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Current and Emerging Therapies for the Treatment of Cystic Fibrosis or Mitigation of Its Symptoms

Mark P. Murphy¹ · Emma Caraher¹

Abstract  Clinical presentation of the chronic, heritable condition cystic fibrosis (CF) is complex, with a diverse range of symptoms often affecting multiple organs with varying severity. The primary source of morbidity and mortality is due to progressive destruction of the airways attributable to chronic inflammation arising from microbial colonisation. Antimicrobial therapy combined with practices to remove obstructive mucopurulent deposits form the cornerstone of current therapy. However, new treatment options are emerging which offer, for the first time, the opportunity to effect remission from the underlying cause of CF. Here, we discuss these therapies, their mechanisms of action, and their successes and failures in order to illustrate the shift in the nature of how CF will likely be managed into the future.

1 Common and Characteristic Pathologies Associated with Cystic Fibrosis (CF)

Cystic fibrosis (CF) is a heritable, chronic condition affecting multiple organs. The loss of or reduction in function of the CF transmembrane regulator (CFTR), a transmembrane ion channel through which chloride and bicarbonate secretion are regulated, interferes with the normal functioning of the epithelia within which it resides.

Functional CFTR is required to adequately hydrate the airway surface liquid (ASL), the continuous transit of which from the respiratory tract into the gastrointestinal tract under ciliary movement aids in the preservation of lung health and removal of inhaled microorganisms and particles. People with CF, however, are unable to clear their dehydrated airway mucous secretions, the prolonged residence of which creates a niche that can be exploited by a number of microorganisms. Many such colonising microorganisms fail to be cleared by the immune system and may persist for many years. Accordingly, this propagates a continuous inflammatory state in the airway which causes bronchiectasis, a progressive destruction of lung tissue architecture. Morbidity of the pulmonary epithelium is most directly responsible for the decreased lifespan experienced by people with CF. Ultimately, mortality results from asphyxiation due to progressive destruction of lung tissue caused by chronic infection and a chronic inflammatory response. Pulmonary insufficiency is the hallmark of CF clinical presentation, and this is reflected by the common use of the FEV₁ (forced airway expiratory volume in 1 s) test as an outcome for efficacy of interventions during clinical trials for CF.

In addition to the airway, pathologies are frequently associated with each of the intestinal, biliary and pancreatic mucosa. In the pancreas, CFTR is localised to the apical surface of pancreatic duct cells [1], where it serves to secrete bicarbonate [2], which facilitates raising of the duodenal pH by pancreatic fluid secretions. CFTR deficiency impairs the adequate exocrine functioning of the pancreas [3], reducing fluid throughput, which serves to concentrate secreted digestive enzymes. This, in turn, leads to ductal occlusion and, hence, pancreatic insufficiency [4].
This leads to pancreatic fibrosis with consequent intestinal malabsorption of nutrients, for which therapeutic supplementation of digestive enzymes is required. Approximately 85% of people with CF have pancreatic insufficiency [4], with 87.3% of patients in the USA requiring pancreatic enzyme replacement therapy (PERT) in 2014 [5].

In addition to mediating compromised digestion, defective CFTR mediates further pathology through the pancreas: up to 50% of people diagnosed with CF may suffer from CF-related diabetes (CFRD). CFRD presents atypically with respect to types 1 or 2 diabetes mellitus in non-CF individuals [6], likely due to ancillary nutritional deficiencies, and this can influence the diagnosis, with the prevalence of the condition being approximately 6% in paediatric patients but rising to ~50% in adults [7]. Glucose-stimulated electrical conductance has long been known to be involved in insulin secretion from pancreatic β cells [8] and, given that CFTR is expressed on the surface of β cells [9], the absence of CFTR has recently been proposed to result in dysregulation of glucose-mediated electrical activity, hence preventing insulin secretion from pancreatic β cells in some people with CF [10].

Disease of the liver is also a notable contributor to the overall pathology of CF. Epidemiological estimates of its prevalence vary, with incidences of 2–41% or more having been reported from different studies [11–15]. CFTR in the liver is localised to the luminal surface of intrahepatic cholangiocytes of bile canaliculi [16], where it infuses biliary secretions with bicarbonate in parallel to its function in the pancreas, also serving to induce the efflux of bile acids [17]. Thus, the absence of bicarbonate secretion causes the retention of harmful bile acids within hepatocytes, inducing an inflammatory response, leading to fibrosis analogous to that seen in the pancreas and, ultimately, cirrhosis and portal hypertension [18].

The morbidities associated with pancreatic and hepatic insufficiency can, in turn, cause a number of gastrointestinal sequelae [19], leading to a complex clinical presentation of people with moderate or severe disease requiring multiple concurrent treatment and management strategies. Indeed, several co-morbidities, such as pancreatic insufficiency and CFRD, correlate with lesser lung function [20].

These morbidities and their ensuing therapies can have adverse secondary effects when administered routinely for chronic illness, with secondary kidney dysfunction being an example of this. The kidney can be damaged by successive rounds of non-steroidal anti-inflammatoryatories, aminoglycoside antibacterials, as well as chronic infection itself, or CFRD and insulin treatment [21].

Each of these primary morbidities may individually or interactively engender a number of potential acute or chronic sequelae themselves requiring management for patients [22].

2 Treatment Strategies

Myriad treatments are prescribed to address the complications engendered by the loss of CFTR function. The majority of these abate the symptoms of this loss but cannot restore the function of the proteins. As our understanding of the structure of native CFTR and its CF-causing mutants has improved, alongside the attendant functional consequences of these, therapeutics that act to express or restore CFTR have begun to emerge. A remarkable diversity of these therapeutics is evident with regard to their mechanisms of action (Fig. 1) and, as discussed here, a number of them are beginning to demonstrate efficacy in trials or in the clinic.

2.1 Small-Molecule Therapies for Improving CF Transmembrane Regulator (CFTR) Function

In excess of 2000 variants of the CFTR gene have been described [23], with the consequence of each for the fate of the protein falling into one of six classes (Fig. 2) [24, 25]. The first class results in CFTR simply being absent from the cell by virtue of deletion of all or part of the gene, a premature stop codon being introduced into the gene, a frame-shifted gene or one which produces alternately spliced messenger RNA (mRNA). The second class of mutations comprise those that generate a protein incapable of being trafficked to the membrane. Such misfolded proteins are targeted for proteosomal degradation or remain in a partially mature state in the cytoplasm or endoplasmic reticulum. It is in this class that the common mutant, p.Phe508del CFTR, lies.

Substitutions of amino acids in either nucleotide-binding domains may yield a protein of sufficient structural normality that it is trafficked to the membrane. However, such mutations may result in a third class of defective proteins, so-called gating mutants, whereby the chloride transport capability of the protein is greatly reduced. Alternately, if it possesses one of the fourth class of mutations, then an incorrect amino acid in one of the membrane spanning domains can alter the transmembrane pore sufficiently to prevent conductance of chloride (or bicarbonate).

Normal physiological function requires not only native CFTR but also sufficient levels of the protein. The fifth class of mutations comprise those that result in the degradation of some proportion of the mRNA, thus reducing the quantity of CFTR produced. Similarly, the sixth class of mutations give rise to CFTR that is recycled from the
plasma membrane with a frequency that cannot sustain normal levels of chloride throughput. The first class of mutations that may afflict the CFTR gene include those that result in the inclusion of a premature termination codon (PTC) in the sequence. This will, in most cases, cause the degradation of the PTC-containing mRNA or the truncated protein translated from it. Small-molecule therapeutics have been developed that can induce the read-through of the aberrant mRNA as normal [26]. Assuming that the PTC did not accompany a frameshift of the ensuing mRNA sequence, a protein of potential normalcy may be translated; the utility of such therapeutics...
extends to improving the likelihood of the correct peptidyltransfer RNA binding at the A site of the ribosome while the PTC is present, overcoming the potentially deleterious effect of the absence of an amino acid which the PTC would otherwise cause.

One of the furthest-progressed PTC correctors in development is PTC124 (generic name ataluren), being brought to market by PTC Therapeutics (South Plainfield, NJ, USA). It has advantageous potency and tolerability relative to small molecules of comparable function and has been shown to have encouraging efficacy in patients amenable to treatment [27]. In assessing its physiological effect in people with CF harbouring a class I mutation on at least one allele, two open-label clinical trials demonstrated an effect on nasal potential difference—a metric of CFTR function—in the majority of patients over the durations of study (Table 1) [28, 29]. No change in percentage FEV1 was apparent, though this was unsurprising given treatment extended for only 6 weeks.

Ataluren has recently been subject to a randomised controlled phase III trial in 238 patients who received the drug for 48 weeks [30], with the primary outcome of the study being lessening of the rate of decline in FEV1. Those patients receiving treatment experienced a marginally lesser decline in FEV1 with respect to the control group (2.5 % decline vs. 5.5 % decline; P = 0.12; Table 1). The results of the study do not seem immediately impressive; however, the ability of the drug to preserve patients’ lung function over an extended period of time (years or decades) would be a more informative and useful metric and a halving of the rate of decline over 1 year, as this study demonstrated, is encouraging.

Moreover, post hoc subgroup analysis demonstrated that those patients who had received ataluren, but who were not concurrently taking aminoglycoside antibacterials demonstrated significantly less decline in FEV1 with respect to their counterparts who had received placebo (0.7 vs. 6.4 %; n = 44, P = 0.0082). This subgroup benefit likely stems from the mechanism of aminoglycoside action in which such compounds bind to ribosomal RNA [31]. Here, it may interfere with the ability of ataluren to effect readthrough of PTC-harbouring CFTR mRNA, positing discontinuation of aminoglycoside treatment as an indication for positive response to ataluren. This hypothesis is presently under investigation in the form of a repeat study of the aforementioned phase III trial specifically recruiting patients who are not undergoing treatment with aminoglycoside antibacterials [120].

For the majority of mutations, CFTR protein is produced, albeit in a non-functional form. This has provided the opportunity for development of therapeutics that can restore the normal functioning of the protein. Broadly speaking, such therapeutics fall into one of two categories: potentiators of CFTR open-conformation (enabling throughput of greater quantities of chloride) and correctors of CFTR structure (and, hence, function). Perhaps the most prominent example of a therapeutic targeting the defective CFTR itself is the small molecule, ivacaftor (brand name Kalydeco®), developed by Vertex Pharmaceuticals (Boston, MA, USA).

Ivacaftor was designed to potentiate the channel opening of CFTR bearing a class III single-nucleotide polymorphism, Gly551Asp, and received US FDA approval for the treatment of people with CF bearing this mutation—representing ~5 % of patients—in 2012 [32]. It had demonstrated statistically significant therapeutic efficacy, with respect to control subjects who continued standard treatment, in two randomised controlled phase III trials and a subsequent open-label extension over 48 weeks and up to 144 weeks, respectively (Table 2) [33–35]. Each trial utilised the common primary endpoint of improvement or lesser decline in FEV1. The drug not only halted decline in FEV1 but improved it relative to the baseline established at the trials’ outset, displaying an ~10 % improvement in FEV1 [36].

Given its efficacy when administered to patients homozygous for the Gly551Asp mutation, ivacaftor was then trialled in patients homozygous for Phe508del; however, it failed to demonstrate a significant change in FEV1 relative to control subjects [37], reflecting the difference in the nature of the defect affecting CFTR. Nonetheless, ivacaftor treatment appears to be a potent and efficacious therapy for adults with CF who harbour homozygous Gly551Asp mutant CFTR alleles; it improves FEV1 and weight gain as well as reducing incidence of infection by Pseudomonas aeruginosa and prolonging intervals between pulmonary exacerbations [38]. The drugs’ efficacy in patients who have other class III mutations has also been demonstrated clinically [39], leading to its approval as a treatment in patients harbouring such mutations, as well as in patients with p.Arg117His CFTR [32]. It may also prove useful for patients who have other ‘partial function’ mutations (classes III–V), further extending its reach.

Moreover, lesser doses are being trialled in paediatric patients to assess tolerability, as CF pathology is often apparent in infancy [40, 41]; ivacaftor treatment is presently approved to commence from 2 years of age [32]. While the requirement for early intervention is apparent, this is accompanied by several caveats. As mentioned, tolerability is an issue, given the load placed particularly on the hepatic cytochrome, cytochrome P450 3A (CYP3A), by ivacaftor [40]. In addition, the yearly cost per patient of ivacaftor treatment is considerable—£182,000 per patient per year in the UK—leading to stringent assessment of its clinical benefit [42, 43], which may limit the breadth of patients to whom it will be administered.
Table 1  Selected data reported from clinical trials of ataluren administration in people with cystic fibrosis harbouring a class I mutation in the *CFTR* gene on at least one allele. Percentage predicted FEV$_1$ is reported at baseline and following treatment for both those in receipt of medication and control subjects who had received placebo treatment, where applicable

| Study type                  | No. of patients completing (% female) | Median age, years (range) | Treatment regimen                                                                 | % FEV$_1$ at baseline (range) | Study duration, weeks | Change in % FEV$_1$ (SD) | No. of patients undergoing aminoglycoside treatment [change in % FEV$_1$ (SD)] | References |
|-----------------------------|--------------------------------------|---------------------------|-----------------------------------------------------------------------------------|-------------------------------|-----------------------|------------------------|---------------------------------------------------------------------------------|------------|
| Phase III                   |                                       |                           |                                                                                   |                               |                       |                        |                                                                                  |            |
| Dual-arm RCT                | 100 (48)                             | 22.8 (6–49)               | Ataluren 10 mg/kg morning, 10 mg/kg midday and 20 mg/kg evening                   | 62.1 (38.4–90.3)             | 48                    | −2.5$^a$ (13.3)         | 38 [−0.7 (11.9)]                                                            | [30]       |
|                             | 103 (50)                             | 23.2 (8–53)               | Matching placebo                                                                   | 60.2 (36.2–92.6)             |                       | −5.5 (12.56)            | 40 [−6.4 (12.6)]                                                            |            |
| Other                       |                                       |                           |                                                                                   |                               |                       |                        |                                                                                  |            |
| Single-arm, open-label study| 23 (52)                              | 25 (18–56)                | Ataluren 4 mg/kg morning, 4 mg/kg afternoon and 8 mg/kg evening per day for 14 days | 65 (41–117)                  | 6$^b$                 | −$^c$                  | 0                                                                               | [28]       |
|                             |                                       |                           | Ataluren 10 mg/kg morning, 10 mg/kg afternoon and 20 mg/kg evening per day for 14 days |                               |                       |                        |                                                                                  |            |
| Open-label crossover Study  | 14                                   | 12 (6–18)                 | Ataluren 4 mg/kg morning, 4 mg/kg afternoon and 8 mg/kg evening per day for 14 days | 90 (40–103)                  | 6$^d$                 | −$^e$                  | 0                                                                               | [29]       |
|                             |                                       |                           | Ataluren 10 mg/kg morning, 10 mg/kg afternoon and 20 mg/kg evening per day for 14 days |                               |                       |                        |                                                                                  |            |

*CFTR* cystic fibrosis transmembrane regulator, FEV$_1$ forced airway expiratory volume in 1 s, RCT randomised controlled trial, SD standard deviation

$^a$ Relative change with respect to baseline

$^b$ Inclusive of 14-day washout period between treatment cycles

$^c$ Figure 5 in original report, no apparent difference day 0 vs. day 42

$^d$ Cohorts received treatment for 14 days and, following a 14-day washout period, received the other treatment listed

$^e$ Reported as “no statistically significant change”
Table 2  Selected data reported from clinical trials of ivacaftor administration to people with cystic fibrosis heterozygous or homozygous for alleles giving rise to a gating mutation in CFTR. Percentage predicted FEV$_1$ is reported at baseline and following treatment for both those in receipt of medication and control subjects who had received placebo treatment, where applicable.

| Study type              | No. of patients enrolled (% female) | Mean age, years (range) [SD] | Treatment regimen | Mean % FEV$_1$ at baseline (range) [SD] | Treatment duration, weeks | Mean absolute change in % FEV$_1$ (95% CI) [SD] | CFTR genotype                    | References |
|-------------------------|-------------------------------------|------------------------------|-------------------|----------------------------------------|--------------------------|-----------------------------------------------|-----------------------------------|------------|
| **Phase II**            |                                     |                              |                   |                                        |                          |                                               |                                   |            |
| Dual-arm RCT            | 8 (62)                              | 23$^a$ (18–40)               | 150 mg/12 h       | 65 (53–112)                            | 4                        | 8.7$^{b,}\,^{c}$ (2.3–31.3) $^{d}$            | Gly551Asp, ≥1 allele              | [75]       |
|                         | 4 (25)                              | 24$^a$ (18–42)               | Placebo/12 h      | 77 (42–122)                            | 16                       | 1.5 $^{f}$ (–0.6 to 4.1) $^{f}$               | Phe508del homozygous DISCOVER;     | [37]       |
| Dual-arm RCT            | 112 (48)                            | 22.8 (12–52)                 | 150 mg/12 h       | 79.7 (40–129)                          | 16                       | $^{g}$                                       |                                   |            |
|                         | 28 (43)                             | 25 (12–39)                   | Placebo/12 h      | 74.8 (43–127)                          | 4                        | $^{h}$                                       |                                   |            |
| Dual-arm RCT-crossover$^a$ | 20 (50)                      | 16.6 (8–43)                  | Ivacaftor 150 mg/12 h | 97.2 [9.7]                           | 4                        | 7.1 (1.8–12.2)$^{l}$ $^{p}=0.0117$          | Gly551Asp, ≥1 allele              | [121]      |
|                         |                                     |                              | Placebo/12 h      |                                          |                          |                                               |                                   |            |
| **Phase III**           |                                     |                              |                   |                                        |                          |                                               |                                   |            |
| Dual-arm RCT            | 83 (53)                             | 26.2 (12–53)                 | 150 mg once daily | 63.5 (37.3–98.2)                       | 48$^e$                   | 10.4 (8.6–12.6)$^{f}$ $^{p}=0.0001$          | Gly551Asp, ≥1 allele              | STRIVE;    [33] |
|                         | 78 (51)                             | 24.7 (12–53)                 | Placebo once daily | 63.7 (31.6–97.1)                       |                          | $^{g}$                                       |                                   |            |
| Dual-arm RCT            | 26 (65)                             | 8.9 (6–11)$^f$               | 150 mg/12 h       | 84.7 (52.4–133.8)                      | 24                       | 12.6 (6.6–18.3)$^{f}$ $^{p}=0.001$           | Gly551Asp, ≥1 allele              | ENVISION;  [34] |
|                         | 26 (38)                             | 8.9 (6–11)$^f$               | Placebo/12 h      | 83.7 (44.0–116.3)                      | 24                       | 0.1                                          |                                   |            |
| Dual-arm RCT            | 34 (56)                             | 29.2 [16.6]                  | 150 mg/12 h       | 70.2 [18.9]                            | 24                       | 2.6 [1.2]                                    | Arg117His, ≥1 allele              | KONDUCT;  [122] |
|                         | 35 (57)                             | 32.7 [17.4]                  | Placebo/12 h      | 75.7 [19.3]                            |                          | 0.5 [1.1]                                    |                                   |            |
| **Other**               |                                     |                              |                   |                                        |                          |                                               |                                   |            |
| OLE$^g$                 | 77$^a$ (53)                         | 27.7 [9.8]                   | 150 mg/12 h       | 71.9 [18.5]                            | 144$^b$                  | 9.4 [10.8]                                    | Gly551Asp, ≥1 allele              | PERSIST;  [35] |
|                         | 67$^b$ (52)                         | 26.0 [9.6]                   | Placebo/12 h      | 62.2 [18.7]                            |                          | 9.5 [11.2]                                    |                                   |            |
|                         | 26$^c$ (65)                         | 9.8 [1.9]                    | Placebo/12 h      | 94.9 [14.5]                            |                          | 10.3 [12.4]                                  |                                   |            |
|                         | 22$^d$ (41)                         | 9.8 [1.8]                    | Placebo/12 h      | 83.6 [7.4]                             |                          | 10.5 [11.5]                                  |                                   |            |
| Dual-arm RCT-crossover with OLE | 19$^e$ (52.6) | 21.7 (6–47)                  | 150 mg/12 h       | 79.1 (42.9–104.1)                      | 24                       | 13.5 (–6.9 to 36.5)                          | Non-Gly551Asp gating mutation     | KONNECTION; [39] |
| Observational study     | 151 (46)                            | 21.1 [11.4]                  | 150 mg once daily | 82.6 [25.6]                            | 26                       | 6.7 (4.9–8.5)                                | Gly551Asp, ≥1 allele              | GOAL;     [38]  |
Concern over the costings of new investigational drugs for ostensibly orphan conditions may be tempered somewhat by the nature of the re-distribution of revenue. The arrangement in place between Vertex Pharmaceuticals and the Cystic Fibrosis Foundation—the US-based not-for-profit CF research organisation, whose model of venture philanthropy has seen it receive ~US$3.3 billion in drug royalties in 2014 [44]—may help to ensure continued high levels of research into the condition.

Vertex Pharmaceuticals has also progressed to clinical trials with a therapeutic, generic name lumacaftor, designed to assist in the trafficking of Phe508del CFTR to the epithelial membrane [45]. Despite in vitro mechanistic evidence, phase II trials failed to show a benefit of taking lumacaftor for patients homozygous for the Phe508del mutation (Table 3) [46], which represents the genotype of approximately 50% of CF patients in both the EU and USA [22]. Accordingly, it was decided to combine lumacaftor treatment with ivacaftor treatment on the basis that ivacaftor may prove effective once Phe508del CFTR had been trafficked. Phase II trials of several dosage permutations of this combined therapy showed a modest improvement in FEV1 at high doses of each therapy: 600 mg of lumacaftor once daily and 250 mg of ivacaftor once daily [47]. Patients in this treatment arm demonstrated a 5.6% improvement in % FEV1, and, as such, this regimen was progressed to two phase III trials which are currently undergoing open-label extensions having compared 600 mg lumacaftor once daily and 400 mg lumacaftor twice daily vs placebo, respectively [48]. The aforementioned phase III trials each demonstrated significant improvement in percentage FEV1 and other metrics (Table 3). Accordingly, licensing for a combination therapy of lumacaftor and ivacaftor (trade name Orkambi™) has been approved by the FDA [49].

There are other novel therapeutics following in the developmental pipeline of Vertex Pharmaceuticals, focused on the major mutations in CF. The furthest progressed of these is another corrector of Phe508del CFTR, designated VX-661. This has shown an ability to improve CFTR operation in vitro and seems to be well-tolerated in patients with some efficacy when administered in conjunction with ivacaftor [50].

The same defects are currently being targeted by other companies or institutions. For example, Novartis have developed a series of correctors for Phe508del CFTR that possess similar functional activity to those of the Vertex therapeutics [51]. Many other candidate chemical entities have so far been declared to have efficacy in restoring CFTR function in vitro and, undoubtedly, further useful therapies will emerge during clinical trials [36].

The current high cost of the ivacaftor regimen had raised doubts as to whether it would be supported by public health
Table 3  Selected data reported from clinical trials of lumacaftor administration to people with cystic fibrosis homozygous or heterozygous for the c.1521_1523delCTT CFTR allele which gives rise to mistrafficked p.Phe508del CFTR. The percentage predicted FEV₁ is reported at baseline and following treatment for both those in receipt of medication and control subjects who had received placebo treatment, where applicable.

| Study type                  | No. of patients enrolled (% female) | Mean age, years (range) [SD] | Treatment regimen                                      | % FEV₁ at baseline (range) | Treatment duration, weeks | Mean absolute change in % FEV₁ (95 % CI) | Phe508del status | References |
|-----------------------------|-----------------------------------|------------------------------|--------------------------------------------------------|-----------------------------|--------------------------|----------------------------------------|-----------------|------------|
| Phase II RCT, multiple arms³ | 20 (40)                           | 28.5 [9.8]                   | Lumacaftor 200 mg once daily + ivacaftor 150 mg/12 h   | 75.1 (42.4–117.1)           | 1                        | 3.1 (0.1–6.1)                         | Homozygous      | [47]       |
|                             | 21 (62)                           | 28.6 [9.1]                   | Lumacaftor 200 mg once daily + ivacaftor 250 mg/12 h   | 57.0 (39.1–93.3)            | 1                        | 0.5 (–2.8 to 3.8)                               | Homozygous      |            |
|                             | 21 (48)                           | 30.1 [10.3]                  | Placebo once daily + placebo/12 h                     | 69.1 (32.8–100.8)           | 1                        | 0.3 (–2.6 to 3.1)                               | Homozygous      |            |
|                             | 23 (48)                           | 28.1 [9.0]                   | Lumacaftor 200 mg once daily + ivacaftor 250 mg/12 h   | 72.4 (43.3–99.1)            | 4                        | 2.0 (–0.8 to 4.8)                               | Homozygous      |            |
|                             | 21 (43)                           | 29.2 [8.5]                   | Lumacaftor 400 mg once daily + ivacaftor 250 mg/12 h   | 67.4 (40.4–91.1)            | 4                        | 2.0 (–0.9 to 4.8)                               | Homozygous      |            |
|                             | 21 (52)                           | 26.7 [6.5]                   | Lumacaftor 600 mg once daily + ivacaftor 250 mg/12 h   | 65.6 (37.0–98.4)            | 4                        | 6.2 (3.3–9.0)                                | Homozygous      |            |
|                             | 11 (45)                           | 25.5 [6.7]                   | Lumacaftor 400 mg/12 h + ivacaftor 250 mg/12 h         | 63.7 (41.8–93.4)            | 4                        | 6.1 (2.0–10.2)                                | Homozygous      |            |
|                             | 17                                | 30.8 [12.4]                  | Placebo once daily + placebo/12 h                     | 72.0 (36.4–107.1)           | 4                        | −1.6 (–4.2 to 1.1)                              | Homozygous      |            |
|                             | 4                                 |                             | Placebo/12 h + placebo/12 h                           |                             |                          |                                        |                 |            |
|                             | 6                                 |                             | Placebo once daily + placebo/12 h                     |                             |                          |                                        |                 |            |
|                             | 21 (38)                           | 27.5 [7.2]                   | Lumacaftor 600 mg once daily + ivacaftor 250 mg/12 h   | 68.5 (38.3–101.7)           | 4                        | 2.3 (–0.8 to 5.4)                               | Heterozygous    |            |

References:
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schemes or insurance providers. However, given the larger cohort of patients eligible to receive Orkambi™, the latter regimen has been offered at a cost lower than that for the ivacaftor monotherapy [52], which may facilitate its prescription to patients. Longer term, however, a proliferation of personalised medicines for CF seems unsustainable from an economic standpoint and there are numerous calls for action to be taken to ensure that costs are controlled, while preserving research investment [43, 53].

2.2 Gene Therapy for the Restoration of Normal CFTR Function

While antibacterial use is frequent in clinical treatment of CF, arguably the most direct route to treating CF, as well as CFTR-related diseases, would be to integrate the native gene into airway stem cells in vivo. Such integrating vectors exist [54] but have been demonstrated to be oncogenic in some settings, justifiably giving rise to concerns over the use of integrating gene therapy [55]. The next best option would be to introduce copies of the native CFTR gene into the cells of the affected epithelia. Encouraging work has been carried out in vitro in this regard. Introduction of the native CFTR gene into CFTR-deficient airway epithelial cell lines—via a transformed human parainfluenza virus—has been shown to result in the expression of the protein in those cells [56]. Furthermore, that study highlighted that normal ASL height was restored above the cell layer following induction of expression in as few as 25% of the cells. Lessening the bioavailability and transfection efficiency of the vector—by including adenoassociated viral vectors [57]—has emerged as one avenue to address these issues. As such, a viral vector that could impart tolerance to repeated administration, while being amenable to repeated delivery, would be preferable, and a next-best approach would be to minimize any potential impact of the virus on the virus-delivery vector itself. Hence, the potential for future clinical utility of viral vectors is dependent on the ability of these viruses to infect the airway epithelium, without adverse consequences, has been demonstrated in vivo [58].

| Study type | No. of patients enrolled (% female) | Mean age, years (range) [SD] | Treatment regimen | % FEV\textsubscript{1} at baseline (range) | Treatment duration, weeks | Mean absolute change in % FEV\textsubscript{1} (95% CI) | Phe508del status | References |
|------------|-----------------------------------|-----------------------------|-------------------|------------------------------------------|-------------------------|-----------------------------------------------|---------------|-----------|
| Phase III triple-arm RCT\textsuperscript{b} | 368 (49.3) | 25.3 (12–54) | Lumacaftor 600 mg once daily + ivacaftor 250 mg/12 h | 60.8 (31.1–92.3) | 24 | 5.6 (3.8–7.3)\textsuperscript{c} | Homozygous Traffic and Transport; [48] |
|            | 369 (49.5) | 24.5 (12–57) | Lumacaftor 400 mg/12 h + ivacaftor 250 mg/12 h | 60.5 (31.3–96.5) | 4.8 (3.0–6.6)\textsuperscript{c} | NA |
|            | 371 (48.8) | 25.4 (12–64) | Placebo/12 h | 60.4 (33.9–99.8) | | |

\textsuperscript{b} Reported here as pooled data arising from TRAFFIC and TRANSPORT studies

\textsuperscript{c} Difference in percentage change in % FEV\textsubscript{1} from baseline with respect to placebo
not been described. This has, however, been overcome by the generation of chimeric viruses possessing each of these traits: the well-tolerated lentivirus, simian immunodeficiency virus, was transformed with coat proteins of the respiratory infectious agent, Sendai virus, producing a virus that possessed the favourable traits of both [59].

Given the complexities inherent in developing viruses as vectors for gene therapy, alternate vectors for the delivery of CFTR DNA are under investigation. For example, pH-responsive peptides may be used. These peptides are bound to nucleic acids and remain so during endocytosis, subsequently enabling the escape of the nucleic acid from the endosome through a pH-dependent conformational change which disrupts the endosomal membrane [60]. Such a delivery vector avoids much of the immunogenicity inherent in a virus.

It should be noted that, while CF is a disease affecting multiple organs, correction of defects in the airway will provide a significant improvement in the quality of patients’ lives as well as an extension of their lifespan and has been pursued most extensively for this reason, in conjunction with the supposed ease of delivery of therapies via inhalation. However, the mucous layer presents an obstacle to delivery of therapeutics in this setting. For (relatively) small-molecule therapeutics, access to the epithelium may be improved by coating them in a non-mucous-adhesive compound such as polyethylene glycol (PEG), the efficacy of which can be further improved by coadministration with a mucolytic agent such as N-acetyl cysteine [61].

Combining a non-viral vector with each of these traits would represent a promising gene therapy for the treatment of CF lung disease. To that end, the UK CF Gene Therapy Consortium have collectively progressed research into such a vector. A plasmid harbouring CFTR was depleted of pro-inflammatory CpG motifs and encapsulated within a proprietary cationic lipid, GL67 (developed by Genzyme, Cambridge, MA, USA), in conjunction with the neutral lipid dioleoylphosphatidylethanolamine and a PEG-containing fusion lipid (Fig. 1). This has been shown in a murine study to have a favourable tolerability and induce persistent expression of CFTR, and each of these characteristics were maintained throughout the repeated-dose study [62].

The outcome of this study gave rise to a phase IIb clinical trial of the formulation. It was demonstrated that administration of a once-monthly dose of the formulation detailed above over 12 months arrested the decline in FEV1 percentage for treated patients with respect to those who had received nebulised saline as placebo (−0.4 vs. −4.0 %, respectively; Table 4) [63]. While modest in size, it is notable that some treated patients had better responses than others, which indicates that a study powered to identify subgroup effects may lead to further improvement in the utility of the therapy. Moreover, adverse events were comparable to the placebo-treated cohort and response did not depend on the type of CFTR mutation borne by patients (with respect to Phe508del status).

Successful transfection of airway cells with the CFTR gene subsequently requires it to be expressed. The plasmid being utilised by the UK CF Gene Therapy Consortium harbours a humanised promoter to ensure expression, but a number of other groups have sought to correct the sequence that already exists. The recent advent of genome editing tools has made this feasible. One such tool, which has been applied to the introduction of CFTR into the genome, is zinc finger nuclease (ZFN) technology. This comprises a fusion protein, one domain of which specifically recognises

| Study type          | No. of patients completing (% female) | Mean age, years (SD) | Treatment regimen | % FEV1 at baseline (range) | Study duration, weeks | Mean response (95 % CI) | References |
|---------------------|--------------------------------------|----------------------|-------------------|---------------------------|------------------------|-------------------------|------------|
| Phase II dual-arm RCT | 62 (50)                              | 23.6 (10.8)          | pGM169/GL67A 5 mL/28 daysa | 69.9 (49.6–89.9)           | 52                     | −0.4b (−2.8 to −2.1)    | [63]       |
|                     | 54 (46)                              | 26 (13)              | Placebo: 0.9 % saline/28 days | 69 (49.6–89.9)           |                        | −4.0b (−6.6 to −1.4)    |            |
| Phase III dual-arm RCT | 334 (44.6)                           | 21.3 (10.7)          | Inhaled mannitol 400 mg twice daily | 63.6 (25–105)      | 26                     | 3.6c                    | [78]       |
|                     | 232 (49.8)                           | 21.6 (10.5)          | Inhaled mannitol 50 mg twice daily | 61.9 (30–100)       |                        | NA                      |            |

CI confidence interval, FEV1 forced airway expiratory volume in 1 s, NA not applicable, RCT randomised controlled trial, SD standard deviation

a ±5 days
b Relative change with respect to baseline
c Difference between groups in absolute % FEV1

Δ Adis
short (9–12 bp) sequences of DNA, while the other possesses non-specific endonuclease activity capable of introducing double-strand breaks into the DNA at the targeted site, allowing the sequence of interest to insert [64]. Such ZFN complexes form heterodimers to carry out this activity, enabling the recognition of ~18 bp sequences, hence providing a high degree of specificity [65]. This approach has been demonstrated in vitro to correct the defective CFTR sequence in epithelial cells having the c.1521_1523delCTT genotype, which gives rise to the Phe508del mutant CFTR [66].

3 Indirect Therapies for CF Management

3.1 Putative Mediators of Improved Lung Physiology

Concurrent efforts are also ongoing to correct the defective physiology evident in CF by compensating for the lack of functional CFTR, a strategy that has the advantage of being applicable to all patients with CF. Most directly, increasing the activity of alternative chloride channels could, in principle, restore the native phenotype of the mucosal epithelium. For example, the calcium-activated chloride channel, TMEM16A, is expressed on airway goblet cells (which secrete mucins) and contributes to mucin release [67]. Mouse ASL height is also regulated by TMEM16A [68]. A direct-acting, long duration pharmacological modulator of TMEM16A is currently being sought [69].

Alternatively, modulation of sodium absorption, which is hyperactive in CF, may be considered [22]. The epithelial sodium channel (ENaC) has been explored in this context, with some therapeutics having reached clinical trials before ultimately being rejected on the basis of lack of efficacy due to insufficient potency or duration of effect or adverse effects such as pulmonary oedema [70, 71]. Efforts in this regard are continuing to pursue longer-acting modulators of ENaC, with some demonstrating increased ASL height and mucociliary clearance in animal models [72]. More recently, however, additional regulators of ENaC have been described, offering targets for candidate drugs with more finely tuned effects [73]. Similarly, the chaperones that guide the folding and trafficking of CFTR itself have been proposed for therapeutic action, which would be of particular benefit to class II mutations that do not reach the plasma membrane and class V mutations where CFTR is recycled from the membrane too quickly [74].

Given the attractiveness of ostensibly curing a chronic, debilitating, life-shortening condition, combined with the apparent requirement to take the aforementioned therapeutics for the duration of life [75], there is considerable research endeavour taking place into their further improvement. Presumably, the combined efforts of various entities in this regard will eventually yield an effective, persistent correctional treatment, but in the intervening years palliative therapy remains the best available option.

One such therapy concerns emulating the effect of ASL hydration, which is lacking in CF, by inducing osmosis of water from the airway cells into the luminal space through the inhalation of nebulised hyperosmolar solutions or dry powders. Two such osmolytes are in use: hypertonic saline, which overcomes ENaC-mediated hyper-absorption of sodium, and mannitol, which also establishes an osmotic driving force; both of which also induce coughing, thereby helping to dislodge deposited mucus. In concentrations as high as 6%, hypertonic saline has been demonstrated to improve lung function after 16 weeks of therapy [76]; extension of therapy to 48 weeks failed to confirm improved lung function but did extend the duration between pulmonary exacerbations [77], highlighting the utility of clearing the lungs of deposited material. By comparison, two recent phase III trials of inhaled mannitol demonstrated improved lung function for up to 26 weeks (the duration of the studies) and prolonged the interval between exacerbations (Table 4) [78].

There is potential for incidental effects of mannitol administration, with in vitro evidence suggesting that the presence of mannitol can induce sensitivity to the antibacterial tobramycin in P. aeruginosa biofilm persistor cells [79], presumably due to carbon source recognition by the bacteria leading to their ‘awakening’. Similarly, mannitol may enhance adhesion by CF-associated Burkholderia multivorans strains [80], illustrating a potential need for concurrent antibacterial treatment with mannitol therapy.

Treatment with recombinant human deoxyribonuclease (rhDNase), also known as dornase alfa or pulmozyme, represents another measure commonly employed to aid breathing by clearance of pulmonary obstructions. rhDNase treatment degrades the DNA derived from lysed neutrophil infiltrates, enabling easier clearance by airway clearance techniques such as saline therapy or thoracic agitation to induce coughing [81]. This treatment can improve lung function, though its efficacy in reducing exacerbations is less clear [82]. It may also be useful for disruption of microbial biofilms in the airways [83].

Given that lung damage driven by inflammation is the major contributor to mortality in people with CF, inhaled corticosteroids are often prescribed to lessen the extent of inflammation experienced by patients. Oral prednisone had been tested under clinical trials, lasting up to 48 months, and has shown improvements in patients’ lung function [84, 85]. However, an increase in adverse events compared with the placebo group was evident and age-matched growth impairment was noted among males for up to several years following discontinuation of treatment [86].
A recent systematic review of trials of inhaled corticosteroids \( (n = 13) \) failed to find a significant improvement in patients’ health while participating in those studies \([87]\). The authors noted that withdrawal from corticosteroids often took place without issue and highlighted possible negative side effects from taking them, in a parallel to the experience with oral prednisone. As a counterargument to the lack of trial evidence, such trials were conducted for only a limited period of time in patients who have already experienced prolonged inflammation. They cannot reflect any benefit that early, prolonged intervention with corticosteroids may have.

Improvement of patient health can not only enable better outcomes to treatment and immunity to exacerbations but can improve the quality of patients’ lives. To that end, pancreatic or hepatobiliary insufficiency are often compensated for by supplementation with digestive enzymes and vitamins specific to the needs of the patient, alongside maintenance of a high-calorie diet that emphasises high intake of fat and protein \([88]\).

PERT is widely used; porcine-derived pancrelipase has demonstrated tolerability and mediates improvements to absorption of fats and nitrogenous compounds, hence contributing to restoration of normal growth and maintenance of adequate weight \([89]\). Porcine-derived native enzymes do, however, present challenges regarding dosage and efficacy during gastrointestinal transit \([90]\). Accordingly, non-animal-derived PERT agents are being pursued with liprotamase, for example, having demonstrated clinical efficacy and presently undergoing further investigation \([91, 92]\). Similarly, microbially derived lipases may be effective as PERT agents and are under clinical investigation \([93]\). Bile acids are also administered for those patients experiencing CF liver disease, though their efficacy is questionable \([94]\) and probably dependent on the residual functioning of the liver among other factors such as age at intervention \([95]\).

### 3.2 Antimicrobial Agents

Strategically related to amelioration of inflammation is the removal of its cause, namely the colonisation of patients’ lungs by microorganisms. Numerous species of microorganisms are frequently isolated from CF lung sputum samples, most commonly \( \textit{P. aeruginosa} \), \( \textit{Staphylococcus aureus} \), \( \textit{Haemophilus influenzae} \), as well as the fungal pathogen \( \textit{Aspergillus fumigatus} \) and members of the \( \textit{Burkholderia cepacia} \) complex of bacteria (Bcc), but a host of others are also frequently detected by routine culture including \( \textit{Stenotrophomonas maltophilia} \), non-tuberculous mycobacteria, etc. \([96, 97]\).

\( \textit{P. aeruginosa} \), for example, has a prevalence ranging from 13 to 75 % of CF patients, depending on age and geographic location \([96, 98]\). Its prevalence increases steadily with age (from \( \sim 25 \% \) at the onset of adolescence to \( \sim 75 \% \) at adulthood in the US population), reflecting the tendency for many patients to become chronically colonised with a given strain with worsening airway conditions, often defined as greater than 50 % of their sputum samples being culture positive over a 12-month period \([99]\). A recent study in the USA indicated that as many as 48 % of people with CF become colonised with \( \textit{P. aeruginosa} \) by the age of 6 years \( (n = 3608) \) \([100]\). Concurrently, the rate of pulmonary exacerbations significantly increases in patients following acquisition \([101]\).

The overall prevalence of \( \textit{P. aeruginosa} \) is falling, as evident in US patient registry data, which can be attributed to the success of early and frequent intervention with antibacterials such as inhaled tobramycin or aztreonam \([102, 103]\). A number of trials have demonstrated the utility of inhaled antibacterials for improving lung function and lessening the rate of pulmonary exacerbations in patients with long-term colonisation by \( \textit{P. aeruginosa} \) \([104]\). Moreover, if treatment with antibacterials of proven efficacy is commenced soon after acquisition of the bacterium, it is possible to effectively eradicate \( \textit{P. aeruginosa} \) \([103]\).

While many patients for whom \( \textit{P. aeruginosa} \) is successfully eradicated following early intervention were shown to sustain the absence of the bacterium for at least 5 years, many failed to demonstrate an improvement in their rate of pulmonary exacerbation \([105]\). This may suggest that other microorganisms then dominate, emphasising that effective antimicrobial regimes against a broad range of CF-associated pathogens is needed. This presents a challenge where microorganisms of low prevalence are concerned, as trials of the efficacy of candidate therapies may be under-powered.

Considering the example of colonisation by species of \( \textit{Burkholderia} \) bacteria, their multi-drug-resistant status has precluded the instigation of a standard course of treatment. This is brought into sharp relief by the failure of a recent review, carried out by the Cochrane collaboration, to find even a single eligible trial of effective interventions against Bcc bacteria \([106]\). Clinical trials are also lacking interventions against the increasingly prevalent methicillin-resistant \( \textit{S. aureus} \) (MRSA) \([107]\), \( \textit{S. maltophilia} \) \([108]\) or non-tuberculous mycobacteria \([109]\).

Such endeavours are not trivial, however, as evidenced by findings from systematic reviews of trials of antibacterial interventions against chronic \( \textit{P. aeruginosa} \) colonisation. A recent review of trials of oral antibacterials for the treatment of \( \textit{P. aeruginosa} \) failed to find a notable improvement to either lung function or quality of life \([110]\). The authors highlighted both the subjectivity of quality-of-life assessments, as well as the differential response of different patients to a given treatment (into which...
contribute myriad factors). This situation is echoed in a review of studies of inhaled tobramycin for treatment of chronic *P. aeruginosa* colonisation [102], in which the ability to improve clinical condition in the majority of patients was clear, but the failure of some patients to respond confounded large-scale analyses of antibacterial efficacy.

In a more general sense, the preferential route of antibacterial administration has proven equally difficult to confirm, with none of oral, intravenous or nebulised administration emerging as clearly more efficacious in treating pulmonary exacerbations [111]. However, for long-term therapy, inhalation may have an advantage, particularly in the case of inhaled tobramycin [112, 113]. It is also recognised that the propensity of successful colonisers to form biofilms may necessitate the administration of types and concentrations of antibacterials with anti-biofilm efficacy, though solid confirmation of this has not yet been provided [114].

Hence, treatment decisions are informed by prior experience and case reports. In the case of *Burkholderia* bacteria, there are isolated case studies of successful eradication of *Burkholderia* from CF patients and from settings other than CF, such as successful intervention with aztreonam in non-CF bronchiectasis [115]. However, while early eradication is often attempted, using combined intravenous and nebulised antibacterials, the success rate is low, with many infections remaining in situ [116].

### 3.3 Transplant

Management of the CF pulmonary physiology and microbiome is a matter of delaying the decline in lung function, but inevitably patients are rendered unable to achieve sufficient oxygen intake through breathing and, as they approach this point, bilateral lung transplantation is considered. In a retrospective study of 101 transplantations in CF patients in Italy, the 1-, 5- and 10-year survival rates for patients post-transplantation were 79, 58 and 42 %, respectively, while FEV$_1$ increased from a pre-operative mean of 22 to 85 % 1 year after the operation [117]. Interestingly, these figures match those of a UK cohort of transplant recipients, with mean FEV$_1$ for those patients improving from 21 to 78 % at 1 year, while survival rates were 82 % at 1 year, 62 % at 5 years and 51 % at 10 years ($n = 176$) [118].

Hence, there is a substantial survival and life-quality advantage to be gained through transplantation. Although there was sizable post-operative mortality (14.8 % of patients in the Italian clinic), the potential benefits may be decisive in listing patients for transplant. Supplementary to this, many clinics view colonisation by certain bacteria, such as *Burkholderia*, as a contra-indication for transplantation. In a UK CF clinic, ~10 % of CF patients who underwent lung transplantation had colonising *Burkholderia*. Of these, ~40 % died within a year following the operation, with the most frequent cause of death being sepsis [119]. This greatly exceeded the mortality rate for patients who did not harbour these bacteria, and these outcomes led to that clinic ceasing to list CF patients who harbour *Burkholderia* for transplantation.

### 4 Concluding Remarks

CF causes chronic morbidity in multiple organs, leading to a substantial reduction in the lifespan of those affected. Loss of lung function, mediated by tissue destruction pursuant to microbial colonisation and the attendant inflammatory response, is the most pronounced driver of mortality for people with CF. As such, multiple avenues of therapeutic interventions are being pursued. Ultimately, restoration of the airways to a normal phenotype holds the most promise for meaningful extensions of survival for people with CF, whether by allowing the epithelium to produce normal CFTR or by restoring the normal function of existing CFTR. In the meantime, diminishing the microbial burden in the airways is an important focus of research, but requires potent antibacterials and better delivery of them. These interventions are greatly aided by a therapeutic strategy that addresses as many of the symptoms of CF as possible; the resulting improvements to organ function and, hence, quality of life are instrumental in continuing to extend the lives of people with CF.

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