drugs to enable people with CF to live full and active lives*" [44, 45]. In addition, scientists are comparing and trying to elucidate the robustness of current methods and markers used to evaluate the benefit of these new modulation therapies [27, 46, 47]. Specific nasal potential difference measurements [46], circulating inflammatory proteins [48], for example, have been highlighted as reliable biomarkers of CFTR activity [46] or lung disease severity [48] in the clinical setting.

In contrast to non-responding patients, there are many people with CF experiencing a great benefit under the mentioned CFTR modulator therapies [49]. As a recent study identified, in these cases, the goal would be to study the effects of withdrawing one or more chronic treatments to reduce the CF treatment burden [50].

Management of CF lung infections

Even in the era of CFTR modulation therapies, the detection, diagnosis, and treatment of some CF micro-organisms remains challenging, especially for patients with more advanced stages of lung disease [51]. The Cystic Fibrosis Foundation has put forth efforts through the Infection Research Initiative to tackle these issues, including the development of new anti-infective therapies. It has been demonstrated that *in-vitro* exposure of *Pseudomonas aeruginosa* (*P. aeruginosa*) reduced Orkambi-mediated rescue of CFTR function in human bronchial epithelial cells and stimulated the expression of pro-inflammatory cytokines (IL-6 and IL-8) [52, 53]. Thus, differences in the type of microbial infections across patients and even within a single patient over time could explain the low efficacy in some cases and the high patient-to-patient variability in Orkambi response. However, treatment with antibiotic tobramycin and antimicrobial peptide, has been shown to restore Orkambi-mediated rescue of F508del-CFTR function in human bronchial epithelial cells infected with clinical strains of *P. aeruginosa* [54]. Recent studies are evaluating the impact that CFTR modulators such as lumacaftor/ivacaftor have on infections, including patients with severe lung disease [55]. In this study, although treatment with lumacaftor/ivacaftor reduced exacerbations, the unacceptably frequent adverse events resulted in a very high discontinuation rate [55]. Research efforts to evaluate the clinical effectiveness and impact on infections is ongoing in future triple-combination CFTR modulator therapy studies [51].

CF is not only a lung disease

In addition to the lung disease symptoms, CF usually affects pancreas, liver and digestive system, often leading to pancreatic insufficiency, CF-related diabetes, CF liver disease, severe malabsorption and meconium ileus [56–58]. Thus, in addition to focusing on the lung disease paradigm, clinical measures to predict the effect of new CFTR modulators on other CF related symptoms [59, 60] and even on establishing organ function early in life [61] are needed. It may be foreseeable that additional therapies or changes to standard of care are needed for these patients, in hopes of changing the scenario from a life-shortening disease to a treatable chronic condition. Finally, as the median survival for CF continues to increase and the CF population ages, new models for CF care will need to be adopted to tackle an increasing CF population with both CF morbidities and additional diseases of ageing [8].

Summary

This last decade has created historical moments for CF, primarily driven by the development of CFTR modulators. First for patients with gating mutations who benefited from Kalydeco, then for those patients with one F508del copy who could benefit from Orkambi, and most recently, patients with at least one F508del copy who can benefit from Trikafta. Despite heterogeneity in patient response, the majority of CF patients will be greatly impacted by using a CFTR modulator therapy, thus changing the trajectory of their life. Furthermore, it remains to be determined whether the next generation of modulators will be effective for individuals bearing rare mutations that are Orkambi resistant. However, it should not be forgotten that there still remains 10% of the CF population who do not have a targeted CFTR modulator treatment. In addition, even with these novel drug therapies, managing infections will continue to be a challenge, thus the CF community will need to adapt the standards for an improving, but ageing CF population.

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Conflict of interest

None declared.

References

1. Riordan JR, Rommens JM, Kerem B, *et al.* Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989; 245: 1066–1073.
2. Bear CE, Li CH, Kartner N, *et al.* Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* 1992; 68: 809–818.
3. Gray MA, Plant S, Argent BE. cAMP-regulated whole cell chloride currents in pancreatic duct cells. *Am J Physiol* 1993; 264: C591–C602.
4. The Hospital for Sick Children. Cystic fibrosis mutation database. http://genet.sickkids.on.ca/Home.html (2011).
5. CFF. The Clinical and Functional TRanslation of CFTR (CFTR2). https://cftr2.org/ (2011).
6. Molinski S, Eckford PDW, Pasyk S, *et al.* Functional rescue of F508del-CFTR using small molecule correctors. *Front Pharmacol* 2012; 3: 160.
7. Paterson SL, Barry PJ, Horsley AR. Tezacaftor and ivacaftor for the treatment of cystic fibrosis. *Expert Rev Respir Med* 2020; 14: 15–30.
8. Elborn JS. Adult Care in Cystic Fibrosis. *Semin Respir Crit Care Med* 2019; 40: 857–868.
9. Ramsey BW, Davies J, McElvaney NG, *et al.* A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011; 365: 1663–1672.
10. Van Goor F, Yu H, Burton B, *et al.* Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J Cyst Fibros* 2014; 13: 29–36.
11. Nick JA, St Clair C, Jones MC, *et al.* Ivacaftor in cystic fibrosis with residual function: Lung function results from an N-of-1 study. *J Cyst Fibros* 2020; 19: 91–98.
12. Lumacaftor/ivacaftor for cystic fibrosis. *Aust Prescr* 2019; 42: 170–171.
13. Wainwright CE, Elborn JS, Ramsey BW. Lumacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. *N Engl J Med* 2015; 373: 220–231.
14. Laselva O, Marzaro G, Vaccarin C, *et al.* Molecular mechanism of action of trimethylangelicin derivatives as CFTR modulators. *Front Pharmacol* 2018; 9: 719.
15. Loo TW, Bartlett MC, Clarke DM. Corrector VX-809 stabilizes the first transmembrane domain of CFTR. *Biochem Pharmacol* 2013; 86: 612–619.
16. Cholon DM, Quinney NL, Fulcher ML, *et al.* Potentiator ivacaftor abrogates pharmacological correction of ΔF508 CFTR in cystic fibrosis. *Sci Transl Med* 2014; 6: 246ra296.
17. Veit G, Avramescu RG, Perdomo D, *et al.* Some gating potentiators, including VX-770, diminish ΔF508-CFTR functional expression. *Sci Transl Med* 2014; 6: 246ra297.
18. Taylor-Cousar JL, Munck A, McKone EF, *et al.* Tezacaftor-ivacaftor in patients with cystic fibrosis homozygous for Phe508del. *N Engl J Med* 2017; 377: 2013–2023.
19. Tezacaftor/ivacaftor for cystic fibrosis. *Aust Prescr* 2019; 42: 174–175.
20. Middleton PG, Mall MA, Dřevínek P, *et al.* Elexacaftor-tezacaftor-ivacaftor for cystic fibrosis with a single Phe508del allele. *N Engl J Med* 2019; 381: 1809–1819.
21. Heijerman HGM, McKone EF, Downey DG, *et al.* 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.
22. Vertex.Pharmaceuticals.Incorporated. (*Vertex press release August 20 2019*, 2019).
23. Hoy SM. Elexacaftor/ivacaftor/tezacaftor: First approval. *Drugs* 2019; 79: 2001–2007.
24. Orenti A, Zolin A, Naehrlich L, *et al.* ECFS Patient Registry Annual Data Report 2016. https://www.ecfs.eu/sites/default/files/general-content-images/working-groups/ecfs-patient-registry/ECFSPR_Report2016_06062018.pdf.
25. Eckford PD, Ramjeesingh M, Molinski S, *et al.* VX-809 and related corrector compounds exhibit secondary activity stabilizing active F508del-CFTR after its partial rescue to the cell surface. *Chem Biol* 2014; 21: 666–678.
26. Dekkers JF, Berkers G, Kruisselbrink E, *et al.* Characterizing responses to CFTR-modulating drugs using rectal organoids derived from subjects with cystic fibrosis. *Sci Transl Med* 2016; 8: 344ra384.
27. Pranke IM, Hatton A, Simonin J, *et al.* Correction of CFTR function in nasal epithelial cells from cystic fibrosis patients predicts improvement of respiratory function by CFTR modulators. *Sci Rep* 2017; 7: 7375.
28. Chevalier B, Hinzpeter A. The influence of CFTR complex alleles on precision therapy of cystic fibrosis. *J Cyst Fibros* 2020; 19: Suppl. 1, S15–S18.
29. Paranjapye A, Ruffin M, Harris A, *et al.* Genetic variation in CFTR and modifier loci may modulate cystic fibrosis disease severity. *J Cyst Fibros* 2020; 19: Suppl. 1, S10–S14.
30. Sharma N, Cutting GR. The genetics and genomics of cystic fibrosis. *J Cyst Fibros* 2020; 19: Suppl. 1, S5–S9.
31. Secunda KE, Guimbellot JS, Jovanovic B, *et al.* Females with cystic fibrosis demonstrate a differential response profile to ivacaftor compared to males. *Am J Respir Crit Care Med* 2019. 201: 996–998.
32. Awatade NT, Wong SL, Hewson CK, *et al.* Human primary epithelial cell models: Promising tools in the era of cystic fibrosis personalized medicine. *Front Pharmacol* 2018; 9: 1429.
33. Eckford PDW, McCormack J, Munsie L, *et al.* The CF Canada-Sick Kids program in individual CF therapy: A resource for the advancement of personalized medicine in CF. *J Cyst Fibros* 2019; 18: 35–43.
34. Berkers G, van Mourik P, Vonk AM, *et al.* Rectal organoids enable personalized treatment of cystic fibrosis. *Cell Rep* 2019; 26: 1701–1708.
35. Clancy JP, Cotton CU, Donaldson SH, *et al.* CFTR modulator theratyping: Current status, gaps and future directions. *J Cyst Fibros* 2019; 18: 22–34.
36. Joshi D, Ehrhardt A, Hong JS, *et al.* Cystic fibrosis precision therapeutics: Emerging considerations. *Pediatr Pulmonol* 2019; 54: Suppl. 3, S13–S17.
37. Durmowicz AG, Lim R, Rogers H, *et al.* The U.S. Food and Drug Administration's experience with ivacaftor in cystic

fibrosis. Establishing efficacy using in vitro data in lieu of a clinical trial. *Ann Am Thorac Soc* 2018; 15: 1–2.
38. DeStefano S, Gees M, Hwang TC. Physiological and pharmacological characterization of the N1303K mutant CFTR. *J Cyst Fibros* 2018; 17: 573–581.
39. Han ST, Rab A, Pellicore MJ, *et al*. Residual function of cystic fibrosis mutants predicts response to small molecule CFTR modulators. *JCI Insight* 2018; 3: e121159.
40. Awatade NT, Uliyakina I, Farinha CM, *et al*. Measurements of functional responses in human primary lung cells as a basis for personalized therapy for cystic fibrosis. *EBioMedicine* 2015; 2: 147–153.
41. Laselva O, Eckford PDW, Bartlett C, *et al*. Functional rescue of c.3846G>A (W1282X) in patient-derived nasal cultures achieved by inhibition of nonsense mediated decay and protein modulators with complementary mechanisms of action. *J Cyst Fibros* 2019; 19: 717–727.
43. Valley HC, Bukis KM, Bell A, *et al*. Isogenic cell models of cystic fibrosis-causing variants in natively expressing pulmonary epithelial cells. *J Cyst Fibros* 2019; 18: 476–483.
44. Kleizen B, Hunt JF, Callebaut I, *et al*. CFTR: New insights into structure and function and implications for modulation by small molecules. *J Cyst Fibros* 2020; 19: Suppl. 1, S19–S24.
45. Amico G, Brandas C, Moran O, *et al*. Unravelling the regions of mutant F508del-CFTR more susceptible to the action of four cystic fibrosis correctors. *Int J Mol Sci* 2019; 20: E5463.
46. Kyrilli S, Henry T, Wilschanski M, *et al*. Insights into the variability of nasal potential difference, a biomarker of CFTR activity. *J Cyst Fibros* 2019; 19: 620–626.
47. Masson A, Schneider-Futschik EK, Baatallah N, *et al*. Predictive factors for lumacaftor/ivacaftor clinical response. *J Cyst Fibros* 2019; 18: 368–374.
48. Sagel SD, Wagner BD, Ziady A, *et al*. Utilizing centralized biorepository samples for biomarkers of cystic fibrosis lung disease severity. *J Cyst Fibros* 2019; 19: 632–640.
49. Volkova N, Moy K, Evans J, *et al*. Disease progression in patients with cystic fibrosis treated with ivacaftor: Data from national US and UK registries. *J Cyst Fibros* 2020; 19: 68–79.
50. Gifford AH, Mayer-Hamblett N, Pearson K, *et al*. Answering the call to address cystic fibrosis treatment burden in the era of highly effective CFTR modulator therapy. *J Cyst Fibros* 2019; in press; [https://doi.org/10.1016/j.jcf.2019.11.007].
51. Saiman L. Improving outcomes of infections in cystic fibrosis in the era of CFTR modulator therapy. *Pediatr Pulmonol* 2019; 54: Suppl. 3, S18–S26.
52. Trinh NT, Bilodeau C, Maillé É, *et al*. Deleterious impact of Pseudomonas aeruginosa on cystic fibrosis transmembrane conductance regulator function and rescue in airway epithelial cells. *Eur Respir J* 2015; 45: 1590–1602.
53. Maillé É, Ruffin M, Adam D, *et al*. Quorum sensing down-regulation counteracts the negative impact of Pseudomonas aeruginosa on CFTR channel expression, function and rescue in human airway epithelial cells. *Front Cell Infect Microbiol* 2017; 7: 470.
54. Laselva O, Stone TA, Bear CE, *et al*. Anti-infectives restore ORKAMBI® rescue of F508del-CFTR function in human bronchial epithelial cells infected with clinical strains of P. *aeruginosa*. *Biomolecules* 2020; 10: 334.
55. Tong K, Barker D, France M, *et al*. Lumacaftor/ivacaftor reduces exacerbations in adults homozygous for Phe508del mutation with severe lung disease. *J Cyst Fibros* 2019; 19: 415–420.
56. Rowntree RK, Harris A. The phenotypic consequences of CFTR mutations. *Ann Hum Genet* 2003; 67: 471–485.
57. Boëlle PY, Debray D, Guillot L, *et al*. Cystic fibrosis liver disease: Outcomes and risk factors in a large cohort of French patients. *Hepatology* 2019; 69: 1648–1656.
58. Aksit MA, Pace RG, Vecchio-Pagán B, *et al*. Genetic modifiers of cystic fibrosis-related diabetes have extensive overlap with type 2 diabetes and related traits. *J Clin Endocrinol Metab* 2020; 105: 1401-1415.
59. Christian F, Thierman A, Shirley E, *et al*. Sustained glycemic control with ivacaftor in cystic fibrosis-related diabetes. *J Investig Med High Impact Case Rep* 2019; 7: 2324709619842898.
60. Kelly A, De Leon DD, Sheikh S, *et al*. Islet hormone and incretin secretion in cystic fibrosis after four months of ivacaftor therapy. *Am J Respir Crit Care Med* 2019; 199: 342–351.
61. Sun X, Yi Y, Yan Z, *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.