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Ataluren in cystic fibrosis: development, clinical studies and where are we now?

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1 Abstract

Introduction: Cystic fibrosis (CF) is one of the most common genetically-acquired life-limiting conditions worldwide. The underlying defect is dysfunction of the cystic fibrosis transmembrane-conductance regulator (CFTR) which leads to progressive lung disease and other multi-system effects. Around 10% of people with CF have a class I nonsense mutation that leads to production of shortened CFTR due to a premature termination codon (PTC).

Areas covered: We discuss the discovery of the small-molecule drug ataluren, which *in vitro* has been shown to allow read-through of PTCs and facilitate synthesis of full-length protein. We review clinical studies that have been performed involving ataluren in CF. Early-phase short-term cross-over studies showed improvement in nasal potential difference. A follow-up phase III randomised controlled trial did not show a significant difference for the primary outcome of lung function, however a *post-hoc* analysis suggested possible benefit in patients not receiving tobramycin. A further randomised controlled trial in patients not receiving tobramycin has been reported as showing no benefit but has not yet been published in full peer-reviewed form.

Expert opinion: A small-molecule approach to facilitate read-through of PTCs in nonsense mutations makes intuitive sense. However, at present there is no high-quality evidence of clinical efficacy for ataluren in people with CF.

1.1 Keywords

ataluren; CFTR; cystic fibrosis; nonsense mutation; PTC 124; read-through agent

2 Introduction

Cystic fibrosis (CF) is the most common life-limiting autosomal recessive disease in Europe and North America [1]. In the United Kingdom for example it affects over 10,000 individuals [2]. CF is characterised by progressive lung disease that ultimately results in end-stage bronchiectasis and respiratory failure. Other clinical features include pancreatic insufficiency, CF-related diabetes, nutritional compromise, liver dysfunction and male infertility [1]. The genetic basis of CF are pathogenic mutations in the *CF transmembrane conductance regulator (CFTR)* gene, which encodes for the CFTR transmembrane chloride and bicarbonate channel that is predominantly expressed at the apical membrane of epithelial cells [3, 4] . CF is most common in populations of Northern European ancestry but also occurs at a lower frequency across ethnically diverse populations globally.

Around 2,000 *CFTR* mutations have been identified of which a small number account for the majority of disease [5]. *CFTR* mutations are classified according to the underlying mechanisms involved in CFTR protein dysfunction, synthesis and trafficking (Table 1 and Figure 1) [4]. Mutations causing defective expression of CFTR protein are classified as class I mutations. Class II mutations describe dysfunctional CFTR processing and failure of CFTR trafficking to the cell surface. In class III mutations, CFTR is expressed at the cell surface but defective nucleotide binding leads to impaired channel gating and abnormal epithelial chloride transport. The CFTR channel pore is defective in class IV mutations resulting in impaired channel conductance. Splicing defects in class V mutations decrease the quantity of correctly transcribed CFTR protein at the cell surface. Reduced stability of functional CFTR results in high cell surface turnover in class VI mutations. Despite this classification, some mutations are not exclusive to a specific class as exemplified by the most common *CFTR* mutation, Phe508del, which belongs to both classes II and III. Furthermore, although classes I to III are associated with a severe disease, phenotypic variation within classes and genotypes indicates involvement of multifactorial environmental and non-*CFTR* genetic factors [6].

Class I mutations are comprised of a number of mutation types. These include nonsense, frameshift and large deletion or insertion mutations that all result in total or partial absence of CFTR protein expression[7]. Class I mutations are therefore functionally characterised by the absence of chloride conductance in affected epithelia and a resultant severe disease phenotype. Individual *CFTR* mutation frequency varies globally amongst different ethnic groups [7]. The Phe508del *CFTR* variant accounts for around 70% of mutations in Northern Europe whereas in people of Ashkenazi Jewish ancestry class I *CFTR* mutations are the most common [8].

Development of coordinated multidisciplinary care delivered by specialist centres and guided by evidence-based guidelines has yielded incremental improvements in survival as understanding of the condition has steadily improved since its first recognition in 1938 [9-11]. When first described, CF was associated with poor survival limited to early childhood; however, median survival is now over 40 years in countries with well-funded health care systems [12]. Traditionally the mainstays of treatment have targeted symptoms and attempted to delay disease progression using intensive chest physiotherapy, life-long antibiotics, pancreatic enzyme replacement therapy and inhaled mucolytics [1]. Although clinically successful, none of these strategies have corrected the underlying CFTR defect, but rather attempted to ameliorate downstream effects.

Ivacaftor is a small molecule CFTR potentiator that rescues function in class III mutations, most notably the Gly551Asp mutation that accounts for around 3-5% of mutant *CFTR* alleles worldwide [13]. It improved lung function by around 10 percentage points of predicted forced expiratory volume in 1 second (FEV_1) in multicentre randomised controlled trials with significant benefits in sweat chloride, respiratory symptoms, body mass index and quality of life [14, 15]. Accordingly, ivacaftor is now prescribed as a mutation-specific treatment in many countries. Ivacaftor has provided a substantial breakthrough over the last decade for the small cohort of CF patients with eligible mutations, is one of the best examples to date of precision medicine and crucially provides ‘proof-of-concept’ that targeting CFTR dysfunction is likely to be a successful and potentially

transformative therapeutic strategy [4, 16]. Subsequent research and development has focused on small molecule approaches to restore function in other common *CFTR* mutations, most notably with the combination treatment (Orkambi™) of lumacaftor, a CFTR corrector, and ivacaftor in patients homozygous for Phe508del. However clinical benefits in randomised controlled trials have only been modest compared to ivacaftor in Gly551Asp patients and the biology of Phe508del *CFTR* remains complex [17].

Ataluren, also known as PTC-124, is a drug that was identified to specifically target class I nonsense *CFTR* mutations and lead to 'read-through' of mRNA resulting in expression of full length CFTR protein. This article will review and discuss the background to the development of ataluren, its mechanisms of action, and evidence from clinical trials with regard to ataluren as a potential treatment in people with CF.

2.1 Overview of the market

Around 10% of people with CF worldwide have at least one class I nonsense *CFTR* mutation [18]. There is, however significant geographical variation; for example in Israel more than 45% of patients with CF have a nonsense mutation compared to less than 15% in the UK [7]. The most common class I mutation is Gly542X [19-21].

2.2 Introduction to the compound – What is ataluren?

Nonsense mutations give rise to inframe premature termination codons (PTCs) that result in the interruption of ribosomal translation and subsequent production of shortened and non-functional CFTR protein [7, 22]. This activates a regulatory mechanism called nonsense-mediated mRNA decay (NMD) that degrades PTC-containing mRNA, reducing further production of shortened proteins. Nonsense mutations are defined by PTCs and its regulation via NMD, both of which are intrinsically linked [18, 23]. Due to an overall lack of functional protein, it follows that patients with this class of mutation normally have a severe clinical phenotype.

Therapeutic approaches to CF caused by nonsense mutations include both PTC read-through and NMD inhibition [24] to promote production of full-length CFTR, however this article will focus specifically on advances in the understanding of PTC read-through with regard to ataluren.

Aminoglycosides were the first class of drugs to demonstrate ribosomal read-through of PTCs [25]. It is thought that their selectivity to binding-sites on ribosomes causes insertion of a random amino acid at the PTC position of mRNA, thus allowing it to read-through the effectively 'masked' PTC producing a full-length, functioning protein. This exciting observation was first made in the 1960s following demonstration of phenotypic repair of defective bacterial genotypes with streptomycin [26]. From the 1970s, further supporting evidence emerged as this activity of aminoglycosides was reproduced in mammalian cells [27]. Finally, in 1996, in a pivotal study using CF as a disease model, PTC read-through was tested as a novel therapeutic approach by demonstration of full-length and functioning CFTR protein in cell lines expressing PTCs treated with aminoglycosides [28]. Indeed, a number of studies since then have shown improvement in CFTR function at a cellular level following treatment with gentamicin [29-31]. However, parenteral administration and significant otic and renal toxicities of this class of drugs limit their clinical usefulness for the purpose of restoring CFTR function as a chronic treatment.

Ataluren was identified via high-throughput screening as a compound that similarly promoted read-through of PTCs [32]. It is structurally distinct from aminoglycosides and the first drug in its class (Figure 2). The initial discovery was based on demonstration of read-through activity in firefly luciferase (FLuc) reporters.

Ataluren has also been investigated in nonsense mutation Duchenne muscular dystrophy (nmDMD) and was granted conditional approval for marketing in 2014 by the European Medicines Agency (EMA) for this indication following a study showing some clinical benefit in slowing the rate of decline in those with advanced disease [33, 34]. It is still awaiting US Food and Drug Administration (FDA) approval for this indication [35] having initially been filed a Refuse to File letter

in February 2016 due to lack of convincing evidence of its effectiveness [36]. Although more data is needed, it provides hope and marks a significant advancement in drug research for this group of patients with otherwise no known disease-modifying treatment.

2.3 Chemistry

Ataluren is a 284 Dalton non-aminoglycoside molecule and its chemical structure (Figure 2) is known as 3-[5-(2-fluorophenyl)-[1,2,4]oxa-diazol-3-yl]-benzoic acid ($C_{15}H_9FN_2O_3$). Its anhydrous free carboxylic acid form is orally bioavailable when prepared in aqueous suspension [32].

2.4 Pharmacodynamics

Several studies in the last decade have studied ataluren's pharmacodynamic properties and mechanism of action with differing results. In a study using cell lines transfected with a firefly luciferase gene engineered to have a PTC at codon 190, ataluren demonstrated dose-dependent read-through of all three known PTCs (UGA, UAG, UAA) as demonstrated by the amount of luciferase activity observed. Compared to gentamicin, ataluren demonstrated potency at much lower concentrations [32]. In this study, ataluren's nonsense suppression was demonstrated to be due to promotion of PTC read-through activity with minimal effect on the NMD regulatory mechanism. Figure 3 schematically summarises the proposed mechanism of action. Ataluren demonstrated selectivity to premature but not normal termination codons. Unlike gentamicin, it did not demonstrate any antibacterial activity.

A further study published in 2007 shortly after its discovery affirmed its use as a read-through agent. In a CF mouse model expressing the Gly542X mutation ataluren treatment resulted in expression of CFTR protein at the apical surface of mice intestinal glands, consistent with effective *in vivo* nonsense mutation suppression activity [21].

There are conflicting reports in the scientific literature around the methodology used in ataluren's discovery and mechanisms of action. Two studies used alternative reporter assays and did not

reproduce evidence of the read-through activity of ataluren [37, 38]. These authors suggest that the initial observation of ataluren's read-through ability was instead due to its off-target activity as a FLuc inhibitor [37, 38]. These studies have faced recent rebuttal however by Roy *et al* who demonstrated read-through activity in several non-luciferase reporter assays. Further evidence was provided elucidating ataluren's selectivity for the ribosomal A site and that it promotes insertion of near-cognate tRNAs at the PTC during protein synthesis [39]. Roy *et al* also demonstrated that tobramycin (an aminoglycoside with similar ribosomal selectivity) is a strong inhibitor of ataluren, consistent with a previous hypothesis proposed by Kerem *et al* in a *post-hoc in vitro* study as part of a phase III trial (see further discussion below) [40].

The *ex vivo* human intestinal organoid model has also been used to further investigate the functional effects of ataluren. In this model, intestinal epithelial cells derived from rectal biopsy tissue are cultured to produce organoids that contain crypt-like structures and a central lumen lined by a CFTR-expressing differentiated apical epithelium. Addition of forskolin, a potent activator of CFTR, can be used to assess the effects of CFTR activity on fluid secretion via assessment of organoid swelling. In this study, intestinal organoids were derived from compound heterozygous patients for 5 nonsense mutations in combination with either a frameshift mutation or Phe508del [41]. Pre-treatment with ataluren in isolation or combination with ivacaftor and lumacaftor did not result in either total organoid or luminal swelling in all donor organoids, indicating its failure to induce detectable read-through in this model [41].

2.5 Pharmacokinetics and metabolism

Phase I and II studies have demonstrated rapid oral absorption of ataluren and dose-proportional increases in pharmacokinetic parameters with absence of any significant gender or age effects [42-44]. Peak ataluren plasma levels are achieved at approximately 2 hours after dosing and its half-life ranges between 3 to 6 hours. With multiple dosing, there was no evidence of drug accumulation or metabolic auto-induction. Phase I studies have also observed that the percentage of ataluren excreted in the urine as the parent drug is low [42].

2.6 Clinical efficacy

As with all studies discussed in this section, participants included those with two disease-causing *CFTR* mutations with at least one being a class I nonsense mutation.

2.61 Phase II studies

An initial phase II study was performed in 23 adults [43]. In this prospective open-label crossover trial, participants received 2 cycles of treatment. 23 participants were included in the first cycle but only 21 in the second. Each cycle comprised a 14-day treatment period (first: 16mg/kg/day; second: 40mg/kg/day) followed by a 14-day washout period. No placebo was used during the washout period.

The primary outcome was treatment response as measured by nasal PD. Nasal PD was used as a surrogate measure of the presence and function of CFTR protein on respiratory epithelial cell surfaces. Measurement of nasal PD involves sequential perfusion of compounds across the nasal epithelial surface, initially blocking absorption of sodium and subsequently augmenting CFTR-mediated chloride transport. This sequence results in dynamic changes in potential difference, with eventual hyperpolarisation. The most consistent abnormality in CF is the absence of this hyperpolarisation [45]. A good response was pre-defined as an increase in total chloride transport by -5mV or more. Normal chloride transport was predefined as nasal PD that is at least as electrically negative as -5mV. Patients were used as their own control based on the premise that all participants had an abnormal baseline nasal PD and that fewer than 5% of nonsense mutation CF patients have been found to have nasal PD values corresponding to that of 'hyperpolarisers' [29, 43].

Improvement in total chloride transport was seen in the majority of patients in both cycles, see Table 2. In a proportion of these, total chloride transport entered the normal range: 13 patients (57%) in the first cycle (p=0.0003) compared to 9 patients (43%) in the second cycle (p=0.02). These improvements reverted to baseline values during 14 days off treatment, suggesting that the

effect observed was mediated by ataluren. A small increase in FEV_1 (actual numerical values not included in the original paper; p=0.037) and bodyweight with a mean change of +0.6kg (SD 0.6; p<0.0001) were seen only in the first phase of treatment.

A follow-on open-label study was performed in 19 of the initial participants of the above trial evaluating the effects of 12 weeks of continuous treatment [46]. Patients were allocated to the dosing regimen in which their best response was observed in the prior study. They demonstrated statistically significant ongoing improvement in total chloride transport, see Table 2. This improvement was progressive with increasing duration of treatment but was not dose-dependent.

A subsequent phase II prospective crossover trial was the first to look at the same primary outcome in children involving 30 participants aged 6 to 18 years [44]. They were randomised to two cohorts (high-to-low dosing versus low-to-high dosing) and were assessed similarly in 2 cycles of 14 days on treatment and 14 days off. Using nasal PD, a total chloride response was seen in 50% of patients (n=15). 47% of patients (n=14) were observed to enter the normal range of total chloride transport, with higher rates observed in higher dose levels in both cohorts. Using immunohistochemistry, this study was also the first to observe increased apical CFTR protein expression on nasal epithelial cells post-treatment although this could not be correlated with changes in nasal PD due to small sample size and variability in measurements. Again, no statistically significant changes were seen in clinical outcomes such as FEV_1 and body weight. Encouragingly, this study also demonstrated its safety for use in a paediatric cohort.

In each of these studies no association was demonstrated between treatment response and any particular genotype or genotype combination. Notably, all the phase II studies had a small sample size and with a relatively short trial period and crossover design, they are not ideal for studying long-term clinical outcomes of a potentially disease-modifying drug.

2.62 Phase III studies

The above studies paved the way for a phase III multi-centre randomised controlled trial that was performed in a larger cohort of 238 patients aged 6 years or over [40]. Patients were randomised to receive ataluren (40mg/kg/day in three divided doses) or placebo for 48 weeks. The primary outcome was change in percentage predicted FEV_1 with rate of pulmonary exacerbations as a secondary outcome. Overall there was no significant difference in mean relative change in FEV_1 at 48 weeks between the treatment groups with a mean difference of 1.76% in favour of ataluren (95% CI -0.43, 3.95) [40, 47]. The mean rate of pulmonary exacerbations was lower in the ataluren-treated group compared to placebo (rate ratio 0.77; p=0.099) across the 48 weeks but this was not statistically significant. Similar to the phase II studies, there were also no changes seen in sweat chloride concentrations, which was an exploratory outcome of this study [43, 44, 46]. No significant difference in total chloride transport, as measured by nasal PD was detected post-treatment.

A *post-hoc* analysis was performed on the subgroup of patients receiving chronic inhaled tobramycin versus those not receiving it. This analysis, which a Cochrane review identified as being at high risk of bias for selective reporting, suggested that in patients not receiving tobramycin, the mean relative change in percentage predicted FEV_1 at week 48 was 5.7% in favour of ataluren (95% CI 1.5 to 10.1; p=0.024) [47]. It was also demonstrated *in vitro* using a luciferase reporter assay that ataluren-induced readthrough is diminished when cells are co-incubated with a combination of ataluren and the aminoglycoside antibiotics gentamicin or tobramycin [40].

To investigate these observations a further phase III study (ClinicalTrials.gov identifier NCT02139306) involving 279 patients not receiving chronic inhaled tobramycin was performed. In March 2017 an announcement was made via a PTC Therapeutics press release stating that the results of this trial showed similar failure to reach the same primary and secondary endpoints. Note that at the time of writing, the full data remains unpublished. However, the announcement reported in brief that the change in percentage predicted FEV_1 was 0.6% in favour of ataluren (p=0.534) and the rate of pulmonary exacerbations was 14% lower in the ataluren group (p=0.401)

but neither of these results were statistically significant. In light of these discouraging results PTC Therapeutics have recently decided to discontinue their clinical development of ataluren for CF [48] .

2.7 Safety and tolerability

Preclinical pharmacology studies in rats and dogs have demonstrated that oral administration of ataluren at high doses (up to 1500mg/kg) did not induce any neurological, pulmonary or cardiovascular toxicities [42]. All human phase II studies have not shown any clinically significant adverse effects potentially attributed to ataluren, however none of these studies were placebo-controlled. Most adverse events were reported as mild to moderate, and none showed a dose-dependent increase in frequency or severity. In the first phase II trial in adults, the most common adverse event was dysuria (n=4) but this was not accompanied by any urinary abnormalities [43] . This observation was not seen in the ensuing trial in children [44]. Blood markers of renal and liver function, such as creatinine and levels of aminotransferases were stable throughout the phase II studies.

Importantly in the first phase III trial there were significantly more episodes of acute kidney injury, reported as renal failure, acute renal failure, renal impairment or hyper-creatininaemia, in the ataluren group (RR 17.70 CI 1.28 to 244.40) [40]. The authors attributed cases of acute kidney injury in the ataluren arm to concomitant use of systemic nephrotoxic antibiotics (aminoglycosides and vancomycin) and dehydration. This was addressed by amendment of the study protocol, at 7 months, to prohibit ataluren use with these systemic antibiotics and encouraging hydration.

Ataluren was generally well tolerated across all studies by participants and compliance was not a major issue.

2.8 Regulatory affairs

The rights to ataluren are owned by PTC Therapeutics and it is marketed under the trade name Translarna™. Ataluren is approved within the European Union Member States for the treatment of

nmDMD in ambulatory patients aged over 5 years. It is designated as an orphan medicinal product by the EMA and the US FDA has granted orphan drug designation to ataluren for the treatment of nmDMD.

2.9 Conclusion

Class I nonsense *CFTR* mutations are present in around 10% of people with CF worldwide and are associated with a severe clinical phenotype due to absence of full-length functional CFTR protein. Ataluren is an orally administered small-molecule drug that facilitates the read-through of mRNA beyond a PTC thereby allowing expression of full-length protein. There is *in vitro* evidence that ataluren facilitates read-through in cellular models expressing nonsense *CFTR* mutations. Early phase cross over studies in patients with nonsense *CFTR* mutations also showed favourable changes in electrophysiology in terms of nasal PD with short-term ataluren treatment. However, phase III randomised, blinded and placebo-controlled clinical trials on two occasions have not shown benefit from ataluren treatment and at present there is no high-quality evidence of clinical efficacy in patients. It is important to note that the most recent RCT has not been formally published in a peer-reviewed format and we base our comments on a press release from PTC Therapeutics [48].

2.10 Expert opinion

The story to date of the identification of ataluren and subsequent development and evaluation as a potential treatment in people with CF caused by class I nonsense *CFTR* mutations is a fascinating one from an academic and clinical perspective. It began with exciting discovery science and the identification of a small-molecule able to facilitate the translation of full-length protein and nullify the basic problem caused by pathogenic PTCs. In the laboratory/basic science world there was subsequently some controversy about its true mechanism of action and how best to study this with the most recent publication confirming *in vitro* efficacy.

Using changes in nasal PD as a read out of CFTR function short-term cross over studies in adults and children with CF caused by class I nonsense *CFTR* mutations showed some positive effects. Appropriately large multi-centre RCTs were subsequently performed with the first study essentially being negative aside from a *post-hoc* analysis of participants not receiving tobramycin. The scientific rationale as to why tobramycin may be relevant here provided fresh hope and a further RCT was performed in patients not receiving tobramycin. The full results of this trial are yet to be formally published in a peer-reviewed journal but we understand based on a press release from PTC Therapeutics that they are negative and that the company are no longer pursuing ataluren as a treatment in CF. It has to be our opinion therefore that there is no good-quality evidence of clinical efficacy for ataluren in CF. With the benefit of hindsight, the current evidence would now suggest that data associated with the *post-hoc* analysis from the group not receiving tobramycin was not strong enough to base a further phase III study on.

We suspect that from a patient's perspective it is a particularly frustrating and disappointing story. The therapeutic approach of using a small-molecule to facilitate read-through and expression of full-length functional CFTR protein is sound and worthy of continued investigation in our opinion. On reflection the ataluren experience to date illustrates a number of key points for CF research; firstly, the importance of ongoing basic science using the most valid experimental models and secondly the crucial importance of careful study design in translational CF research. There are ongoing challenges around which endpoints are most relevant in early phase versus later phase studies in CF and these feed in to the difficulties in conducting appropriately powered phase III studies in subsets of people who have what is regarded as an orphan disease.[49]

2.11 Drug summary box

Drug name	PTC-124 or Ataluren
Phase (for indication under discussion)	Second Phase III trial (awaiting publication of results;

	ClinicalTrials.gov identifier NCT02139306)
Indication (specific to discussion)	Patients with nonsense mutation cystic fibrosis
Pharmacology description/mechanism of action	Enables ribosomal read-through of PTCs hence restoring production of full length and functional CFTR protein
Route of administration	Oral
Chemical structure	3-[5-(2-fluorophenyl)-[1,2,4]oxa-diazol-3-yl]-benzoic acid ($C_{15}H_9FN_2O_3$)
Pivotal trials	Phase II trials • Kerem et al 2008 [43] • Sermet-Gaudelus et al 2010 [44] • Wilschanski et al 2011 [46] Phase III trials • Kerem et al 2014 [40] • ACT CF Trial (ClinicalTrials.gov identifier NCT02139306) [48]

Table 1. Different functional classes of *CFTR* pathogenic mutations and relevant therapeutic strategies.

Mutation class	**Nature of defect**	**Example**	**Relevant therapeutic strategies**
Class I	Protein synthesis	Gly542X	Read-through compounds (Ataluren)
Class II	Protein processing and trafficking	Phe508del	Corrector plus potentiator (Lumacaftor + Ivacafor))
Class III	Channel gating	Gly551Asp	Potentiator (Ivacaftor)
Class IV	Channel conductance	Arg117His	Potentiator (Ivacaftor)
Class V	Reduced protein production	3849 + 10 kb C → T	Non-approved strategies: Antisense oligonucleotides Correctors Potentiators
Class VI	High turnover of unstable protein at cell surface	c. 120del23	None available

Table 2. Summary of published clinical trials of ataluren in people with cystic fibrosis

Study	Kerem *et al* 2008 [43]	Sermet-Gaudelus *et al* 2010 [44]	Wilschanski *et al* 2011 [46]	Kerem *et al* 2014 [40]
Design	Phase 2 Non-randomised Open-label Single group assignment Crossover	Phase 2 Randomised Open-label Parallel assignment Crossover No placebo	Phase 2 extension Non-randomised Open-label Parallel assignment No placebo	Phase 3 multicenter Randomised Double-blind Parallel assignment Placebo-controlled
Sample size	23	30	19	238
Duration	2 x 28 day cycles Cycle 1: 16mg/kg/day for 14 days; no treatment for 14 days Cycle 2: 20mg/kg/day for 14 days; no treatment for 14 days	2 x 28 day cycles 14 days on treatment 14 days off treatment Cycle 1 treatment Either 16mg/kg/day or 20mg/kg/day Cycle 2 treatment Opposite regimen to that in 1st cycle	12 weeks Group 1 16mg/kg/day Group 2 20mg/kg/day	48 weeks Group 1 40mg/kg/day Group 2 Placebo
Inclusion criteria	Age ≥ 18 years Sweat chloride > 40mEq/L Nasal PD > -5mV 2 disease *CFTR* mutations, ≥1 nonsense FEV_1≥40% O_2 saturations ≥92% in air	Age 6-18 years Sweat chloride > 40mEq/L Nasal PD > -5mV 2 disease *CFTR* mutations, ≥1 nonsense FEV_1≥40% O_2 saturations ≥92% in air	Age ≥ 18 years Sweat chloride > 40mEq/L Nasal PD > -5mV 2 disease *CFTR* mutations, ≥1 nonsense FEV_1≥40% O_2 saturations ≥92% in air	Age ≥ 6 years Sweat chloride > 40mEq/L Nasal PD > -5mV 2 disease *CFTR* mutations, ≥1 nonsense 40%≤FEV_1≤90% O_2 saturations ≥92% in air

		Weight ≥25kg		Weight≥16kg
Primary outcome	CFTR activity (nasal PD)	CFTR activity (nasal PD)	CFTR activity (nasal PD)	FEV_1
Mean FEV1	Small increase in 1st cycle of treatment P=0.037	No improvement	No significant difference	No significant difference
Total chloride transport (measured by nasal PD)	Cycle 1 -7.1mV (p<0.0001) Cycle 2 -3.7mV (p=0.032)	Low to high dosing -4.6mV (p=0.037) High to low dosing -3.9mV (p=0.046)	Group 1 -6.8mV (p=0.004) Group 2 -3.4mV (p=0.025)	No significant difference
Sweat chloride	No change	-	-	No significant difference
Pulmonary exacerbations	-	-	-	Ataluren 1.42 Placebo 1.78 Rate ratio 0.77 (p=0.0992)
Weight	Mean change +0.6kg (p<0.0001)	No improvement	-	No significant difference

Figure legends

Figure 1. Schematic diagram illustrating different functional classes of *CFTR* mutation.
CFTR mutations are classified into 1 of 6 classes based on the mechanisms underlying CFTR protein dysfunction. Class I mutations lead to the formation of a truncated CFTR protein, Class II mutations result in dysfunctional CFTR processing and transport. Class III – VI mutations are expressed apically; however, Class III mutations feature defective CFTR gating, in Class IV mutations the CFTR channel pore is defective, Class V mutations result in a splicing defect limiting the amount of CFTR protein produced and Class VI mutations result in increased CFTR protein turnover.Figure adapted from Brodlie *et al*, 2015 [22]

Figure 2. Chemical structure of ataluren.

Figure 3. Schematic diagram of mechanism of action of ataluren.
In health **a)** full length CFTR protein is transcribed from the CFTR mRNA, processed and transported to the apical membrane of airway and other epithelial cells. At the apical membrane CFTR modulates chloride, and other, ion transport maintaining ASL homeostasis; promoting mucociliary clearance and anti-microbial activity inhibiting pathogen colonisation. Class I *CFTR* mutations (e.g. Gly542X) **b)** result in the translation of a truncated CFTR protein, due to nonsense, frameshift or insertion/deletion mutations introducing a premature stop codon. This truncated CFTR is non-functional and is rapidly degraded. The absence of CFTR at the apical membrane leads to a lack of chloride, and other ion, transport altering ASL homeostasis; leading to mucostasis, impaired anti-microbial activity giving rise to the pathology of CFLD. Ataluren **c)** is thought to interact with the ribosome to promote read-through of premature stop codons. In Class I *CFTR* mutations this read-through capacity is proposed to facilitate the translation of full length, functional CFTR, thereby restoring a proportion of its apical activity.

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