Cochrane systematic reviews of mutation specific therapies for patients with cystic fibrosis

1. CFTR potentiators (specific therapies for class III and IV mutations) in CF.
2. CFTR correctors (specific therapies for class II mutations) in CF.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master in Philosophy

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I. Abstract

Background

Cystic Fibrosis (CF) is the most common inherited condition amongst people of European descent. It is caused by a mutation in the CFTR gene located on chromosome 7, and codes for the CFTR protein. Mutations in the CFTR gene lead to the characteristic features of CF, namely progressive and irreversible lung disease which is responsible for the majority of premature deaths in CF. Recently, research into the management of CF has shifted towards personalised genomic medicine. New classes of drugs called mutation-specific-therapies have been designed to target the mutation specific defects in CFTR protein synthesis or function. They have shown to restore the gating defect of class III mutations (CFTR potentiators) and intracellular processing defect of class II mutations (CFTR correctors) in cell studies. Both CFTR potentiators and CFTR correctors have progressed to human clinical trials. Cochrane systematic reviews are the gold standard for establishing the current evidence base for interventions.

Objective

To conduct two Cochrane systematic reviews to evaluate the benefits and harms of 1) CFTR potentiators (for class III-IV mutations) and 2) CFTR correctors (for class II mutations) on clinically important outcomes in children and adults with CF.

Methods

Systematic reviews were conducted according to peer assessed and published review protocol. Relevant studies were identified by searching the Cochrane Cystic Fibrosis Trials Register, compiled from electronic database searches and hand searching of journals and conference abstract books. We also searched the clinical trial registries maintained by the EMA, the US NIH and the WHO and contacted leading researches and industry for relevant trials. We included only RCTs of parallel design that met pre-defined eligibility criteria. Two authors independently extracted data and assessed the risk of bias in included trials. If appropriate, data were combined in a meta-analysis.

Results

Studies with patients with the G551D mutation demonstrated a clinically relevant impact of Ivacaftor on outcomes at 24 and 48 weeks providing evidence for the use of Ivacaftor (CFTR potentiator) in patients with G551D mutation (Class III). There is no evidence to support the short-term use of CFTR correctors, CFTR potentiators, or both as combination therapy in patients with the ΔF508 mutation (class II mutation). As patients with Class II mutations comprise a significant proportion of all CF patients, identifying an efficacious CFTR corrector (or CFTR corrector and CFTR potentiator combination) will have a profound impact on the field.

II. Dedications

I would like to dedicate this Master's thesis to all patients, families and friends whose lives are affected by Cystic Fibrosis.

III. Acknowledgements

This master's Thesis would not have been possible without the help and support from my supervisors, family and friends.

First and foremost, I would like to thank Dr Kevin Southern my primary supervisor who provided me with the opportunity to conduct these systematic reviews, and for the help and support throughout the year. I would also like to thank Dr Ian Sinha for his constant support and encouragement.

I would also like to thank Nikki Jahnke, Kerry Dwan and Natalie Hall from the Cochrane Cystic Fibrosis and genetic disorder groups for their continued advice throughout the year. Despite my persistent e-mails and nagging, they were always happy to help and guide me the right direction.

Finally I would like to thank my family and friends for their support throughout the year.

IV. Overview of papers

This MPhil thesis is based on the following three papers:

Paper 1: Sinha Ian, P., et al. (2014) Correctors (specific therapies for class II CFTR mutations) for cystic fibrosis (Protocol). Cochrane Database of Systematic Reviews DOI: 10.1002/14651858.CD010966

Paper 2: Sinha Ian, P., et al. (2014) Potentiators (specific therapies for class III and IV mutations) for Cystic Fibrosis. Cochrane Database of Systematic Reviews (unpublished)

Paper 3: Patel Sanjay, et al (2014) Correctors: Correctors (specific therapies for class II mutations) for Cystic Fibrosis. Cochrane Database of Systematic Reviews (unpublished)

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VI. Table of Abbreviations

ASL	Airway Surface Liquid
cAMP	Cyclic adenosine monophosphate
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Regulator
CFQ-R	Cystic Fibrosis Questionnaire – Revised
CPX	8-cyclopentyl-1,3-dipropylxanthine
DIOS	Distil Intestinal Obstruction Syndrome
EMA	European Medicines Agency
EPI	Exocrine Pancreatic Insufficiency
ER	Endoplasmic reticulum
ENaC	Epithelial sodium channel
ERAD	Endoplasmic reticulum associated degradation
FEV_1	Forced expiratory volume in 1 second
HRQoL	Health related Quality of Live
HS	Hypertonic Saline
IL	Interleukin
MI	Meconium ileus
NBD	Nucleotide Binding Domain
MPhil	Masters in Philosophy
NIH	National Institute of health
NPD	Nasal Potential Difference
PRO	Patient Reported Outcome
P. aeruginosa	*Pseudomonas aeruginosa*
QoL	Quality of Life
WHO	Wold Health Organisation
UPS	Ubiquitin Protease System
WT	Wild-type (referring to Wild type CFTR)
US NIH	United States National Institute of Health
95%CI	95 percent confidence interval
4PBA	Sodium phenylbutyrate

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Chapter 1

Introduction

1.1 Cystic Fibrosis

Cystic Fibrosis (CF) is the most common genetically inherited condition amongst individuals of European descent with a carrier status of 1 in 25 individuals (occurs in 1 in 2500 births). An estimated 28, 804 people in the US and 8284 people living in the UK are currently affected by this disorder.[1, 2] It is caused by a mutation in the gene that codes for the CFTR protein. This protein is expressed throughout the body and when defective causes the characteristic phenotypic consequences of CF, namely an elevated sweat chloride concentration, recurrent lung infections and bronchiectasis. Despite outstanding advances in management of CF, patients with CF continue to die prematurely. In the majority of CF patients, chronic infection with *P. aeruginosa* leads to progressive and irreversible airway obstruction which, with time leads to the loss of lung function and respiratory failure.[3] In the UK, CF has a mean life expectancy of 43.5 years.[4]

1.1.1 Progression of research in CF

Since the discovery of CF, advances in characterisation of its pathophysiology and in its management have been remarkable and this is demonstrated by the improved life expectancy of affected individuals. Statistics released by the Cystic Fibrosis Foundation show that since 2002 the median survival age of a patient with CF has increased by almost 10 years from age 31.3 in 2002 to 41.1 in 2012.[2]

The history of CF can be better discussed if divided into three eras; pre gene discovery, gene discovery and post gene discovery.[5]

Pre gene discovery

The pre gene discovery era spans half a century and begins in 1938 when Dorothy Anderson, a paediatric clinician working in New York, was the first to recognise the pathology and disease pattern of CF. She identified and described mucus plugging of glandular tissues in the pancreas and recognised this as a distinct pathological process to coeliac disease.[6] Her pioneering work did not stop there. She then investigated the aetiology of CF and recognised it to be an autosomal recessive disorder .[7] At this stage the prognosis of CF was extremely poor with many children dying in their first year of life. In 1949, Lowe postulated that because CF is inherited in an autosomal recessive pattern the defect must lie with a single gene.[8] But before the putative gene was identified, the

molecular basis of the disease was characterised when high levels of salt were detected in the sweat of patients with CF, on a hot New York day.[9] This discovery instigated development of the diagnostic sweat chloride test through pilocarpine iontophoresis by Gibson and Cooke and also helped distinguish milder CF phenotypes.[10] Sweat salt concentration remains an important diagnostic test even today. In 1983, Quinton isolated sweat glands and demonstrated that chloride ion channels were unusually impermeable to chloride ions.[11] Defective chloride conductance and increased sodium reabsorption were then identified at the apical membranes of epithelial cells lining the airways, characterising the molecular defect of CF.[12, 13] At this point in the history of CF, the three pillars of CF management; nutritional repletion, relief of airway obstruction and antibiotic therapy of lung infection were established.[14]

Gene discovery

In 1989, the putative 'Cystic Fibrosis Transmembrane Regulator' gene (CFTR gene) was discovered through innovative chromosome identification techniques (chromosome 'walking and jumping').[15-17] It was located on chromosome 7 and coded for the CFTR protein; a cAMP-regulated chloride ion channel located at the apical surface of epithelial cells. Defects in the CFTR gene resulted in either insufficient amounts of CFTR protein within the cell membranes or in CFTR that embedded in the cell membrane but displayed defective ion conductance.[15-17]

Post gene discovery and the era of personalised genomic medicine

23 years post gene discovery, the mainstay of CF management is still focused on aggressive symptomatic management. However research into the putative gene has provided the CF community with a greater understanding into the how the gene influences CFTR production, expression and function. With this knowledge, new personalised therapies targeting the underlying defect of CF have started to emerge, igniting an exciting era of personalised genomics in CF.[18]

Before we can understand how these therapies aim to work, we must appreciate how normal CFTR protein (also known as Wild-type (WT) CFTR) is synthesised and functions, how this is affected by mutations in the CFTR gene, and the impact of CFTR gene mutations on the body.

1.1.2 CFTR synthesis, structure and function

The production of CFTR from gene to protein

The CFTR gene is located on the long arm of chromosome 7 at position q13.2. The process of CFTR synthesis and maturation is highly monitored as it is subject to many sites of quality control.[19, 20] Extracellular signals stimulate transcription of the CFTR gene into single stranded messenger RNA (mRNA) in the nucleus of epithelial cells located in sweat ducts, airways, pancreatic ducts, intestine,

biliary tree and vas deferens. Single stranded mRNA escapes the nuclear pores and is translated by ribosomes situated principally in the endoplasmic reticulum (ER) but also in the cell cytoplasm. With transfer RNA (tRNA) donating amino acids, a fully extended 'nascent' CFTR glycoprotein is produced. Further maturation involves a process of folding in the lipid bilayer of the ER. This process however is inefficient. Approximately 60-75% of CFTR are improperly folded and are subject to degradation via the ER-associated degradation (ERAD) system. This is a quality control system involving various molecular chaperones and ensures only properly folded CFTR progress.[21] What specifically happens is that the misshapen CFTR are detected by the Ubiquitin Protease System (UPS) (part of the ERAD) which signals the 26S proteasome system to eliminate all defective CFTR. It is however possible for misshapen CFTR to escape this degradation process. Once nascent CFTR has successfully progressed (or escaped) beyond this stage, it undergoes further post-translational modification in the Golgi body before being trafficked to the cell membrane in vesicles. At the cell membrane, WT CFTR has a half-life of 12-24 hours and is subject to on-going quality control. The plasma membrane protease quality control system either recycles CFTR back to the plasma membrane or commits CFTR to degradation by lysosomes.[19] Approximately 10-35% of CFTR cellular activity is required to prevent significant morbidity in CF.[22]

Structure of CFTR

CFTR is a member of a larger group of ATP-binding cassette transporters of which CFTR is the only to function as an ion transporter.[23] It is composed of five domains comprising two homologous halves. There are two intracellular nucleotide binding domains (NBD1/NBD2) that each contribute an ATP-binding sites, two membrane spanning domains (MSD1/MSD2) that form the channel pore and a central intracellular regulatory 'R' domain that is susceptible to phosphorylation (Figure 1)[24]. Phosphorylation of the 'R' domain by cAMP dependent protein kinases, principally protein kinase A induces further binding of ATP to both NBD sites. These sites therefore influence the activation state of the channel and ion gating through CFTR.[19, 25] It remains unclear whether ATP hydrolysis is required at both sites (NBD1 and NBD2) for CFTR channel activation.[26]

Figure 1 Structure of CFTR

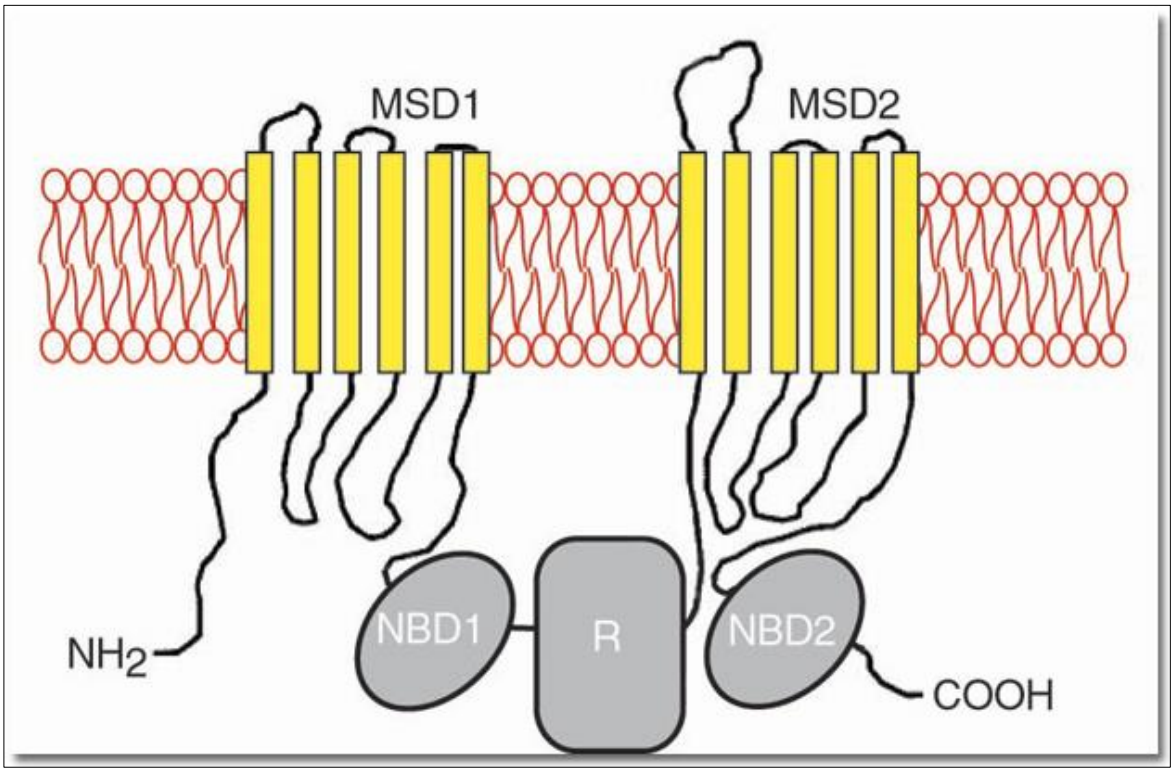


Figure 1: A diagram of CFTR. Starting inside the cell, there are two nucleotide binding domains (NBD1/NBD2) with a centrally located 'R' domain between them. Within the cell membrane lies the membrane spanning domains (MSD1/MSD2). When the nucleotide binding domains and R domain are activated, the channel pore is activated. Dephosphorylation of the R domain causes subsequent closure of the channel.[24]

Function of CFTR protein

CFTR functions as a chloride ion transporter at the apical cell membrane of epithelial cells located in the airways, pancreas, liver, intestine, sweat glands and vas deferens (Figure 2).[27, 28] Phosphorylation with cAMP and binding of ATP induces activation of the CFTR channel pore and results in the movement of chloride ions out of the epithelial cells.[29] CFTR is also responsible for regulating ENaC; a sodium transporter channel and bicarbonate transporter channels. The functions of CFTR maintain salt, fluid and pH balance within the cell.[30, 31] CFTR has also been shown to influence its host's susceptibility to *P. aeruginosa* lung infection.[31]

Figure 2 Diagrammatic representation of CFTR Function

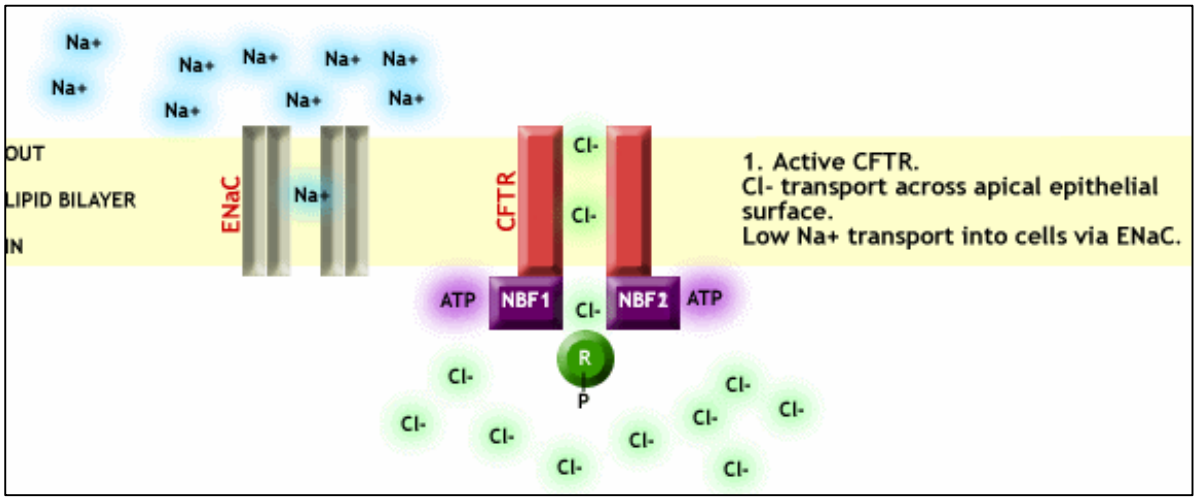


Figure 2: A diagrammatic representation of normal CFTR function. Activation of the CFTR channel pore results in the movement of chloride ions out of the apical epithelial cells. CFTR also down regulates ENaC, a sodium transporter channel. Under normal circumstances, there is minimal transport of sodium ions into cells.[27]

1.1.3 Classification of CFTR Gene mutations

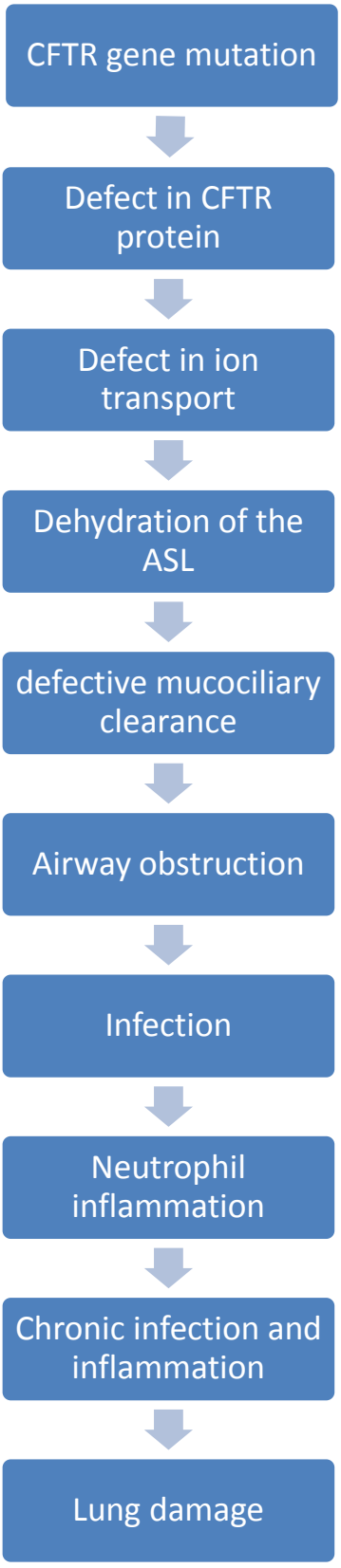
Mutations in the CFTR gene affect either the synthesis or function of CFTR (or both) and are responsible for initiating a cascade of events that ultimately leads to the morbidity and mortality associated with CF (Figure 3). To date, over 1500 variations of the CFTR gene have been identified. The majority (80%) of these are represented by six mutation classes which are based on whether the mutations in the CFTR gene impair 1) mRNA translation 2) CFTR processing and folding or 3) chloride channel gating (Figure 5).[32, 33] Some mutations, for example the ΔF508 mutation, exhibit multiple defects and therefore can be characterised by more than one mutation class.

Class I

Class I mutations are characterised by nonsense, frameshift and splicing mutations that prevent CFTR biosynthesis. These mutations result in the presence of an early stop codon (UAG, UAA or UGA) within the mRNA nucleotide sequence which has one of two resultant consequences: either a truncated protein or mRNA that cannot be translated (unstable mRNA). The truncated proteins or unstable mRNA are degraded by the ERAD before they can be trafficked to the cell membrane. Therefore patients with only class I mutations do not express functional CFTR at cell membranes, and demonstrate the most severe CF phenotypes.[32, 34]

Class 1 mutations are designated an X (e.g. W1282X) and account for approximately 10% of loss-of-function alleles worldwide.[19] W1282X mutation accounts for slightly greater than 1% of CF cases worldwide but has a vastly increased frequency (greater than 50%) amongst Israeli Ashkenazi Jews.[35]

Figure 3 Pathophysiologic cascade of CF Lung disease



Class II

Class II mutations can be represented by the common ΔF508 -CFTR mutation. This loss-of-function mutation accounts for 70% of homozygous patients and at least one allele of 90% of heterozygotes, making it the most common loss of function allele in Caucasians.[36, 37] The ΔF508 mutation causes deletion of three base pairs (T-A-G) that code for the amino acid Phenylalanine at position 508 of the CFTR glycoprotein (Figure 4). Loss of Phenylalanine generates a misfolded protein, which is mostly (99%) identified by the ER quality control system, tagged with Ubiquitin and degraded by protease.[38] This is known as the intracellular processing defect and is characteristic of class II mutations. With ΔF508-CFTR minute amounts of have been shown to escape the degradation system and are expressed on the apical cell membrane of epithelial cells.[39] Here however, they display defective anion transport (like class III/IV mutations) and have been demonstrated to have reduced half-lives (<4hours) due to degradation by the plasma membrane protease quality control system.[38, 40]

Figure 4 Site of ΔF508 mutation

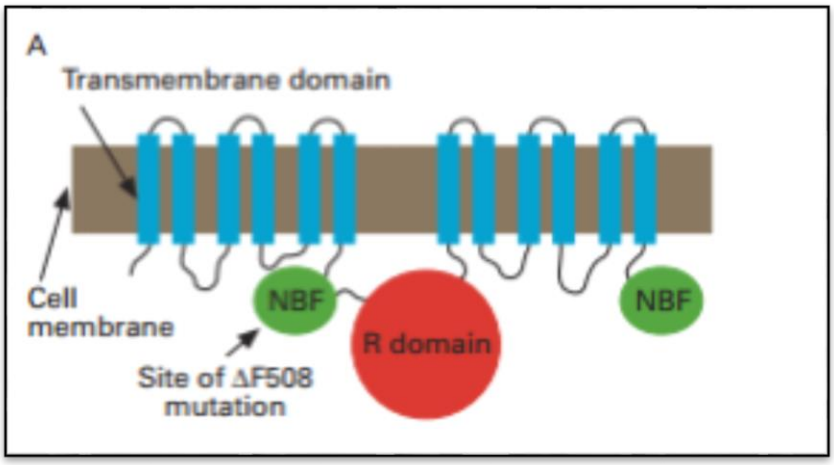


Figure 4 demonstrates the structure of the CFTR protein, and the site of ΔF508 mutation within the nucleotide Binding Fold (also known at the nucleotide binding domain).[37]

Class III

Class III mutations are characterised by a disrupted pattern of channel gating. The CFTR protein products of class III mutations are synthesised, trafficked and embedded within the cell membrane normally, but fail to respond to normal intracellular cAMP stimulation. This prevents phosphorylation of the 'R' domain and binding of ATP to the NBDs, reducing activation of the channel pore.[41] Therefore, Class III CFTR can only function minimally.

The G551D mutation is the most common Class III mutation, and the third most common CFTR mutation, accounting for 4% of loss-of-function alleles worldwide.[24] It is caused by the substitution

of glycine for aspartic acid at codon 551 found in NBD1 and results in the inability of NBD to utilize ATP.[42] The G551D mutation is the first, and only current, allele that has a licensed mutation specific therapy in the UK.[43]

Class IV

Class IV mutations are characterised by compromised chloride ion conductance. The protein product of class IV mutations are embedded within the apical cell membrane of epithelial cells and respond normally to intracellular stimuli, however demonstrate either 1) reduced permeation to chloride ions or 2) reduced channel opening time, meaning chloride ion conductance is significantly reduced.[44]

The R117H mutation is a class IV CFTR mutation. During protein synthesis, arginine is substituted for histidine at position 117 and this results in a reduced channel opening time. It has a worldwide frequency of approximately 2% amongst patients with CF.[24]

Class V

Class V mutations are characterised by errors in RNA splicing resulting in fewer mRNA transcripts and reduced quantity of functional CFTR.[44] Gene splicing mutations accounts for 11.6% of known mutations in the CFTR mutation database but are rarely found in patients with CF (<1% of patients with CF).[24]

Class VI

The protein product of class IV mutations are truncated leading to increased cell surface turnover and degradation.[45]

1.1.4 Summary

Cystic Fibrosis is the most commonly inherited condition amongst people of European decent. It is caused by mutations in the CFTR gene, located on chromosome 7. Under normal circumstances the CFTR gene codes for a CFTR protein product responsible for maintaining ion, fluid and pH balance within the cell. Mutations of the CFTR gene lead to inadequate amounts of CFTR or defective CFTR function (Figure 5) and are therefore responsible for clinical features on CF (Figure 3). The mutation classes are summarised in Table 1.

Figure 5 A diagrammatic representation of how mutation classes affect CFTR protein synthesis or function.

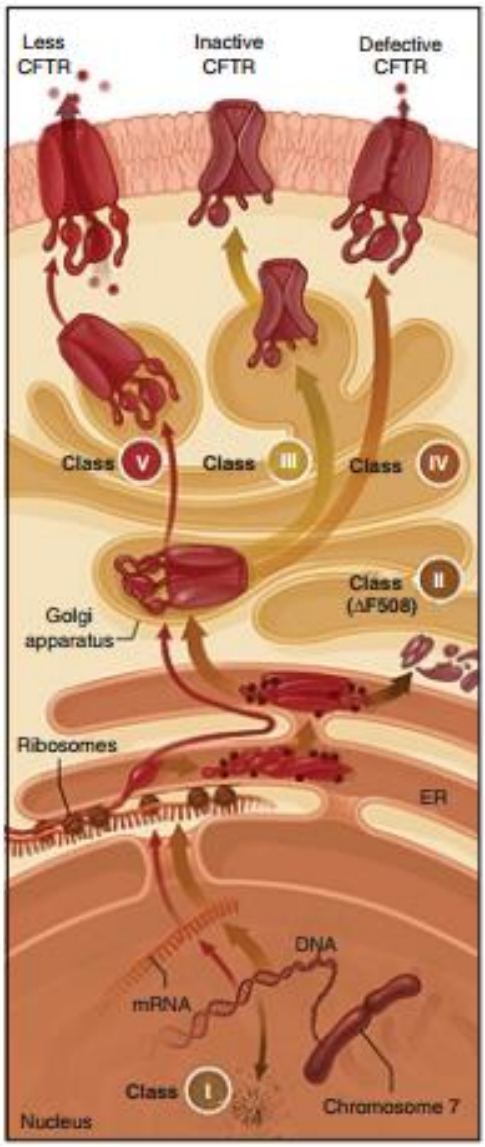


Figure 5: A diagrammatic representation of how CFTR mutation classes affect CFTR synthesis or function. Class I mutations are characterised by the presence of a premature stop codon in the mRNA sequence, coding for a truncated or unstable CFTR protein. Class II CFTR proteins are folded incorrectly whilst in the ER bilayer and subsequently degraded by ERAD. Unlike Class I-II mutations, Class III-VI CFTR are synthesised and trafficked to the cell membrane normally. At the cell membrane however, Class III CFTR demonstrate defective response to normal stimuli and class IV CFTR demonstrate inactive chloride ion gating. Class V mutations are characterised by splicing mutations, whereby reduced functional CFTR is trafficked to the cell membrane. Class VI CFTR is subject to increased cell surface turnover and degradation at the cell membrane.[33]

Table 1 Summary of the CFTR gene mutations

	Example mutation	Mutation type	Pancreatic function and phenotype	Worldwide frequency	Nature of the defect
Class 1	W1282X	Nonsense	Insufficient	10% worldwide (>50% Ashkenazi Jews with CF possess W128X allele)	Defect in Biosynthesis – no functional CFTR expression
Class 2	ΔF508	Missense (amino acid deletion)	Insufficient	70% homozygous and at least one allele in 90% of heterozygotes.	Intracellular trafficking defect – minimal functional CFTR expression
Class 3	G551D	Missense (amino acid substitution)	Insufficient	4%	Defect in regulation by phosphorylation and ATP. – minimal CFTR expression
Class 4	R117H	Missense (amino acid substitution)	Sufficient	2%	Defect in chloride ion conductance – residual CFTR function
Class 5	3849+10kb C->T	Missense (amino acid substitution)	Sufficient	<1%	Reduced synthesis of functional CFTR - variable CFTR function
Class 6	Q1412X				Increased cell surface turnover and degradation of CFTR

1.2 Pathophysiology and clinical features of CF

In CF, pathological changes are seen in all organs that express defective CFTR. The most profound impact of defective CFTR is demonstrated in the lungs, which are responsible for the majority of mortality associated with CF. The pathological process of airway disease starts with dehydration of the lining of the lungs (Figure 3).

1.2.1 Airway disease

Dehydration of ASL

The airway surface liquid (ASL) lining the airways is composed of two layers; an inferiorly positioned pericilliary layer and a superiorly positioned hydrated mucus layer. The mucus layer is mainly composed of water (98%) and facilitates distal to proximal shifting of surface components by the action of cilia; a process known as the mucociliary clearance system. This system is dependent on the hydration status of the mucus layer and is crucial for eliminating invading pathogens.[46]

In CF, there are two theories behind the progression of airway disease from defective CFTR to chronic infection and inflammation; the 'low salt' theory and the high salt theory.' The 'low salt' theory is the most well established theory. Here, defective CFTR is unable to down regulate ENaC permitting the transepithelial movement of sodium ions from the ASL back into the cell. Water then follows by osmosis leaving the mucus layer dehydrated. This has a detrimental impact on mucociliary clearance. Thick viscous mucus, secreted by submucosol glands and goblet cells cannot be shifted and therefore remains stagnant in the airways. This obstructs the airways and makes them vulnerable to repeated infections and increased contact time with bacteria.[47]

The 'high salt' theory suggests that defective CFTR creates a high concentration of salt in the ASL. This is proposed to predispose CF airways to infection by reducing the effectiveness of antimicrobial peptides within the ASL and disrupting mucociliary transport.[47]

Infection - Airway microbiology

Dehydration of ASL increases the propensity of the CF airways to be infected by bacteria. This is evident as early as infancy and early childhood when a large proportion of patients develop intermittent infections with *P. aeruginosa* and other bacteria such as *Staphyloccocus aureus (S. aureus)* and *Haemophilus influenza (H. influenza)*. When treatment regimens fail to eradicate the infection, often following repeated intermittent infection, the patients are said to have developed chronic infection.

Chronic *P. aeruginosa* dominates the CF airways and is detected in more than 50% of UK CF patients by the age of 20-23 years. The change in prevalence of infective organisms identified from CF airways with increasing age is demonstrated in Figure 6.[48]

The increased propensity of *P. aeruginosa* to chronically infect CF airways is multifactorial. *P. aeruginosa* has the ability to mutate in response to environmental stresses (for example hypoxia created by a mucus plug) into mucoid variants, during the years following initial colonisation. Mucoid variants produce alginate, which surrounds them and protects them from external challenges such as mucociliary clearance, host immune response and antibiotic agents. Therefore conversion to the mucoid phenotype allows for persistent infection that is difficult to eradicate.[49] The high prevalence of *P. aeruginosa* in CF lungs has also been associated with the lack of wild-type (normal) CFTR. Under normal circumstances wild-type CFTR receptors are thought to bind to *P. aeruginosa* antigens and stimulate internalisation of the pathogen by epithelial cells.[50] Mutant CFTR is unable to bind to *P. aeruginosa* so it is allowed to freely reside within the CF airways. In addition to this, *P. aeruginosa* also forms biofilms, which make the prospect of its eradication even more challenging.[51] Chronic infection with *P. aeruginosa* is associated with a faster decline in lung function, worse nutrition and ultimately reduced survival rates.[52]

Figure 6 Prevalence of infective organisms

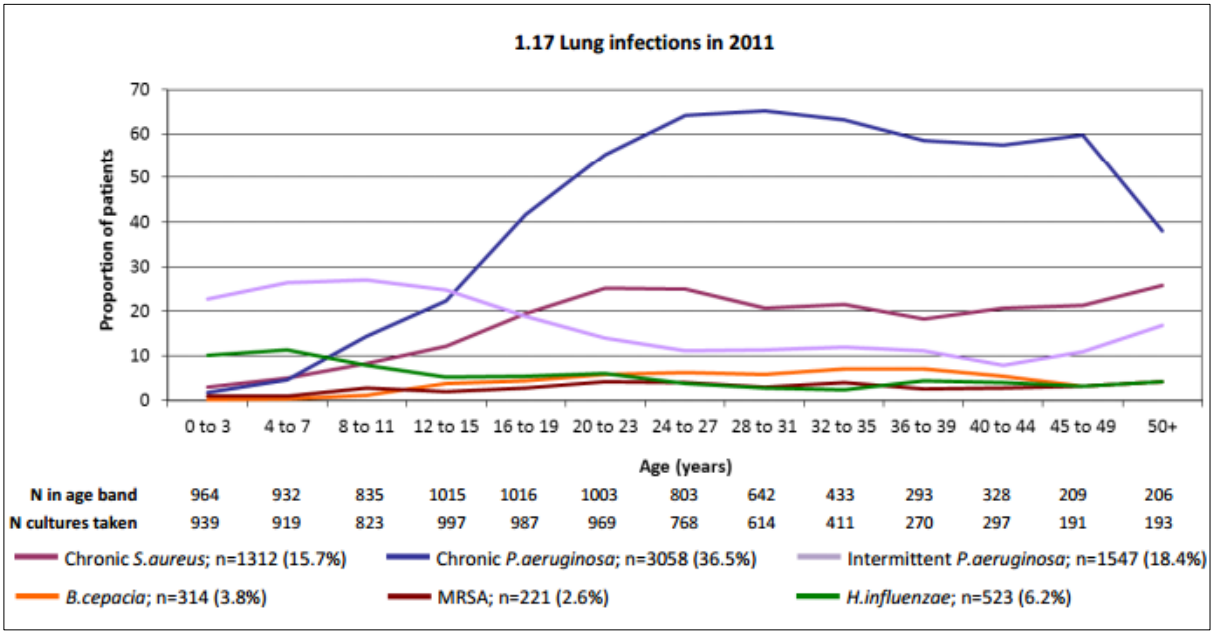


Figure 6 demonstrates the change in prevalence of infective organisms identified from CF airways with increasing age. It is evident from this graph that from approximately 16-19 years of age, chronic infection with *P. aeruginosa* dominates the CF airways.[48]

Airway inflammation

In chronically infected CF airways, the picture is that of a prolonged primary inflammatory response.[53] Neutrophils predominate and are the principal inflammatory cells responsible for tissue damage and disease progression in CF.[54] They secrete various pro-inflammatory cytokines (including interleukin (IL) -8, IL-6 and IL 1**β)**, oxidants and proteases (such as neutrophil derived elastase (NE)) that damage the lungs. Neutrophil cytokines and oxidants injure the respiratory epithelium, and the former incite further neutrophil accumulation at the site of infection. Proteases also weaken airway structures by digesting structural proteins.[55] The presence of NE in sputum or serum is associated with a faster lung function decline and onset of bronchiectasis.[56] Under normal circumstances, antiprotease defences in the airways oppose the effects of proteases. Protective antioxidants prevent free radicals placing oxidative stress on airways and anti-inflammatory factors, such as IL-10, oppose the action of pro-inflammatory factors. In the CF airways however, the effect of these protective factors are overwhelmed, leading to the excessive and continuous inflammatory response seen in patients with CF.[54, 55]

Chronic infection by bacteria is the leading trigger of lung inflammation in CF and as the bacterial burden increases the inflammatory response intensifies. Chronic infection with *P. aeruginosa* in particular elicits an aggressive inflammatory response; upon infection with *P. aeruginosa* cells lacking functional CFTR secrete higher amounts of IL-1, IL-8, tumour necrosis factor (TNF)-a and macrophage inflammatory protein (Mip)-2.[57] Viruses also contribute to airway inflammation. They pre-dispose the airways to bacterial infection and change the antibiotic resistance pattern which incites a greater inflammatory response.[58]

It is well recognised that lung inflammation starts early and that CF airways deficient of WT CFTR exhibit a pro-inflammatory state and over-respond to infectious agents.[59] The relationship between infection and inflammation however is hazy. It was previously considered that airway infection initiated the process of inflammation but there is emerging evidence demonstrating dissociation between infection and inflammation.[60]

Lung damage

As a consequence of chronic infection and inflammation, patients with CF may develop bronchiectasis and episodes of pulmonary exacerbations.

Bronchiectasis

Patients with cystic Fibrosis develop bronchiectasis, defined as the abnormal dilation of the bronchi due to the loss of elastic and muscular components of the wall. It is considered to be a result of destruction of elastin, muscle and cartilage by proteases from neutrophils during inflammation.

Patients with bronchiectasis develop increased mucus production and persistent lower respiratory tract infection.[61]

Pulmonary exacerbations

CF patients with chronic airway infection and inflammation usually exhibit on-going symptoms such as cough and sputum production, but some may be symptom free. Episodes of worsening symptoms or new symptoms that require additional antibiotic therapy are termed 'exacerbations.' These episodes are intermittent and unpredictable and occur due to the presence of chronic lower respiratory infection, usually *P. aeruginosa*. In many cases they are triggered by additional factors, for example non adherence to maintenance treatment or viral infection (RSV, Influenza).[62] Pulmonary exacerbations are associated with worse pulmonary function and a more rapid rate of lung function decline and also have a significant detrimental impact on psychological health of the patient.[63-65]

Other complications

In addition, patients can develop other life threatening airway complications that must be recognised early and appropriately management. These include: haemoptysis, pneumothorax and respiratory failure.[66]

1.2.2 Pancreatic, hepatobiliary and GI tract manifestations

CFTR is also expressed throughout the entire GI system, including the pancreatic (exocrine and endocrine) and the hepatobiliary systems. In these systems, the picture is similar to that of the CF airways whereby thick, viscous mucus obstructs lumen lined by epithelial cells possessing defective CFTR. The most pronounced effects of defective CFTR occur in the intestines, pancreas and hepatobiliary system which carry their own risks and also contribute to the poor nutritional status seen in patients with CF.

CF Intestine

Meconium ileus (MI), Distil Intestinal Obstruction Syndrome (DIOS) and constipation form the obstruction syndromes that burden the CF intestines. They are all consequences of increased viscosity of luminal mucus and reduced transit time.[67] MI is characterised by thick, adhesive and dehydrated meconium that completely obstructs the intestinal lumen and fails to pass. It occurs in 20% of CF infants, with the majority of cases (13-17% of CF infants) present at birth. Once diagnosed, prompt management is required to reduce the risk of bowel perforation and sepsis.[68] After neonatal/infancy period, DIOS emerges as the major cause of obstruction. It can be either complete or incomplete faecal obstruction of the ileocecum and occurs more commonly in adults than

children.[67] In the long term, thick viscous mucus within the lumen of the intestines contributes to development of constipation and contributes to malabsorption of nutrients.

CF Exocrine and Endocrine Pancreas

Pancreatic exocrine insufficiency (PEI) is responsible for the majority of malnutrition in people with CF. It affects 85-90% of the CF population and is characterised by inadequate secretion of digestive enzymes and alkaline fluid.[69] Consequently, people with PEI are burdened by maldigestion, malabsorption and increased energy loss, in particular of fats, which clinically manifests as steatorrhoea in the short term and the consequences of malnutrition in the long term long term.

The pathophysiology EPI relates to the short and long term effects of defective CFTR. Within the pancreatic ducts, mucus containing a high concentration of digestive enzymes is created.[70] This obstructs the ductal lumen and prevents digestive enzymes and alkaline fluid from being secreted. After prolonged stagnation of mucus within the ducts, the digestive enzymes (present in high concentrations) start to degrade secretory acinar cells and lead to fibrosis of the pancreas (hence "cystic fibrosis of the pancreas") and fatty infiltration.[71]

When progressive acinar fibrosis and fatty infiltration progress to the islet of Langerhans cells, patients with PEI develop CF related diabetes (CFRD).[72] The diagnosis of CF is commonly made around 20 years of age and population studies have demonstrated that 76% of CF patients have CFRD by the age of 30.[72]

CF Hepatobiliary system

CFTR is expressed at the apical membrane of cholangiocytes and gall bladder epithelial cells but not hepatocytes. Therefore hepatic damage in CF is a consequence of pathological processes occurring in the biliary system.[73] Mucous that obstructs the biliary lumen leads to 1) injury of bile duct cells 2) bile duct plugging making the bile duct epithelium susceptible to destruction by cytotoxic agents secreted in bile and 3) malabsorption of fats. After long-standing biliary obstruction and progressive fibrosis, focal biliary cirrhosis develops. This process burdens a third of patients with CF and is becoming more prevalent as patients with CF live longer.[74] CF hepatobiliary disease has a negative impact on nutritional status through malabsorption, has a detrimental impact on long term lung function and carries its own risk for mortality.[74]

Nutritional status

Reduced nutrient intake, malabsorption, increased loss of nutrients and increased energy expenditure all contribute to worsen nutritional status in patients with CF.[75] Poor nutritional status during childhood is associated with a failure to grow, developmental delay, reduced quality of life and reduced pulmonary function when older.[76]

1.2.3 Other clinical manifestations of Cystic Fibrosis

Sweat Glands

In the CF sweat glands, there is reduced re-absorption of sodium, chloride and potassium ions due to the absence or dysfunction in CFTR. The resultant salty skin is characteristic of CF and sweat chloride levels exceeding 60 mmol/L is diagnostic of CF.[77]

Reproductive System

The majority of males with CF have congenital bilateral absence of the vas deferens (CBAVD). This is a developmental defect whereby transport of spermatozoa from the testes or epididymis to the vas deferens is blocked. The resultant azoospermia means that 99% of males are impotent.[78] Women on the other hand with CF have structurally normal reproductive systems. With aggressive nutritional and airway management and normal hormonal levels, CF women should develop into sexually mature adults. The only possible obstacle to successful fertilisation is thickened cervical mucus.[79]

CFTR-related disease

Single organ conditions that have been related to CFTR mutations are called CFTR-related diseases. They have been given a separate classification to typical and atypical CF as patients do not show the classic or mild phenotypes of CF, yet are single organ disorders caused by CFTR related mutations. Disorders include bilateral absence of the vas deferens, idiopathic pancreatitis and allergic bronch0-pulmonary aspergillosis.[46]

1.2.4 Genotype – phenotype correlation

There is an association between different mutation classes and phenotype.[80] These phenotypes are based on individuals who possess the most common mutations; ΔF508 mutation, present in 90% of individuals; G551D, W1282X, G542X and N1303K present in 1-4% of CF individuals and approximately 20 more that have a frequency of >0.1%.[81] The clinical consequences of specific well characterised mutations are outlined on the Clinical and Functional Translation of CFTR (CFTR2) website (www.cftr2.org).[82] It is difficult to characterise phenotypes for the remaining genotypes that have the propensity to cause disease because of their relatively low frequency, however emerging studies seek to explore these mutations by sourcing data directly from well phenotyped subjects.[83]

CFTR mutations can be categorised into mild or severe depending on disease severity (Table 2).[80, 84] The main differences in phenotype between patients with mild and severe genotypes appears to be linked to pancreatic status, as pancreatic function has been shown to correlate well with CFTR genotype, unlike pulmonary function.[80] Mild genotypes correspond to pancreatic exocrine sufficiency and more severe phenotypes, such as being homozygous for the ΔF508-CFTR mutation,

correspond to PEI.[80] This difference in severity between genotypes correlates with the amount of functional CFTR expressed in the cell membrane (Table 1, Table 2).[34]

Despite this, CFTR genotypes alone cannot be used to determine phenotypes which are subject to much heterogeneity. Patients with identical genotypes have been shown to display variable phenotypes, suggests that the influence of CF phenotypes is most likely multifactorial.[85] Environmental factors (bacterial infection, pollution) and/or modifier genes have been proposed to have a role.[85, 86]

Table 2 Phenotypic characteristics of mild and severe CFTR genotypes

Severe CFTR mutations (classes 1-3)	Mild CFTR mutation (classes 4-6)
Pancreatic insufficiency (>95% of cases)	Pancreatic sufficiency
Liver disease (3-5% of patients)	
Previously young age of diagnosis (Early onset of clinical manifestations)	Later age of diagnosis (>10 years of age) (Late onset of clinical manifestations)
High sweat chloride concentration (>80 mmol/L)	Lower sweat chloride levels
Meconium ileus (approximately 20% of cases)	No meconium ileus
	Milder pulmonary disease

1.2.5 Summary

CF airways are obstructed by thick, viscous mucus that cannot be cleared due to defective mucociliary clearance. This creates an ideal environment for infective agents, namely bacteria to reside and chronically infect the airways. One pathogen in particular, *P. aeruginosa* has an increased propensity to chronically infect the CF airways. This chronic infection of the CF airways is dominated by neutrophil inflammation typically seen in an acute infection. Neutrophil inflammation is progressive and irreversible and ultimately manifests as bronchiectasis and recurrent episodes of pulmonary exacerbations.

Malabsorption of nutrients occurs in a large proportion of the CF population (90%) and is mainly due to pancreatic exocrine insufficiency, but also due to obstruction within the intestines and biliary system. Disease processes in these systems also pose their own risk on the survival of patients with CF.

Certain genotypes have been associated with more severe clinical manifestations (www.cftr2.org), and this is probably due to the amount of functional CFTR that is expressed. Despite this, patients

with the same genotypes often demonstrate different disease severities, suggesting the influences of CF phenotypes are most likely multifactorial.

1.3 Management of Cystic Fibrosis

The management of CF involves a multidisciplinary approach from a specialist CF team, usually at a specialist CF centre. This consists of specialist CF clinicians, nurses, dieticians, physiotherapists, pharmacists, clinical psychologists and microbiologists all working together to promote CF health.[68] The management of CF involves aggressive treatment of airway disease and poor nutrition status and can be very burdensome to the lives of patients.

1.3.1 Airway management

In order to limit the progression of CF airway disease, current therapies target steps in the pathogenesis demonstrated in Figure 3. This includes; restoration of the hydration status of ASL, promotion of airway clearance, eradication of infections and limiting progression of airway inflammation.

Restoration of hydration status of ASL

Restoration of hydration status has been targeted via three angles: therapeutic osmolytes, ion transport regulators and ENaC inhibitors.[87] The strongest evidence lies with hypertonic saline (HS, 7% NaCl), a therapeutic osmolyte. Studies have demonstrated it can reduce pulmonary exacerbations, increased quality of life and improve lung function in the short term. [88, 89] However, a Cochrane review called for additional long term studies to demonstrate its long term effectiveness.[90]

Promotion of airway clearance

Therapies or practices that target increased airway secretions include mucolytics and chest physiotherapy. Mucolytics reduce mucus viscosity facilitating easier clearance of airway secretions. To date, inhaled Dornase alpha (Pulmozyme®) is the only mucolytic for which there is evidence demonstrating improved long term lung function and is recommended in CF airway management.[91] This therapy specifically targets the DNA released by dead neutrophils which contributes to the increased viscosity of mucus in patients with CF.

The principal of chest physiotherapy is to promote expectoration of airway secretions and has been demonstrated to improve mucus clearance in the short term.[92] A specialist CF physiotherapist is required to tailor the technique (chest percussion with postural drainage or high frequency chest wall oscillation) towards the patient's age, preference and adverse events, but the practice itself can be self-administered.[93, 94]

Eradication of Infections

Aggressive antibiotic treatment is a cornerstone of CF airway management. Intermittent infections should be treated promptly (< 4weeks) with a suitable antibiotic regimen. Options include 28 days of

tobramycin solution for inhalation (TIS) and up to 3 months of a combination of nebulised colistin and oral ciprofloxacin.[93] Patients who are chronically infected with *P. aeruginosa* are indefinitely managed with daily antipseudomonals such as inhaled tobramycin (TOBI®) and Colomycin. Inhaled tobramycin is associated with improved lung function and reduced exacerbation rates.[93, 95]

Limiting the progression of airway inflammation

Anti-inflammatory drugs have a role in reducing neutrophil dominated airway inflammation. Corticosteroids have limited the progression of lung disease in several clinical trials in patients with CF.[96] However, due to their significant adverse effects such as glucose intolerance and growth retardation, they are not recommended in patients with CF unless there is concomitant asthma.[93] Another anti-inflammatory drug, Ibuprofen, has also slowed the long term progression of CF lung disease in one trial.[97] However, this evidence is insufficient to guide recommendations. Therefore at present, anti-inflammatory drugs are not recommended for routine management in patients with CF.

Treatment of Pulmonary exacerbations

The treatment of patients with CF should focus on limiting and preventing pulmonary exacerbations. However, when they do occur they should be treated promptly and aggressively to maintain lung function, improve QoL and prolong survival. For upper respiratory tract infections with more than 3-5 days of cough or other lower respiratory tract symptoms, a minimum of 10 days of oral antibiotics that cover both *H. influenza* and *S. aureus* are required. If symptoms do not resolve, or worsen on oral antibiotics, a course of intravenous antibiotics is indicated. Patients chronically infected with *P. aeruginosa* who develop new symptoms are treated with oral antibiotics in conjunction with regular inhaled anti-pseudomonal therapy. If this therapy fails to resolve symptoms, a combination of 2 intravenous antibiotics is recommended for 14 days. This may change depending on individual patient requirements and local guidelines. Patients who require intravenous antibiotics also require the following: more intense, supervised chest physiotherapy and airway clearance, increased nutritional supplementation and closer monitoring of other CF related issues.[98]

1.3.2 Nutritional repletion

Nutritional management

CF patients with good nutritional status have been demonstrated to have better pulmonary function and survival.[99] In order to achieve proper nutritional status CF patients need to follow an unrestricted diet that is high in calories (30%-40% of the calories from fat), have optimal pancreatic replacement enzyme therapy and use supplemental nutrition (orally or enterally) when

appropriate. Bicarbonate supplementation and acid suppressants may also be used to neutralise the acidic duodenal pH of CF patients.[100]

1.3.3 Summary

The mainstay of CF airway management is symptomatic and targets the different stages of events in the cascade of events leading to progressive airway disease (Figure 3). The most important emphasis is placed on prompt and aggressive antibiotic management during intermittent infections to limit the onset of chronic infection. When patients are chronically infected particularly with *P. aeruginosa*, they require daily inhaled antibiotic therapy and regular physiotherapy to increase mucus clearance. Therapies that aim to hydrate the ASL (hypertonic saline) and reduce viscosity of secretions (Dornase alpha) are also recommended for routine airway management. In addition to achieving good nutrition this forms a pivotal part of CF management and has a significantly beneficial impact on long term prognosis of patients with CF.

1.4 New approaches to management

It is clear that to date, the focus of treatment has been aimed at resolving the consequences of defective CFTR through aggressive symptomatic management. This has been very efficacious, and has drastically improved survival of patients with CF over the past decades.[2] However it would be preferable for CF therapies to target the underlying CFTR defect itself and this feat has been made possible with the knowledge attained from discovery of the putative gene in 1989.

First efforts at targeting the gene defect aimed to replace the mutated CFTR gene with correct CFTR DNA through CFTR gene transfer therapy. More recent efforts in this field have focused on restoring mutation specific defects in CFTR synthesis or function (Table 1) and are called mutation specific therapies.

1.4.1 CFTR Gene Transfer Therapy

CFTR gene therapy aims to deliver correct CFTR DNA into CF airway cells. Theoretically this methods of treatment is relevant to CF patients as 1) CF is monogenic 2) heterozygotes are phenotypically normal therefore DNA levels do not need to match that of non-CF people 3) therapy targeting the lungs can be administered topically and 4) normal lungs at birth offer a therapeutic window.[101] If effective, gene transfer therapy could be used to treat patients with all types of gene mutations. CFTR DNA can be delivered through viral or non-viral mediums. Viral gene transfer therapy involves using a viral vector for example an adenovirus to introduce the coding sequence to the target cell so it can be transcribed into mRNA or even insert it into the cell genome. Non-viral methods of delivery involve using non-viral vectors such as liposome-based vectors to introduce the coding sequence to the cell. Laboratory studies have demonstrated successful transfer of correct CFTR DNA to airway cells in cell and animal models.[102] Despite this, CFTR Gene Transfer therapy through both viral and non-viral mediums has not yet demonstrated clinical benefit to humans, owing to difficulties in overcoming host immune responses and physical barriers.[103, 104] Research into the development of a clinically effective gene transferring therapy is on-going and is being led by the UK Cystic Fibrosis gene therapy consortium.[105]

1.4.2 Mutation-specific therapies

CFTR Potentiators

The first strategy, particularly related to mutation classes III-V, but potentially important to mutation class II, was to augment the function of defective CFTR successfully embedded within the cell membrane. Drugs that successfully augment membrane bound CFTR were called CFTR potentiators. Genistein, present in tofu and soy, demonstrated increased chloride channel activity in both

defective and WT CFTR.[106] More recently, a small molecule called Ivacaftor (also called VX-770 and Kalydeco®) was identified through high throughput screening by Vertex Pharmaceuticals.[107] The exact mechanism of action of Ivacaftor is not completely understood but has been demonstrated to increase the open time of activated CFTR channel pores located at the epical cell membrane, in a phosphorylation dependent but ATP independent pattern.[108] Ivacaftor has successfully corrected abnormal salt transport in cell models of both ΔF508 (Class II) and G551D (Class III) CFTR.[109] It has progressed to human clinical trials in patients with both the G551D mutation and ΔF508 mutation and is a licensed therapy for children (6-11 years old) and adults with the G551D mutation in the UK.[43]

CFTR potentiator

A drug that aims to increase the amount of functional CFTR expressed at the epithelial cell apical membrane by enhancing defective CFTR already embedded within the cell membrane.

CFTR corrector

A CFTR corrector is defined as a drug which aims to increase the amount of CFTR expressed at the epithelial cell apical membrane, by reducing or preventing degradation of CFTR by normal intracellular mechanisms seen in Class II mutations.

Stop codon therapies

Therapies that 'skip' premature stop codons found in mRNA of Class I mutations.

CFTR Correctors

In addition to CFTR potentiators, other drugs that aim to correct the underlying defect of mutation classes have been developed. CFTR correctors aim to correct the intracellular processing defect of class II mutations so CFTR can be trafficked to the cell surface. As the ΔF508 mutation (class II) accounts for 90% of alleles in the CF population, these therapies could represent a significant healthcare resource and have received much attention. However, due to the complexity of the trafficking process, identifying effective therapeutics has proved challenging.

Early studies demonstrated that low temperature could increase protein trafficking and function.[110] Following this, therapies that targeted molecular chaperones or therapies approved for other conditions demonstrated ΔF508-CFTR rescue activity.[107] In vitro Studies of glycerol (chemical chaperone) demonstrated increased CFTR expression at the cell membrane and increased function of chloride transport.[111, 112] Subsequently, studies of 4-phenylbutyrate (4PBA), a compound approved for an inherited of urea metabolism, demonstrated increased trafficking of mature ΔF508 CFTR to the cell surface.[113] Miglustat, a drug approved for the treatment of Gaucher disease, has recently been shown to promote ΔF508 CFTR trafficking to the cell membrane and augment chloride conductance and sodium reabsorption.[114] In vitro studies of CPX (8-cyclopentyl-1, 3-dipropylxanthine), an A1 adenosine receptor antagonist, demonstrated increased WT and ΔF508-

CFTR channel activity. [115] Following the positive results achieved with these therapies in vitro, they have all progressed to human clinical trials.[116-120]

Additionally, small molecules with ΔF508 corrector ability, namely Lumacaftor (VX-809) and VX-661, have recently been identified by Vertex Pharmaceuticals through high throughput screening (HTS). So far Lumacaftor has demonstrated the most progress. In vitro studies demonstrated significantly improved chloride transport in ΔF508 CFTR cells to that of 14% of non-CF individuals.[121] Its mechanism of action is not fully understood, but it is proposed to suppress the folding defect of ΔF508- CFTR by modulating the shape of MSD1.[122] Developments of both Lumacaftor and VX-661 have progressed into human clinical trials.[123-125]

In vitro studies have demonstrated that CFTR correctors successfully increase ΔF508-CFTR trafficking and expression within the apical cell membrane. However, this does not resolve the issue of compromised chloride conductance of ΔF508-CFTR when embedded within the cell membrane. Restoration of ΔF508 CFTR is therefore likely to require the addition of CFTR potentiators to augment CFTR function at the cell membrane. In vitro studies have demonstrated almost doubled effect of Lumacaftor on ΔF508 human bronchial epithelial cells when used in combination with Ivacaftor.[126] Studies examining the impact of CFTR corrector and CFTR potentiator combination therapy have progressed to human clinical trials.[127-130]

Stop Codon Therapies

The mRNA product of Class I mutation possess a premature termination codon (PTC) that codes for a truncated, unstable protein (Table 1). Stop codon therapies 'force' read-through of the PTC during translation and aim to produce a full length polypeptide chain. In vitro studies of Gentamicin (an aminoglycoside) demonstrated that two loss of function class I CFTR mutations could be suppressed, which resulted in a fully synthesised CFTR polypeptide chain that restored CFTR function up to 35% of WT levels at the cell membrane. [131, 132] Research into Gentamicin has progressed to human clinical trials but there are concerns about regular and high doses of this therapy given its adverse effects profile includes ototoxicity and nephrotoxicity.[133]

More recently, another stop codon therapy called Ataluren (PTC124), developed by PTC therapies, has made significant progress. It allows the ribosome to read through the PTC but unlike Gentamicin, it does not allow read through of the correct PTC at the end of the mRNA chain.[134] Research into Ataluren has progressed to testing in humans.[133, 135]

Summary

Gene transfer therapy and mutation-specific-therapies are the first therapies to be created in the field of personalised genomics in CF. They have both demonstrated efficacy in vitro, have progressed to human clinical trials and could represent significant healthcare resources for patients with CF. Recommendations for the use of these therapies in humans however must be guided by high quality evidence.

1.5: Cochrane Systematic Reviews

1.5.1 Cochrane Systematic reviews

Cochrane Systematic reviews are the most comprehensive and valid form of establishing the current evidence base.[136, 137] They seek to answer a specific healthcare question in ways that limit bias to selection, critical appraisal and synthesis of studies, and are widely seen at the gold standard resource for up-to-date evidence. In comparison to narrative reviews where the author is able to select the studies to include, systematic reviews involve a more objective assessment of the literature. This is through robust and transparent methodology.[138, 139] All Cochrane systematic reviews start with a protocol stage outlining the proposed methodology for the inclusion, methodological assessment and synthesis of studies. This is peer-assessed and published prior to the literature search being conducted, to minimise risk of bias in the selection of studies. The literature search process involves a thorough search of published and unpublished trials to ensure that the evidence is based on all relevant literature. In Cochrane systematic reviews this is done through a specific Cochrane Registry for example the Cochrane CF registry and by contacting leaders in the field. The identified studies are then screened and assessed for eligibility with pre-specified eligibility criteria. The eligible studies are critically appraised to highlight any systematic error in the included studies that could potentially limit the internal validity of the evidence. Conclusions are formed from results of synthesised data and take into account systematic error within the included studies. This robust and transparent methodological approach, employed by systematic reviews and in particular Cochrane systematic reviews, ensures that bias is kept to a minimum. Cochrane systematic reviews are regularly updated to ensure the evidence base is kept up to date.

Systematic reviews include a meta-analysis. Meta-analysis is the statistical technique used to synthesise data from eligible studies that demonstrate homogeneity, thereby increasing the number of participants that the data were obtained from and thus increasing the sample size. Synthesis of data in a meta-analysis can therefore increase the precision of the overall treatment effect estimate, if trials demonstrate consistency.[140]

Cochrane systematic reviews provide a unique resource for clinicians. They provide a concise summary of large bodies of evidence, which would have otherwise taken an extended period of time to assess. In addition to this they also provide a thorough and methodologically transparent appraisal of the evidence for authors who wish to determine how conclusions were formed, so they can form their own judgments on the validity of the evidence. Cochrane systematic reviews are also a unique resource for patients and their families. They report results in a way that is both relevant and interpretable to lay people, empowering patients and their families to make informed decisions

about treatment plans. They are also readily accessible for free on the Cochrane Library, which is particularly important for patients and their families given they may not have access to medical journals.

1.5.2 Quality of the evidence

In order to provide the strongest current evidence base, studies considered eligible for inclusion in systematic reviews must demonstrate sufficient methodological quality. In Cochrane systematic reviews of interventions, included studies are usually limited to randomised controlled trials (RCTs). RCTs represent the most rigorous way of determining whether a cause-effect relationship exists between treatment and outcomes and are therefore considered the best from of clinical trial with regards to clinical trial methodology. Meta-analysis of homogenous RCTs therefore provide the highest quality of evidence (Figure 7).[136]

The validity of evidence can still be limited by flaws in the conduct, analysis and reporting of included studies. In Cochrane systematic reviews, studies are critically appraised using the Cochrane risk of bias tool to determine the effect of these flaws on the treatment effect estimate.

1.5.3 Summary

Cochrane systemic reviews are the gold standard for establishing the current evidence base for interventions. They aim to reduce bias in the review process, and also identify flaws in included studies that can limit the validity of the evidence. This is done through robust, transparent and reproducible methodology.

Figure 7 Hierarchy of evidence with regards to treatment interventions provided by the Oxford centre for Evidence base medicine.

x

Figure 7 demonstrates a hierarchy of evidence for healthcare interventions. At the top of the hierarchy lies systematic reviews (SR) based on homogenous RCTs.[136]

1.6 Objective this MPhil

Since CFTR potentiators and CFTR correctors are novel therapies, it is important that randomised controlled trials (RCTs) testing these chemicals are critically appraised. This will enable examination of the evidence relating to their benefits and harms. It is also important for funding bodies such as NHS to have a clear evidence-base on which to assess new therapies for CF that aim to correct the basic defect as it is likely that these therapies will represent a significant healthcare resource and cost in the future. It is also important for healthcare practitioners and patients to have a clear evidence base to inform clinical decision making.

The objective of this MPhil is:

To conduct two Cochrane systematic reviews to evaluate the benefits and harms of 1) CFTR potentiators (for class III-IV mutations) and 2) CFTR correctors (for class II mutations) on clinically important outcomes in children and adults with CF.

Chapter 2

Methods

Evidence for the impact of CFTR potentiators and CFTR correctors on clinically important outcomes in CF was assessed by conducting two systematic reviews of published and unpublished research evidence. These reviews adhered to guidelines published in the Cochrane Handbook of systematic reviews on interventions.[138]

2.1 Development of the review questions and review protocol

2.1.1 Development and defining the research questions

The PICO tables clearly define the components of the research question.

Table 3 PICO table for development of review question for CFTR potentiators

Review Question	**Potentiators (specific therapies for Class III and IV mutations) for cystic fibrosis**
Population	Patients with Cystic Fibrosis with class III-IV mutation.
Intervention	CFTR potentiators Not alongside other mutation specific therapies
Comparator	Placebo
Outcomes	Figure 10

Table 4 PICO table for development of review question for CFTR correctors

Review Question	**Correctors (specific therapies for class II CFTR mutations) for cystic fibrosis**
Population	Patients with Cystic Fibrosis with class II mutations.
Intervention	CFTR correctors
Comparator	Placebo
Outcomes	Figure 10

To assess CFTR potentiators, we created the title: Potentiators (specific therapies for Class III and IV mutations) for cystic fibrosis. To assess CFTR correctors we created the title: Correctors (specific therapies for class II CFTR mutations) for cystic fibrosis. In both reviews, we did not limit the studies

to participants with mutation classes highlighted in the title if it was clinically relevant to include patients with other mutation classes.

2.1.2 Development of the Review protocol

These systematic reviews were conducted in accordance with peer assessed and published protocols.[141, 142] The author of this thesis was involved in producing the protocol for the CFTR correctors review; the protocol for the CFTR potentiators review had been published by the other review authors prior to commencement of this MPhil. The purpose of the protocol stage was to introduce the reviews, and demonstrate the planned review methodology. We first set out a clear description of Cystic Fibrosis, the interventions to be assessed, and the objectives. We then outlined the proposed review methodology through pre-defined selection criteria, planned search strategies, planned data extractions and proposed methods of quality assessment and synthesis of results. The published protocols are attached.

2.2 Criteria for considering studies for this review

2.2.1 Types of studies

We only included RCTs of parallel design (published or unpublished). Cross-over trials were considered inappropriate given the potential longer term impact of these therapies on outcomes.

2.2.2 Types of participants

In both systematic reviews, we included trials involving children and adults with CF. CF was confirmed by the presence of two disease causing mutations, or a combination of positive sweat test and recognised clinical features of CF.

In both reviews, we included trials of patients with relevant mutation classes. For example, CFTR potentiators aim to enhance the function of compromised CFTR already embedded in the cell membrane. This is classically seen in class III and IV mutations (Table 1), but also in minute amounts in patients with class II mutations, namely the ΔF508 mutation. We therefore did not exclude studies where CFTR potentiators were examined in participants with class II mutations, as it was important to demonstrate the impact of CFTR potentiators on the minimal amounts CFTR that reaches the cell membrane. We also included participants with all levels of disease severity.

2.2.3 Types of Interventions

CFTR potentiators review:

We included studies comparing CFTR potentiators to either placebo or another intervention. Studies that compared a CFTR potentiator with another mutation specific therapy (for example a CFTR corrector) were excluded given their principal purpose was to potentiate and not correct the defect. We deemed it more appropriate for these studies to be included in a review of CFTR correctors.

CFTR correctors review:

We included studies that compared CFTR correctors either as monotherapy or as combination therapy with other mutation specific therapies (for example a CFTR potentiator), to either placebo or another intervention.

2.2.4 Types of outcomes measures

To date, a core outcome set for systematic reviews in Cystic Fibrosis has not been established. The Core Outcome Measures in Effectiveness Trials (COMET) initiative aims to create a COS for CF but for now the selection of outcomes in this review was created by the review authors.[143] We included clinically meaningful outcomes relevant to patients, clinicians, consumers, the general public, administrators and policy makers. We included all relevant outcomes, even if they were not

reported in the included clinical trials. This ensured that all clinically important data was included and highlighted any gaps in the reporting of outcome measures by trialists.

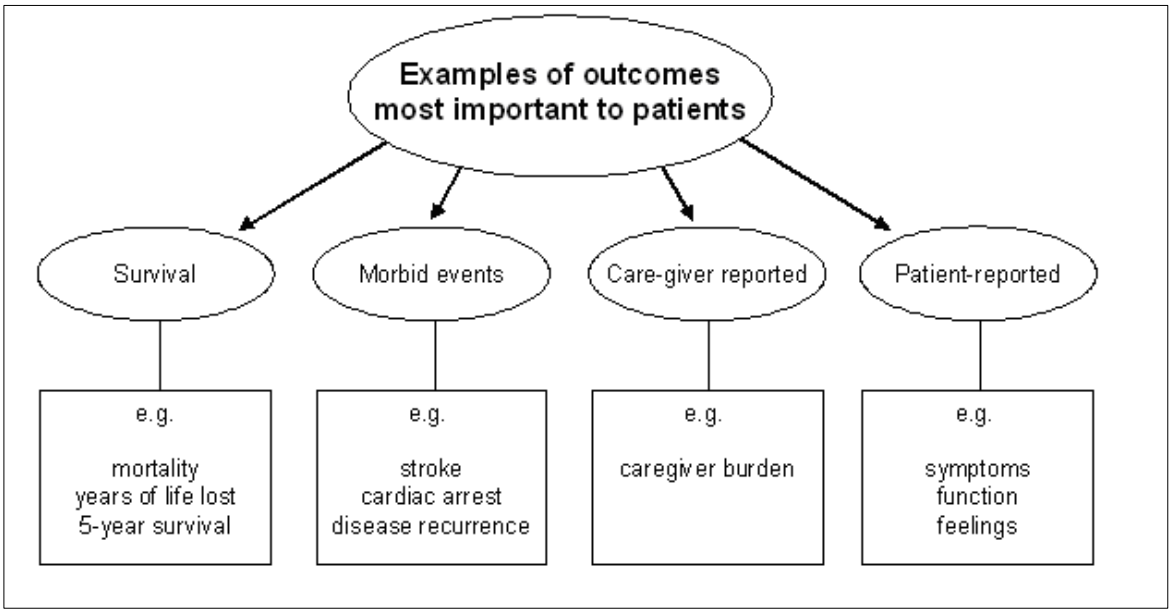
Outcome measures fall into three classes; 1) clinical end-points 2) surrogate endpoints and 3) biomarkers. Clinical outcomes indicate how the patient feels, functions or survives and include the resolution or removal of symptoms (e.g. QoL). A surrogate endpoint is a laboratory measurement used as a substitute for clinical endpoint to determine adverse effects of the therapy or to predict its efficacy (for example FEV_1). It must be associated with a clinical outcome to be clinically meaningful and have a link to survival, long term prognosis or be an accepted measure of treatment effect. The definition of a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic response to a therapeutic intervention." In CF, biomarkers are not yet recognised as surrogate outcome measures, but have become more relevant as with the emergence of personalised therapies..[144]

Primary outcome measures

Survival

The ultimate goal of clinical trials in CF is to increase survival, as despite recent advances, CF remains a life-limiting disease. Therefore, the impact of the intervention on survival is the gold standard efficacy measure in CF clinical trials and systematic reviews.[145] Longer survival rates amongst CF patients has meant that this outcome is having a more limited role in short clinical trials, particularly in children, but It is crucial for all parties whom this review is aimed at, to understand how the intervention may impact survival (Figure 8).[146]

Figure 8 Examples of outcomes most important to patients provided by the Cochrane Handbook



Quality of life (QoL)

Health related quality of life (HRQoL) is a patient reported outcome (PRO). In interventional studies, PROs provide a unique insight the patient's perspective on the benefit or harms of treatment by scoring how patients feel and function with respect to their health.[147] They are essential because they are the only outcome to gather this information. PROs are particularly valuable in CF clinical trials and systematic reviews as patients with CF have to endure aggressive and time consuming treatment on a daily basis.[148]

In CF, there are two disease specific HRQoL instruments; the Cystic Fibrosis Questionnaire – Revised (CFQ-R) and the Cystic Fibrosis Quality of Life Questionnaires (CFQoL). [149, 150] To date, the CFQ-R scale is the most well recognised validated measure of HRQoL in CF with an established minimal clinically important difference (MCID) score of 4.[151] As a test, it has demonstrated reliability and consistent associations with other health outcomes (e.g. FEV_1) in large samples.[152] It is also a feasible test; available for both adults (>14 years of age) and children (6-13 years of age) and can be completed relatively quickly (15 minutes). This QoL score consists of 9 QoL domains; physical functioning, vitality emotional state, social limitations, role limitations/school performance, embarrassment, body image, eating constraints; 3 symptom scales; respiratory, digestive, weight and 1 health perception scale; health status.

Incorporating PROs into Cochrane systematic reviews of interventions for CF is highly valuable for patients and clinicians. PROs directly reflect how the participants of trials felt under the intervention; therefore any concerns highlighted by participants in the trials can be interpreted by patients as issues directly relating to them. This has a number of advantages. It empowers the patient to make informed decisions about personalised treatment plans, maintains the clinical relevance of Cochrane Systematic reviews to patients and provides a unique insight into how interventions can affect the lives of patients for healthcare professionals.

In our systematic reviews we reported on total QoL scores and individual scores for different QoL domains. We reported results on HRQoL scores measured using any validated instrument (e.g. CFQ-R). Both individual and pooled scores for adults and children were included.

Physiological measures of lung function (litres or per cent predicted for age, sex and height)

In our reviews we included the following physiological measures of lung function (litres or percent predicted for age, sex and height)

1. Forced expiratory flow rate at one second (FEV_1) (relative change from baseline)
2. FEV_1 absolute values (rather than change from baseline)

3. Forced vital capacity (FVC) (absolute values and change from baseline)

Forced expiratory flow rate at one second (FEV_1)

FEV_1 (% predicted) is a non-invasive test that measures the maximum amount of air that can be expelled in 1 second. It is then calculated as a percentage of normal, based on age, gender and height. The resultant Figure is used to reflect the degree of airway obstruction within the airways. In CF, the change in FEV_1 is in line with the progression of obstructive airway disease (figure 9). [153] Therefore in clinical trials and systematic reviews, the change in FEV_1 is used to reflect the change in airway obstruction over time and is considered the best possible available method for linking airway disease to survival in patients with moderate to severe lung disease.[153-155] Data for FEV_1 is also used to determine and define disease severity, to inform clinical decisions about changing or intensifying treatment and to form recommendations on new therapies.[156, 157, 158] This outcome is therefore relevant to patients, healthcare professionals and policy makers and is an important outcome in CF systematic reviews.

In both reviews, the relative change from baseline in FEV_1 was a primary outcome of interest. We also reported on the absolute change from baseline in FEV_1.

Figure 9 A bar chart demonstrating the negative correlation between FEV_1 and age in patients with Cystic Fibrosis

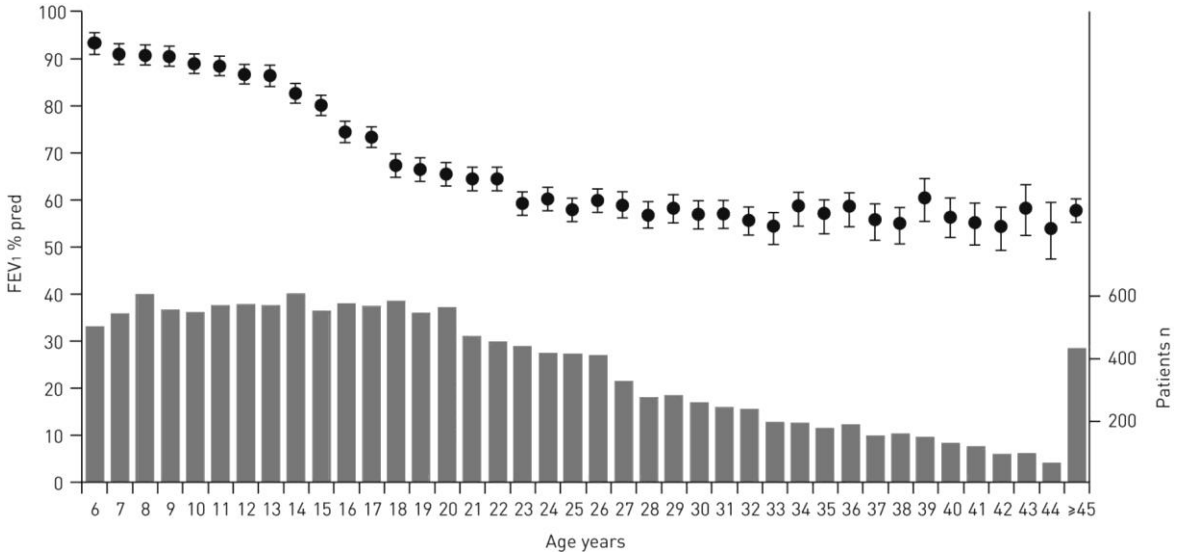


Figure 9 demonstrates the association between increasing age and mean FEV_1 % predicted in patients with CF. The bars represent the number of patients of a particular age group enrolled in the study and the dots represent the mean FEV_1 % predicted for that age. The dots demonstrate the negative correlation between increasing age and FEV_1 in patients with Cystic Fibrosis. This is in line with the progression of obstructive airway disease in CF.[153]

Forced Vital Capacity (FVC)

FVC is the maximum amount of air a person can breathe out after maximum inspiration. As the rate of FVC decline is also related to survival age, it is important to report on this outcome as a reflection of changes in airway disease.[154] We reported on the relative and absolute change from baseline in FVC values.

Secondary outcomes

Adverse effects of therapy

In systematic reviews there should be a balance between the reporting of beneficial and harmful effects of the interventions. This is particularly relevant in systematic reviews of novel therapies, as before an intervention can be recommended its benefits must outweigh potential harms.

We classified adverse effects into three groups by severity:

- Mild: Therapy does not need to be discontinued
- Moderate: Therapy is discontinued and the adverse effect ceases (require study drug interruption)

- Severe: Life threatening or debilitating or which persists even after treatment is discontinued (require study drug discontinuation)

This classification of reporting adverse effects was outlined in the Cochrane Handbook of systematic reviews.[159]

Pulmonary exacerbations

Pulmonary exacerbations and their management are described in the Introduction. To date, there are several definitions of pulmonary exacerbation but there is no consensus regarding its diagnostic criteria. Therefore, trialists can employ different definitions when reporting pulmonary exacerbations. There are two commonly used definitions: physician defined exacerbations and protocol-defined exacerbations. Physician defined exacerbations are based on episodes of worsening symptoms for which the attending physician decides that extra antibiotic are required. Protocol-defined exacerbations are defined on a specified cluster of symptoms and signs to define the clinical need for additional treatment with antibiotics.[98] As there is not yet a set diagnostic criterion or definition for pulmonary exacerbations, they are not considered a standardised outcome in CF systematic reviews. Therefore it was not an individual outcome in these reviews. Data for pulmonary exacerbations were reported under other adverse effects, hospitalisations and extra courses of antibiotics. We stated whether they were physician-defined or protocol-defined.

It is important to report on pulmonary exacerbations because they are associated with worse long term prognosis, reduced QoL and are an important clinical outcome for patients and clinicians.[63-65] Studies have also demonstrated that the rate at which pulmonary exacerbations occurs contribute to lung function decline.[160] Therefore, we also reported on the time to next exacerbation to demonstrate what effect the intervention can have on lung function decline.

Hospitalisation

This clinical outcome was mainly included to account for data on pulmonary exacerbation. We reported on the number of days of hospitalisation and the number of subjects who required hospitalisations. It is important to report on hospitalisations because they represent a chaotic and stressful time for both patients and their families. Patients and family members may have to take extended periods of time off school or work and this can significantly burden social and family life. Studies of the impact of hospitalisations for exacerbations on QoL have demonstrated a decreased QoL amongst inpatients.[161]

School or work attendance (i.e. number of days missed)

Whilst at school, children develop cognitive skills and develop their sense of identity through comparison with their peers. School attendance (lack of) highlights CF-related differences and can impacts the ability of children to form intimate relationships.[162] School and work attendances are particularly important clinical outcomes for patients and their families, as they directly relate to functional ability.

Extra courses of antibiotics (measured as time-to the next course of antibiotics and the total number of courses of antibiotics)

This outcome was included to account for the data on pulmonary exacerbation. We reported on oral, intravenous and inhaled antibiotics.

Radiological measures of lung disease (assessed using any scoring system)

The use of surrogate end-points in clinical trials is particularly limited in young children (less than six years of age). This is because young children do not always co-operate with lung function tests and cannot expectorate sputum. Computerised Tomogram (CT) scan be performed across all ages, assesses structure and is the most sensitive tool for detecting early disease.[163] Chest x-rays are insensitive to the early changes of CF, but can be used to identify later changes such as bronchiectasis and hyperinflation. We therefore included measures of lung disease using 1) chest radiograph scores and 2) computerised tomogram (CT) scores.

Acquisition of respiratory pathogens

In CF, there is much emphasis on preventing/postponing bacterial infection, colonisation and chronic infection because chronic infection with respiratory pathogens, especially *P. aeruginosa*, is associated with significantly poorer long term-prognosis.[52] Therefore in CF systematic reviews it is important to demonstrate how new interventions can affect the rate at which respiratory pathogens are acquired.

Infection with a respiratory pathogen is identified by sputum culture. The procedure of obtaining sputum (expectorated or induced) is standardised and can be conducted in all age groups, except young children, with minimal risk. We reported on the acquisition of pathogens under the following sub-headings: i) *P. aeruginosa*, ii) *S. aureus*, iii) *H. influenza* and iv) any other significant pathogens.

Eradication of respiratory pathogens (as defined by trial authors)

We included this outcome to demonstrate the ability of interventions to help eradicate pathogens. We reported on the eradication of pathogens under the following sub-headings: i) *P. aeruginosa*, ii) *S. aureus*, iii) *H. influenza* and iv) other significant pathogens.

Nutrition and Growth growth (measured as relative change from baseline) (including z scores or centiles)

Poor nutritional status worsens long-term prognosis in CF as outlined in the Introduction.[76] It is therefore important to demonstrate, particularly in children, how a novel therapeutic can impact weight and growth. We reported on i) weight ii) BMI and iii) height.

Sweat chloride (change from baseline) as a measure of CFTR function

Sweat chloride concentration is well-recognised diagnostic test in CF but in CF clinical trials, it is a biomarker; a direct representation of CFTR activity in sweats glands. Although its relevance may not be apparent to patients, it is important in CF clinical trials and systematic reviews to demonstrate changes in CFTR function. The concentration of chloride ions has a number of strengths as an outcome. It can detect rapid changes in CFTR activity, can be measured consistently in patients of young age (non-invasive) and sweat glands do not appear to be susceptible to secondary damage from the disease process (unlike lungs and GI tract).[164] Although sweat chloride concentration is derived from CFTR activity in the sweat glands, results from clinical trials have demonstrate that sweat chloride concentration may be representative of CFTR function expressed elsewhere.[164]

Cost of treatment

This outcome was not part of the original set out outcomes defined in the protocol for the potentiators review. It was added in the review stage because it is likely that the investigated therapies (CFTR potentiators and CFTR correctors) will represent a significant healthcare resource in the future and therefore represents a significant healthcare cost.

2.2.5 Summary of criteria for considering studies for each review

Table 5 Summary of Inclusion/exclusion criteria for the CFTR potentiators review

CFTR Potentiators review		
	Inclusion criteria	Exclusion criteria
Studies	Only RCTs of parallel design	1. RCTs of cross over design 2. Studies that are not RCTs
Participants	1. Children or adults with CF 2. Patients with any clinically relevant class mutation (II-IV) 3. Patients with any disease severity	
Interventions	CFTR potentiator versus placebo or another intervention.	1. Studies where CFTR potentiators are used alongside other mutation specific therapies e.g. (CFTR correctors) 2. Interventions that aim to correct the underlying defect (CFTR correctors) 3. Studies assessing stop-codon-therapies
Outcomes	Studies assessing outcomes relevant to this review as detailed in Figure 10	Studies assessing only outcomes not relevant to this review e.g. NPD and LCI

Table 6 Summary of Inclusion/exclusion criteria for the CFTR correctors review

	CFTR Correctors Review	
	Inclusion criteria	Exclusion criteria
Studies	RCTs of parallel design	1. RCTs of cross over design 2. Studies that are not RCTs
Participants	1. Children or adults with CF 2. Patients with any clinically relevant class mutation (II) 3. Patients with any disease severity	
Interventions	1. CFTR corrector versus placebo or another intervention. 2. CFTR corrector are used alongside other mutation specific therapies e.g. (CFTR potentiator) versus placebo or another intervention	1. Studies examining stop-codon-therapies.
Outcomes	Studies assessing outcomes relevant to this review as detailed in Figure 10	Studies only assessing outcomes not relevant to this review e.g. NPD and LCI

Figure 10 Summary of outcomes measures in our systematic reviews

CFTR potentiators review

Primary outcomes

1. Survival
2. Quality of life (QoL) (measured using validated quantitative scales or scores (e.g. Cystic Fibrosis Questionnaire-Revised)
    a. Total QoL score
    b. Different sub-domains which may be reported
3. Forced expiratory flow rate at one second (FEV_1) (relative change from baseline)

Secondary outcomes

1. Adverse effects
    a. Graded by review authors as mild (therapy does not need to be discontinued)
    b. Graded by review authors as moderate (therapy is discontinued, and the adverse effect ceases)
    c. Graded by review authors as severe (life-threatening or debilitating, or which persists even after treatment is discontinued)
    d. Other adverse effects of therapy (of any severity) that are not classifiable according to these categories
2. Hospitalization
    a. Number of days
    b. Number of episodes
3. School or work attendance (i.e. number of days missed)
4. Other physiological measures of lung function (litres or per cent (%) predicted for age, sex and height)
    a. FEV_1 absolute values (rather than "relative change from baseline", which is specified as primary outcome)
    b. Forced vital capacity (FVC) (absolute values and change from baseline)
5. Extra courses of antibiotics (measured as time-to the next course of antibiotics and the total number of courses of antibiotics)
    a. oral
    b. intravenous
    c. inhaled
6. Radiological measures of lung disease (assessed using any scoring system)
    a. chest radiograph scores
    b. computerised tomogram (CT) score
7. Acquisition of respiratory pathogens
    a. Pseudomonas aeruginosa (P. aeruginosa)
    b. Staphylococcus aureus (S. aureus)
    c. Haemophilus influenzae (H. influenzae)
    d. Other significant pathogen
8. Eradication of respiratory pathogens (as defined by trial authors)
    a. Pseudomonas aeruginosa (P. aeruginosa)
    b. Staphylococcus aureus (S. aureus)
    c. Haemophilus influenzae (H. influenzae)
    d. Other significant pathogen
9. Nutrition and growth (measured as relative change from baseline) (including z scores or centiles)
    a. Weight
    b. Body mass index (BMI)
    c. Height
10. Sweat chloride (change from baseline) as a measure of CFTR function
11. Cost of treatment

CFTR correctors review

Primary outcomes

1. Survival
2. Quality of life (QoL) (measured using validated quantitative scales or scores (e.g. Cystic Fibrosis Questionnaire-Revised)
    a. Total QoL score
    b. Different sub-domains which may be reported
3. Physiological measures of lung function
    a. Forced expiratory flow rate at one second (FEV_1) (relative change from baseline)
    b. FEV_1 absolute values
    c. Forced vital capacity (FVC) (absolute values and change from baseline)

Secondary outcomes

1. Adverse effects
    a. Graded by review authors as mild (therapy does not need to be discontinued)
    b. Graded by review authors as moderate (therapy is discontinued, and the adverse effect ceases)
    c. Graded by review authors as severe (life-threatening or debilitating, or which persists even after treatment is discontinued)
    d. Other adverse effects of therapy (of any severity) that are not classifiable according to these categories
2. Hospitalization
    a. Number of days
    b. Number of episodes
3. School or work attendance (i.e. number of days missed)
4. Extra courses of antibiotics (measured as time-to the next course of antibiotics and the total number of courses of antibiotics)
    a. oral
    b. intravenous
    c. inhaled
5. Radiological measures of lung disease (assessed using any scoring system)
    a. chest radiograph scores
    b. computerised tomogram (CT) score
6. Acquisition of respiratory pathogens
    a. Pseudomonas aeruginosa (P. aeruginosa)
    b. Staphylococcus aureus (S. aureus)
    c. Haemophilus influenzae (H. influenzae)
    d. Other significant pathogen
7. Eradication of respiratory pathogens (as defined by trial authors)
    a. Pseudomonas aeruginosa (P. aeruginosa)
    b. Staphylococcus aureus (S. aureus)
    c. Haemophilus influenzae (H. influenzae)
    d. Other significant pathogen
8. Nutrition and growth (measured as relative change from baseline) (including z scores or centiles)
    a. Weight
    b. Body mass index (BMI)
    c. Height
9. Sweat chloride (change from baseline) as a measure of CFTR function
10. Cost of treatment

2.3 Search Methods for identification of studies

2.3.1 Electronic Searches

A literature search was conducted with help from the Trials Search Co-ordinator from the Cochrane Cystic Fibrosis and Genetic Disorders Group (CFGD). To identify relevant trials, we conducted a search of the group's Cystic Fibrosis trials register. This register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated each new issue of The Cochrane Library), MEDLINE (quarterly searches), EMBASE to 1995 and hand searching two journals - *Pediatric Pulmonology* and the *Journal of Cystic Fibrosis*. Studies were also identified by searching through the abstract books of three major cystic fibrosis conferences: the International Cystic Fibrosis Conference; the European Cystic Fibrosis Conference and the North American Cystic Fibrosis Conference.

After each search of these databases and journals, all relevant trials were tagged with keywords and key terms developed by the CFGD. For the CFTR potentiators review, the keywords were; 'potentiator', 'VX-770' and 'genistein.' Trials relating to one of these keywords were tagged with the appropriate keyword and all trials relating to these keywords were tagged with the key term; 'drugs that augment function of abnormal CFTR protein in the cell membrane.' For example, if a study on VX-770 was identified, it would be tagged with keyword 'VX-770' and also key term 'drugs that augment function of abnormal CFTR protein in the cell membrane.'

To identify all relevant studies for the CFTR potentiators review, we searched the Cystic Fibrosis trials register with the term; 'drugs that augment function of abnormal CFTR protein in the cell membrane.' An Example of the search strategy is demonstrated in Figure 11.

Figure 11 Example of search strategy for trials in the CFTR potentiators review

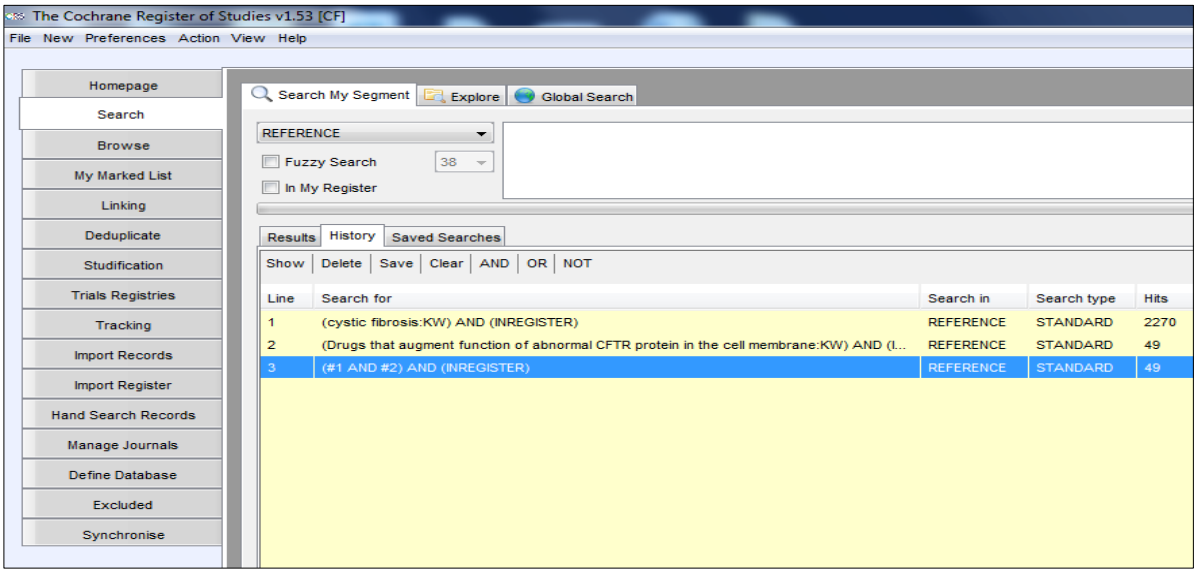


Figure 11 demonstrates the search strategy used for identifying trials for the CFTR potentiators review. All relevant trials had been tagged with the key term 'drugs that augment function of abnormal CFTR protein in the cell membrane.'

The date of the last search of the Cystic Fibrosis trials register for studies to include in the CFTR potentiators review was the 13th February 2014. We also searched clinical trial registries maintained by the European Medicines Agency, the US National Institute of Health (clinicaltrials.gov) and the World health organisation (WHO). We identified on-going studies by using the terms 'Cystic Fibrosis AND Ivacaftor OR VX-770' and 'Cystic Fibrosis AND genestein.' The date of the last search of the trials registries maintained by these organisations was the 6th February 2014.

The same search strategy was employed to search for eligible RCTs of CFTR correctors. For CFTR corrector therapies, studies relevant to the keywords; "corrector", "CPX", "VX-661", "VX-809", "phenylbutyrate", "glycerol" and "miglustat" were identified and tagged and all relevant studies were tagged with the key term ; 'drugs that correct defects in CFTR transcription, translation or processing.' So on conducting the review, we entered the term; 'drugs that augment function of abnormal CFTR protein in the cell membrane' into the Cystic Fibrosis trials register to identify relevant trials.

Figure 12 Example of search strategy for trials in the CFTR correctors review

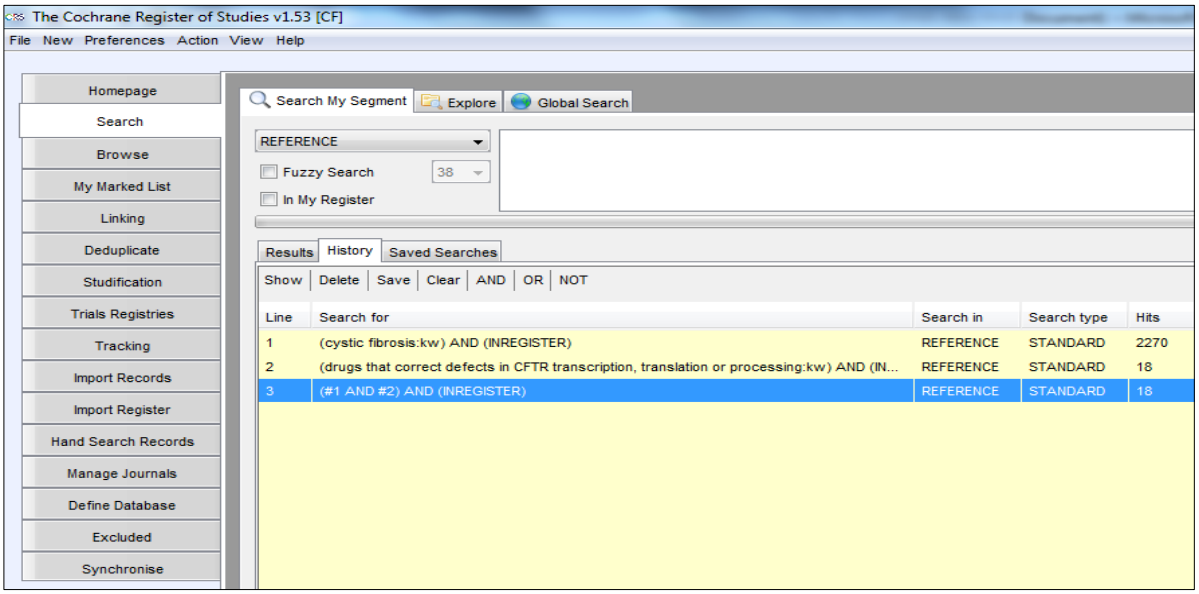


Figure 12 demonstrates the search strategy used for identifying trials for the CFTR potentiators review. All relevant trials had been tagged with the key term 'drugs that correct defects in CFTR transcription, translation or processing.'

The date of the last search of the Cystic Fibrosis trials register for studies to include in the CFTR correctors review was the 13th February 2014. We also searched clinical trial registries maintained by the European Medicines Agency, the US National Institute of Health (clinicaltrials.gov) and the WHO for additional studies using CFTR correctors. We identified relevant on-going studies by using the terms "Cystic Fibrosis and corrector", "Cystic Fibrosis and CPX", "Cystic Fibrosis and VX-661", "Cystic Fibrosis and VX-809", "Cystic Fibrosis and phenylbutyrate", "Cystic Fibrosis and glycerol" and "Cystic Fibrosis and miglustat." The date of the last search of the trials registries maintained by these organisations was the 13th February 2014.

2.3.2 Searching other resources

We also screened references of included trials and contacted the authors of included trials, leaders in the field and pharmaceutical companies to identify any published and unpublished studies that may have been missed by these searches.

2.4 Data collection, risk of bias assessment and statistical analysis

2.4.1 Selection of studies

Two authors (IS and SP) independently assessed the suitability of each trial for inclusion into the review using the pre-defined eligibility criteria. If disagreements arose on the suitability of a trial for inclusion, we attempted to reach a consensus by discussion, failing which a third author arbitrated. Trials were included, excluded or considered on-going.

2.4.2 Data extraction and management

Once we had agreed on eligible studies, we independently extracted relevant data from each included trial using a standardised data extraction form. If disagreement arose on data extraction, we attempted to reach a consensus by discussion, failing which a third author arbitrated.

We reported on our primary outcome 'survival' as a binary outcome as time-to-event data was not available. Where possible, we reported the relative change from baseline in FEV_1, since this way of presenting FEV_1 accounts for variance in baseline characteristics between groups and is very important in clinical practice. If this was not possible, we reported absolute change from baseline in FEV_1 as a secondary outcome. We planned to report on QoL as the relative change from baseline in QoL but it was not possible to do this and insert the result in the analysis. We therefore extracted QoL as the absolute change from baseline.

With regards to the secondary outcome 'adverse effects' we extracted the total number of participants who experienced adverse effects and the total number of participants who required trial drug interruption or termination (Table 7)

Table 7 Example of a data extraction table for moderate and severe adverse effects of therapy

		Study ID	Study ID
Number of participants that required study drug Interruption (moderate)	Intervention group		
	Placebo group		
Total number of participants that required study drug Interruption	Intervention group + placebo group		
Number of participants that require study drug discontinuation (severe)	Intervention group		
	Placebo group		
Total number of participants that require study drug discontinuation	Intervention group + placebo group		

When extracting data on pulmonary exacerbations, we noted whether they were protocol-defined or physician-defined. We reported the number of participants who experienced episodes of pulmonary exacerbation and reported time-to-exacerbation data if possible. We extracted the number of participants who required hospitalisations, the average number of days hospitalised, and the number of participants who required extra courses of antibiotics.

For the secondary outcomes 'change from baseline in weight' and 'change from baseline in sweat chloride concentration', we extracted the absolute change from baseline results. Where data were provided on acquisition or eradication of respiratory pathogens, or radiological measures of lung disease, we reported data using the scoring system employed by the trialists. If data were reported on school or work attendance, we planned to report on the number of days missed.

For continuous outcomes, we extracted means and standard deviations (SDs). Where SDs were not provided, we calculated the standard error of the mean (SEM) from the 95% confidence intervals (CIs) and inserted the results into a generic inverse variance (GIV) analysis. Where outcomes were reported as dichotomous data, we compared the results in the intervention group to the results in the placebo group in the analysis. Where trials with multiple intervention groups reported dichotomous data i.e. adverse effects, we pooled the data to form one intervention group and compared data to the placebo group as recommended by the Cochrane Handbook (section 16.5.4).[165] Where multiple intervention groups were present, and it was not appropriate to combine the data from treatment groups, we presented the data in a table in the text. If the trial author presented non-parametric data, we reported results in the written text and not in the analysis.

For the potentiators review, we reported data at 4 weeks, 16 weeks, 24 weeks and 48 weeks. In the correctors review, we reported results as short term (less than one month), long term (less than six months) and longer term (greater than six months).

2.4.3 Risk of bias assessment (methodological quality)

Bias is defined as *systematic error, or deviation in truth in results that can lead to underestimation or overestimation in the true effect of the intervention*.[166] It is ascribed to flaws in design, conduct or analysis. In systematic reviews, risk of bias (RoB) influences the internal validity of the review, and thus the conclusions that can be drawn from it. Therefore, it is imperative that RoB assessment is part of critical appraisal process.

In Cochrane systematic reviews, bias is assessed using the Cochrane Risk of Bias Tool (Table 8).[167] This tool involves assessment of following six RoB domains; 1) selection bias 2) performance bias 3) attrition bias 4) detection bias 5) reporting bias and 6) other sources of bias. Trials are judged as having either low risk, unclear risk or low risk of bias.[167] The advantage of the Cochrane risk of bias tool over scales or category tools is that the judgements (high risk, low risk or unclear risk) are supported by a narrative explanation so readers can clearly see what methodological features influenced the decision on risk of bias. Also, with this tool, figures can be created to demonstrate graphically the risk of bias across the included trials. The disadvantage of this tool is that the assessments are subjective and therefore are dependent on the skill and experience of the assessors.[168] For this reason, the Cochrane handbook provides examples of methodological features that should influence judgement, and for each review at least two authors are required to carry out the assessment. In these systematic reviews, two authors (IS and SP) assessed the risk of bias for each trial and If disagreement arose, attempts were made to reach a consensus by discussion, failing

which a third author (KWS or MS) arbitrated. Further description of the risk of bias domains is provided.

Table 8 Cochrane Risk of Bias Tool

Domain	Support for judgement	Review authors' judgement
Selection bias.		
Random sequence generation.	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence.
Allocation concealment.	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.	Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment.
Performance bias.		
Blinding of participants and personnel *Assessments should be made for each main outcome (or class of outcomes).*	Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Performance bias due to knowledge of the allocated interventions by participants and personnel during the study.
Detection bias.		
Blinding of outcome assessment *Assessments should be made for each main outcome (or class of outcomes).*	Describe all measures used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Detection bias due to knowledge of the allocated interventions by outcome assessors.
Attrition bias.		
Incomplete outcome data *Assessments should be made for each main outcome (or class of outcomes).*	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors.	Attrition bias due to amount, nature or handling of incomplete outcome data.
Reporting bias.		
Selective reporting.	State how the possibility of selective outcome reporting was examined by the review authors, and what was found.	Reporting bias due to selective outcome reporting.
Other bias.		
Other sources of bias.	State any important concerns about bias not addressed in the other domains in the tool. If particular questions/entries were pre-specified in the review's protocol, responses should be provided for each question/entry.	Bias due to problems not covered elsewhere in the table.

Table 8 demonstrates the different risk of bias domains included in the Cochrane Risk of bias too, factors for review authors to consider when assessing these domains and the bias they can introduce.[168]

Selection bias

Selection bias refers to systematic differences in baseline characteristics in enrolled participants. [169] Baseline characteristics in clinical trials of CF include demographic variables (age, gender, race, genotype) and prognostic variables (FEV_1, BMI, sweat chloride concentration) that act as potentially confounding factors.[170] If participants with particular baseline characteristics are deemed likely to benefit from the intervention, and subsequently assigned to the intervention group, this can lead to an over-estimation of the treatment effect. Assessment of selection bias has two parts; 1) random sequence generation and 2) allocation concealment (random sequence implementation). Random sequence generation refers to the generation of a random sequence to assign participants to either intervention or control group. When steps are taken to secure implementation of the random allocation sequence without foreknowledge of treatment assignments allocation is said to be

concealed.[171] Inadequate randomisation and or allocation concealment have been demonstrated to over-estimate the treatment effect.[172]

Random sequence generation

Proper randomisation ensures that each participant enrolled into the study has an equal chance of being allocated to either the control or the intervention group. Improper randomisation over-estimate the treatment effect and type 1 error (falsely rejecting the null hypothesis).[173] We assessed what methods trialists employed to generate a random sequence and judged the method as either posing a high, low or unclear risk of bias. If, for example, a computer generated a list of random assignments, this would have been deemed low risk of bias. Trials that simply stated that they were 'randomised' and did not provide a method of randomisation were judged to have an unclear risk of bias.

Allocation concealment

Allocation concealment ensures that neither participants nor study personnel are aware of which groups participants will be assigned to. It occurs before the intervention is administered and therefore can always be implemented (unlike blinding).[174] Improper allocation concealment can undermine the effects of random assignment as trialists, who are aware of assignment, can exclude participants based on allocation to the 'inappropriate' group. Results from clinical trials have demonstrated that improper allocation concealment can affect the estimated treatment effect by up to 40%.[171] In these systematic reviews, we assessed what methods trialists employed to conceal allocation and judged the method as posing either a high, low or unclear risk of bias. If for example, participants were allocated through a secure computerised voice system, we considered this method of having a low risk of bias.

Blinding of study personnel and outcome assessors (performance bias and detection bias)

Performance bias relates to differences in the way participants are treated with regards to care and/or exposure to other factors by study personnel.[169] It is minimised by blinding (masking) personal involved with the conduct of the study; for example clinicians after allocation. Detection bias refers to differences in how outcomes are assessed.[169] It is minimised by blinding outcome assessors to treatment assignment. Blinding of study personnel prevents knowledge of treatment assignment and thus favourable care towards participants in a particular group. We assessed whether study personnel and outcome assessors were blinded, and the methods employed to maintain blinding.

Blinding of study participants (Performance bias)

Inadequate blinding of study personnel also makes the trial vulnerable to performance bias.[169] It is particularly important when considering PROs, which are vulnerable to being influenced by patient's

pre-existing beliefs regarding the benefits or harms of the intervention. In addition, surrogate outcomes, for example FEV_1, can differ due to the participant's enthusiasm, driven by their allocation to a particular group.

In both reviews we assessed whether trialists blinded participants to treatment assignments and the methods used to maintain the blind. We specifically assessed for reports on matching of the intervention and placebo for example in taste, colour, size, shape of oral tablet to and searched the trial report for evidence of similar treatment schedules between groups. Any evidence to suggest the possibility of participants finding out about treatment assignment, for example different coloured tablets between groups, was judged to have a high risk of bias.

Attrition bias

Attrition bias refers to systematic differences in withdrawals from the study.[169] It has two components; 1) **withdrawals** - referring the proportion of participants who withdrew from the study and 2) **missing data** - referring to whether an intention to treat analysis (ITT) was employed in the analysis. Participants can withdraw from the trial due to various reasons; adverse effects, loss to follow up and by patient's request. If the differences in characteristics between groups created by withdrawals relates to outcome measures, this can introduce attrition bias.[175] There is no specific proportion of withdrawal at which attrition bias is considered a problem. However, if a high proportion of participants (approximately >20%) withdraw from the study, authors should be concerned about the possibility of attrition bias.[176] In an ITT analysis, data from all randomised participants is used in the analysis, ignoring withdrawals, protocol deviations and noncompliance occurring after randomisation. This approach prevents exclusion of withdrawn participants with poor prognostic characteristics from being omitted from the analysis and thus over-estimation of treatment effect.[177]

In order to assess the impact of withdrawals on attrition bias, we calculated the percentage of participants who withdrew. If <15% of overall participants withdrew, the trials were judged to be of low risk of attrition bias in relation to withdrawals. In order to assess whether an intention-to-treat analysis was conducted, we extracted data on the number of participants with each outcome event, by allocated treated group, irrespective of compliance and whether or not the participant was later thought to be ineligible or otherwise excluded from treatment or follow-up. We also checked to see whether this information was consistent with the data presented on the US online trials registry (ClinicalTrials.gov). If data were missing or unclear, we sought why these participant's data had been excluded, either by scanning the text or by contacting the primary investigators for clarification. If participant data had been excluded from the analysis for reasons that could introduce bias, it was

considered to have a high risk of attrition bias in relation to missing data. For example, if intervention assigned participant data were excluded because they withdrew due to adverse effects, this could have led to an over-estimation of the treatment effect because these participants may have had unfavourable measurements. If the trial was deemed to have a high risk of attrition bias with relation to either withdrawals or missing data, it was judged to be of a high risk of overall attrition bias.

Outcome reporting bias

Outcome reporting bias refers to selective reporting of some outcomes but not others.[178] It is found in a large proportion of published trials and therefore represents a significant problem that affects the conclusions of systematic reviews.[179] The different factors involved in assessment of a trial for selective outcome reporting are shown in table 9.[180, 181]

Table 9 Factors to consider when assessing a trial for selective outcome reporting

1. **Selective omission of outcomes**: outcomes present in review protocol or the methods of the full report are not reported in the results.
2. **Incomplete reporting of outcome data**: outcome data is not reported in sufficient detail for inclusion in meta-analysis (e.g. only reporting P values and not 95%CIs)
3. **Selective choice of measurement for assessing an outcome**: reporting on outcomes using a form of measurement that was not pre-specified
4. **Selective reporting of analysis using the same data:** reporting on outcomes using a form of analysis that was not pre-specified
5. **Selective reporting of subsets of the data**: reporting on outcomes on a subset of participants that was not pre-specified
6. **Selective reporting of data for outcomes not pre-specified in trial protocol**: reporting on an outcome that was not included in the protocol.
7. **Omission of key outcomes**: omission of outcomes considered key and expected to have been reported in such clinical trial.

In these reviews, in order to identify selective outcome reporting, we compared outcomes described in the trial protocol with those reported in the publications. We requested protocols for specific trials from the primary investigators when they were not available. We have recorded the proportion of protocols that were available to us. If the protocol was not available or provided, we checked available information on the trial registry databases (e.g. clinicaltrials.gov). We also compared outcomes listed in the 'Methods' section of the final paper with those presented in the

'Results' section. If the published papers reported negative findings either only partially, or not at all, we contacted primary investigators for these data. If discrepancies in outcome reporting were found we judged the trials to have a high risk of outcome reporting bias because of the association this has with over-estimation of the treatment effect.[182] We also assessed the publications for points 2-7 from table 9, and where potential sources of selective outcome reporting were identified; we contacted the trial authors for clarification. Whether selective reporting in points 2-7 were judged sufficient reason to judge the trial as having a high risk of selecting outcome reporting bias, was based on the subjective assessments of the authors.

We planned to assess publication bias by constructing and assessing the symmetry of a funnel plot. This would have been possible if we had included more than 10 trials in the review.

Other sources of bias

This section is for all other potential sources of bias.[168] The review authors assessed for differences in baseline characteristics.

Overall methodological quality

For each review, we assessed the overall methodological quality of the included studies from the assessments of the risk of bias in each domain for each study.

2.4.4 Statistical analysis

Measurement of treatment effect

Meta-analysis was conducted with RevMan 5.

For binary outcomes, we calculated a pooled estimate of the treatment effect for each outcome using the pooled odds ratio (OR) and 95% CIs. If calculating a pooled OR was not appropriate, we calculated an estimate of the treatment effect for each outcome using the OR and 95% CIs.

For continuous outcomes, we calculated the mean change from baseline for each group or the mean post-intervention values and 95% CIs for each group. We produced a pooled estimate of treatment effect by calculating the mean difference (MD) and 95% CIs. For QoL, CFQ-R was the most frequently used questionnaire and so we calculated the MD and 95% CIs. No other questionnaire was used.

For time-to-event outcomes, such as 'time to first pulmonary exacerbation', we used measures of survival analysis, and calculated hazard ratios (HR) and 95% CIs between different arms of the trial.

Where included trials did not report change data, but instead presented absolute post-treatment data without baseline data so it was not possible to calculate change data, we planned to use absolute post-treatment data instead of change from baseline. However, if the report presented

baseline and post-treatment data for any outcome, we calculated SDs for the change from baseline, for example if the CI was available. If there was not enough information available to calculate the SDs for the changes, we planned to impute them from other trials in the review, where data were available and trials were similar (i.e. when they used the same measurement scale, had the same degree of measurement error and had the same time periods between baseline and final value measurement). If neither of these methods were possible, we planned to calculate a change-from-baseline SD, making use of an imputed correlation coefficient (methods described in section 16.1.3.2 in the Cochrane Handbook of Systematic Reviews of Interventions).[165]

Unit of analysis issues

Within these reviews, we only included results from RCTs of parallel design in which individual trial participants were randomised. We excluded cross-over trials, because they are not appropriate for evaluating therapies that potentially correct the underlying defect.

Heterogeneity

In systematic reviews, data from several trials are synthesised to create an overall pooled estimate. One can expect there to be differences in results between included trials. However it is important to determine whether this variability (heterogeneity) is due to either 'random play of events' (chance), in which case synthesis of data is appropriate, or other factors that further require investigation.[183] Heterogeneity requiring further investigation can be categorised into three types: clinical, methodological and statistical. Clinical heterogeneity refers to differences in the participants, interventions and outcomes between each trial. When there is variability in study design and or risk of bias, it is known as methodological heterogeneity. Statistical heterogeneity considers both clinical and methodological heterogeneity and refers to differences in results being more different than expected if simply due to random chance.[184] In this review, statistical heterogeneity is referred to simply as heterogeneity.

We planned to assess heterogeneity through a visual examination of the forest plots.[185] If the results of studies 'lined up' on the forest plot, we assumed little heterogeneity. We also considered the I^2 statistic together with chi^2 values and their CIs.[184] This reflects the likelihood that the variation of results across trials was due to heterogeneity rather than chance, and we interpreted the I^2 statistic using the following classification:

- 0% to 40%: might not be important;
- 30% to 60%: may represent moderate heterogeneity;
- 50% to 90%: may represent substantial heterogeneity;
- 75% to 100%: considerable heterogeneity.

This test was designed for analysis of heterogeneity in meta-analysis including large number of trials. Therefore we interpreted these test results with caution when only a small number of included trails were included.[184]

Data synthesis

Meta-analysis can use either a fixed effect or a random effects statistical model. A fixed effect model is used when there is no heterogeneity between included studies so the effect estimate of the treatment is the same between including trials and any variability is due to chance alone. A random effects model assumes that estimates of treatment effect vary between included trials due to both real differences in treatment effect and chance.[186] In the potentiators review, we used a fixed-effect model to analyse data from trials that we did not consider to be heterogeneous. When substantial or considerable heterogeneity was present (I^2 greater than 50%), we used a random-effects model to analyse data.

In the correctors review, different interventions were being assessed (clinical heterogeneity) and so we always employed random effects model.

Subgroup analysis and investigation of heterogeneity

We planned to investigate any heterogeneity that we identified using subgroup analyses of potential confounding factors, if sufficient numbers (at least 10 trials) were available. For this review, we planned that these confounding factors would be:

- age (children (defined as younger than 18 years of age) versus adults);
- gender;
- different mutation classes (Table 1)

As we did not seek individual patient data from trial investigators, we did not undertake a subgroup analysis on the basis of disease severity.

Sensitivity analysis

If we had been able to combine a sufficient number of trials (at least 10), we planned to examine the impact of risk of bias on the results examined by comparing meta-analyses including and excluding trials with concerns of high risk of selection or reporting bias due to issues relating to randomisation, allocation concealment, or masking of interventions from participants or trial personnel.

Chapter 3

Results

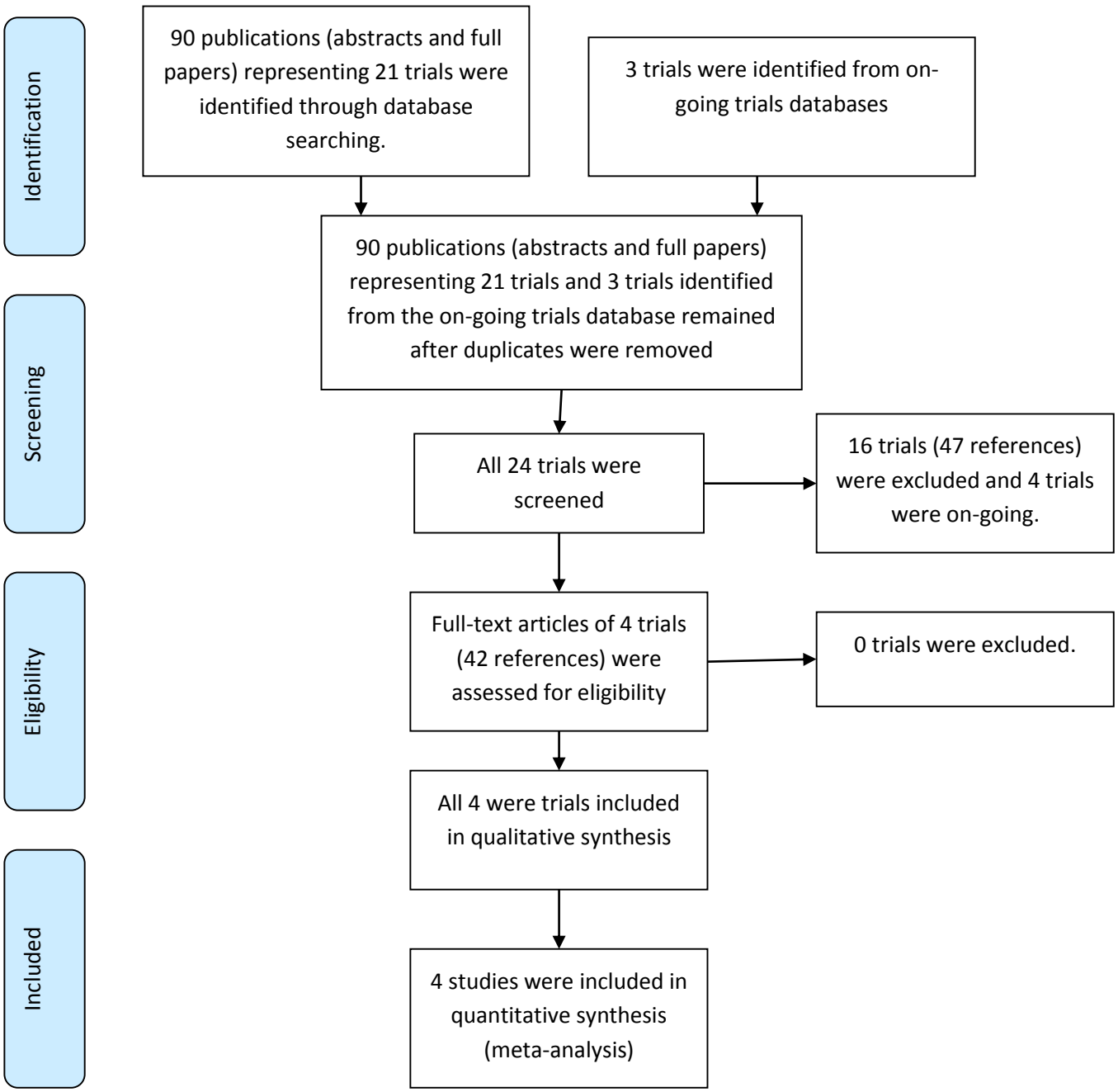
In this section, I have highlighted the main findings of the reviews that are attached to this thesis. I have also report the results of the discussions held between the Cochrane Cystic Fibrosis Systematic Reviews team whilst conducting the reviews. For a more detailed report of the results please refer to result section of the completed reviews, attached in the appendix.

3.1 Summary of findings from the CFTR potentiators review

3.1.1 Results of the literature search

We identified a total of 90 publications representing 21 trials.[117-120, 123, 125, 127, 133, 135, 187-197] All publications were identified from searches of electronic databases and hand searching of conference abstracts. No additional studies were identified by contacting the pharmaceutical company responsible for conducting clinical trials investigating Ivacaftor (E-mail 1). After application of the eligibility criteria to the 21 trials, four trials (42 references) were included[198-201], 16 trials (47 references) were excluded[117-120, 123, 125, 127, 133, 135, 187-192, 197] and four were ongoing[193-196]. Results of the search are displayed in a PRISMA flow diagram (Figure 13). Relevant data were extracted from each of the four eligible trials by two authors (SP and IS) independently and when comparing the two data exertion forms, no disagreements arose. The data extraction forms for each of the 4 studies are included in the appendix (Data extraction Tables 1-4).

Figure 13 A PRISMA flow diagram demonstrating the results of the literature search.



3.1.2 Characteristics of included studies

Four trials with 378 participants met the inclusion criteria for this review.[198-201] All included trials were multicentre RCTs of parallel design, sponsored primarily by Vertex Pharmaceuticals Incorporated and examined the impact of Ivacaftor. In three of the four trials, possessing at least one G551D allele (Class III) was an important eligibility criterion. The first G551D trial was a phase 2 trial enrolling patients 18 years and over (n=19) and measuring a number of outcomes at 14 and 28 days. We called this study the Phase 2 G551D study.[201] This was followed by two phase 3 G551D trials, the first enrolling patients ages 12 years and older (n = 167) (child phase 3 G551D study) and the second enrolled patients aged 6 to 11 years (n = 52) (adult phase 3 G551D study). These trials reported outcomes at 24 and 48 weeks.[198, 200] The remaining trial examined Ivacaftor for patients with ΔF508 and enrolled participants aged 12 years or over (n = 140) for a duration of 16 weeks (Flume 2011). We called this trial the ΔF508 trial.[199] The primary end-points for these studies were safety[201] or change in FEV_1[198, 200] or both.[199] A summary of the characteristics of the included studies (Table 10), on-going studies (Table 11) and reasons for exclusions (table 12) are provided.

We approached all the trial authors of the included studies for data on outcomes reported in our systematic review, but not reported in the published trials (E-mail 1, 4, 7 and 11). Two out of the four authors responded, with no additional data to add (E-mail 2 and 4). In light of the response from Vertex in E-mail 1 we did not approach the authors of the three on-going studies (DeBoeck 2014, Moss 2013, Nick 2014) also sponsored by Vertex for unpublished data. The remaining on-going study had only recently begun and was still recruiting patients.[196]

Table 10 Characteristics of studies included in the CFTR potentiators review

	Study design				Participants			Interventions		Outcomes	
Study	**Study design**	**Commercial research support**	**Treatment period (weeks)**	**Number of sites**	**Number of participants**	**Age group (years of age)**	**Genotype**	**Intervention**	**Control**	**Primary outcome**	**Secondary outcomes**
Accurso 2010[201]	RCT of parallel design	Vertex Pharmaceuticals Incorporated	4	13 sites in North America and Europe	19	> 18	≥ 1 G551D	150mg and 250 mg Ivacaftor BD	Placebo	Safety	NPD. Δ BL QoL, Spirometry, sweat cl
Ramsey 2011[198] (adult G551D)	RCT of parallel design	Vertex Pharmaceuticals Incorporated	48	29 sites in North America, Europe and Australia	167	> 12	≥ 1 G551D	150mg Ivacaftor BD	Placebo	absolute change in FEV_1	Δ BL spirometry, weight, sweat cl, CFQR score & safety
Davies 2013[200] (child G551D)	RCT of parallel design	Vertex Pharmaceuticals Incorporated	48	65 sites in North America, Europe and Australia	52	6-11 years	≥ 1 G551D	150mg Ivacaftor BD	Placebo	absolute change in FEV_1	Δ BL spirometry, weight, sweat cl CFQR score & safety
Flume 2011[199] (ΔF508 study)	RCT of parallel design	Vertex Pharmaceuticals Incorporated	16	34 sites in North America	140	> 12	Homozygous ΔF508	150mg Ivacaftor BD	Placebo	Safety and absolute change in FEV_1	Δ BL sweat cl, weight, CFQR score

Table 11 Characteristics on-going of studies examining CFTR potentiators

Study and title	Sponsor	Methods	Participants. Confirmed diagnosis of CF +:	Interventions	Outcomes (NB Δ BL indicates change from baseline)	Status at literature search
De Boeck 2013 [193] *"Ivacaftor in Subjects With Cystic Fibrosis Who Have a Non-G551D CFTR Gating Mutation (KONNECTION)." (NCT01614470)*	Vertex Pharmaceuticals Incorporated	A phase 3, double-blind, RCT of cross-over design.	1. At least 1 allele of pre-specified gating mutations. 5. ≥ 6 years old	Ivacaftor 150 mg orally 2x daily.	Primary; Δ BL FEV_1. Secondary: Δ BL in BMI, Sweat chloride and CRQ-R score	No data available
Moss 2013[194] *"Study of Ivacaftor in Subjects With Cystic Fibrosis Who Have the R117H-CFTR Mutation (KONDUCT)" NCT01614457*	Vertex Pharmaceuticals Incorporated	Phase 3, double-blind, RCT of parallel design with outcomes measured at 24 weeks	1. At least 1 allele of the R117H-CFTR mutation. 3. ≥ 6 years old	Ivacaftor 150 mg orally 2x daily for 24 weeks.	Primary: Δ BL FEV_1. Secondary: Δ BL BMI , Sweat chloride, CRQ-R score and time to first pulmonary exacerbation	No data available
Nick 2014 [195] *"Study Testing the Effect of Ivacaftor on Lung Function in Subjects With Cystic Fibrosis and Residual CFTR Function"* (*NCT01685801)*	Vertex Pharmaceuticals Incorporated	Phase 2, double-blind RCT of crossover design with 4 treatment arms.	1. Clinical evidence of residual CFTR function 4. ≥ 12 years old	Ivacaftor 150mg orally 2x daily.	Primary: Δ BL FEV_1 (4 weeks) Secondary: LCI (4 and 8 weeks), Δ BL FEV_1, sweat chloride, weight (8 weeks) and Safety.	On-going, no data available
Nielson 2014 [196] "Short-Term Effects of Ivacaftor in Non-G551D Cystic Fibrosis Patients"(NCT01784419)	University of California, San Francisco	Double-blind RCT of cross-over design.	1. Signs of residual CF channel function.	Ivacaftor 150 mg 2x daily.	Primary: Δ BL Sweat chloride. Secondary: spirometry and multi-breath washout testing.	Still recruiting patients

Table 12 Studies excluded from the CFTR potentiators review

Study ID	Reason for exclusion
Accurso 2013[187]	Cross-over trial.
Donaldson 2013[123]	Compared CFTR potentiators with another mutation-specific therapy and not placebo.
Clancy 2012[125]	Intervention to correct molecular defect, not potentiate.
Davies 2012[189]	Cross-over design.
Altes 2011[188]	Cross-over design.
Boyle 2011[127]	Compared CFTR potentiators alongside another mutation-specific therapy.
Sermet-Gaudelus 2010[135]	Intervention for stop codon mutations.
Wilschanski 2008[197]	Intervention for stop codon mutations.
Rubenstein 2006[192]	Intervention to correct molecular defect, not potentiate.
Wilschanski 2003[133]	Intervention for stop codon mutations.
Zeitlin 2002[119]	Intervention to correct molecular defect, not potentiate.
McCarty 2002[120]	Intervention to correct molecular defect, not potentiate.
Pradal 2002[190]	Intervention for stop codon mutations
Romano 2000[191]	Intervention for stop codon mutations
Chadwick 1998[117]	Intervention to correct molecular defect, not potentiate.
Rubenstein 1998[192]	Intervention to correct molecular defect, not potentiate.

3.1.3 Risk of bias in included studies

Two authors (myself and IS) individually assessed each of the four included trials for risk of bias. We found the risk of selection bias amongst the included studies to be low as three of four included studies sufficiently demonstrated how random sequences were generated and how participant allocations were concealed.[198, 199, 201] The same three studies also demonstrated that necessary measures had been taken to blind site personnel and outcome assessors.[198, 199, 201] However, we found that none of the included studies sufficiently demonstrated how participants were blinded. In each study, there was a lack of description on the details of the orally administered Ivacaftor tablet i.e. taste, colour and how it compared to placebo.[198-201]

To assess for attrition bias we calculated the percentage of withdrawals in each trial and determined whether an intention-to-treat (ITT) approach had been employed. In all four studies there was low attrition (less than 15%).[198-201] However, three studies were found to have excluded relevant data from the analysis (not ITT).[198-200] We approached primary authors of these trials for additional information about missing data (E-mail 2, 4, 8). Only study personnel from the sponsoring body involved with the child phase 3 G551D study responded. They stated that an ITT approach had not been employed and instead a modified ITT approach had been employed (not per-protocol) whereby only participant data that were available were included in the analysis (E-mail 5). As withdrawals from this study were due to reasons that could have potentially lead to patients with unfavourable or favourable characteristics from being excluded, (e.g. adverse events, withdrawal of consent), attrition bias remained high in this study.[200] The remaining two studies were also judged to have a high risk of attrition bias because participant data were excluded for no apparent reason.[198, 199, 201]

With regards to selective outcome reporting bias, a high risk was identified in all four trials.[198-201] This was due to either not reporting outcomes pre-defined in the protocol or failure to report key outcomes that would be expected to have been reported. Initially, there was disagreement between the authors in whether failure to report a key CF outcome, that was measured during the trial but not pre-specified in the trial protocol, should be enough reason to judge a trial as having a high risk of selective outcome reporting bias. To resolve this, we first consulted the Cochrane handbook, which included the following statement as a criteria for the judgement of 'high risk' of bias; *"the study report fails to include results for key outcomes that would be expected to have been reported for such a study."* We then discussed this issue with another author (KWS) and considered this to be enough reason to judge a trial as having a high risk of selective outcome reporting bias. We approached all trial authors requesting 1) trial protocol if they were not already available 2) data for outcomes that were pre-defined in the protocol but not reported in the full report and 3) data for

key outcomes that were measured during the trial but not reported (E-mail 1, 3, 4, 6, 7, 9, 11) but no additional study protocols or data were provided.

We did not find any other sources of bias amongst the included trials.

Overall, we judged the methodological quality (risks of bias) in the included studies to be moderate. It was downgraded from high to moderate because of the high risk of attrition and selective outcome reporting bias across included studies (Figures 14 and 15). For more detail on the individual risk of bias assessments for each domain for each study, refer to the characteristics of studies section of the full review (attached).

Figure 14 Risk of bias assessment for each included trial in CFTR potentiators review

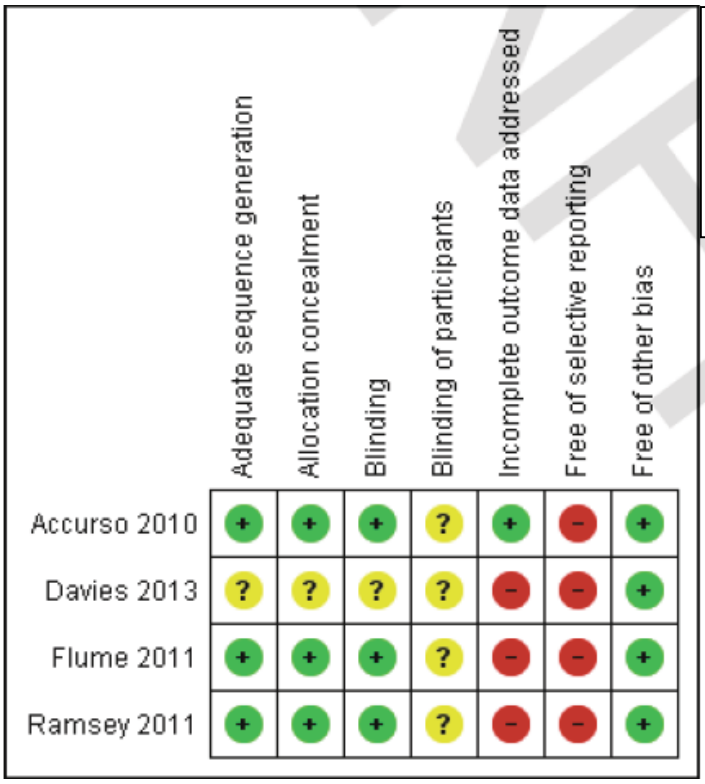


Figure 14 provides a summary of the individual risks of bias assessments for each study included in the CFTR potentiators review.

Risk of bias was based on author's judgements. The symbols represent different risk of bias: + represents low risk of bias, − represents a high risk of bias and ? indicates an unclear risk of bias.

Figure 15 Summary of risk of bias for each included trial using Cochrane Risk of Bias Tool

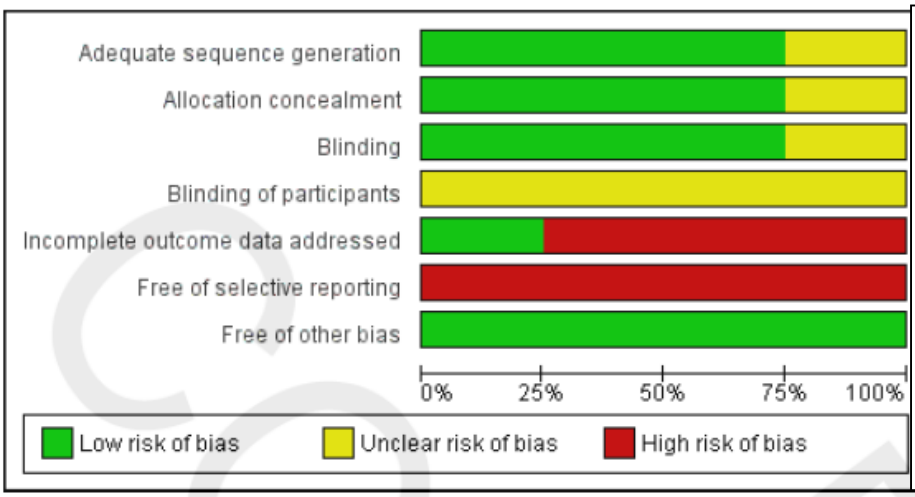


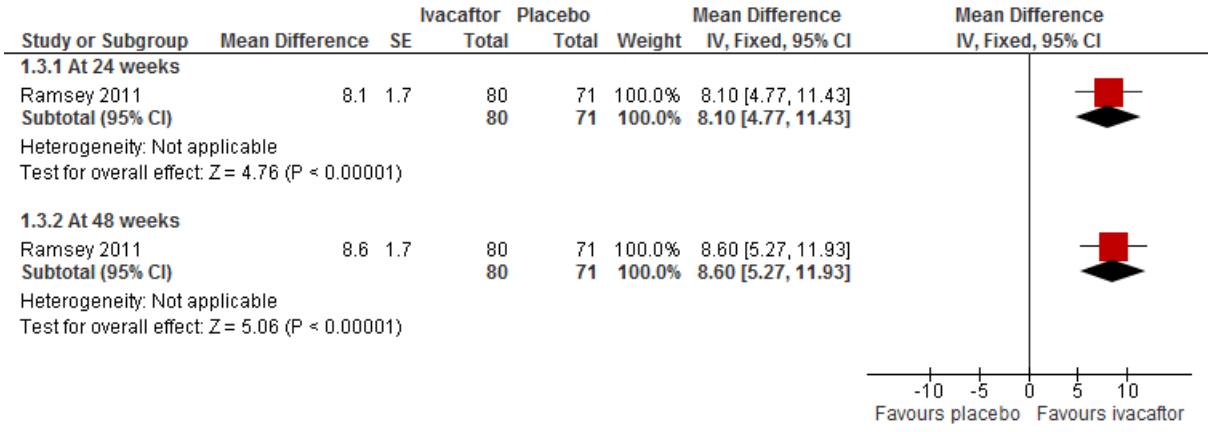
Figure 15 demonstrates the high risks of attrition bias (incomplete outcome data addressed) and selective outcome reporting bias across the included studies.

3.1.4 Effects of the intervention

This summary of the effect of the Ivacaftor on clinically important outcomes in patients with CF has been extracted from the discussion section of the CFTR potentiators review and highlights all the pertinent results.

With respect to this review's primary outcomes for this review, there were no deaths reported in any of the trials and the length and size of the trials precluded valid assessment of the impact of Ivacaftor on survival. For CFQ-R scores, the adults randomised to Ivacaftor in the phase 2 Accurso trial did not report significantly higher CFQ-R respiratory domain scores 28 days.[201] However, adults in the phase 3 Ramsey trial reported significantly higher CFQ-R respiratory scores at 24 weeks, MD 8.10 (95% CI 4.63 to 11.57) and at 48 weeks, MD 8.60 (95% CI 5.27 to 11.93) (Figure 16).

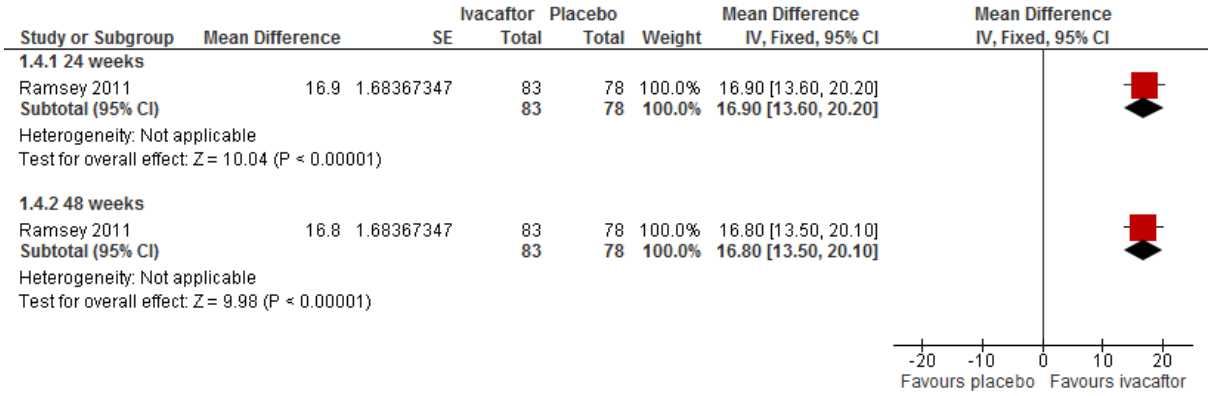
Figure 16 Forest plot demonstrating the change from baseline in CFQ-R respiratory domain scores at 24 and 48 weeks in the adult phase 3 G551D study



The plot in Figure 16 shows the advantage for treatment with Ivacaftor over placebo in CFQ-R respiratory domain scores, in adults with the G551D mutation. The point estimates at 24 weeks and 48 weeks demonstrate that participants reported higher CFQ-R when treated with Ivacaftor. The advantage can also be seen in the mean difference values and the 95%CIs suggest statistically significant advantage of Ivacaftor over placebo.

This finding was not reproduced in the paediatric participants[200] or the homozygous ΔF508 participants[199]. Again, for relative change in FEV_1, the phase 2 trial in G551D patients did not report treatment with Ivacaftor resulting in a significant improvement[201]. However, in the adult G551D phase 3 study, significant improvements in relative change in FEV_1 were seen early (after 15 days) and maintained through to 48 weeks, MD 16.80% (95% CI 13.50 to 20.10) (Figure 17).[198]

Figure 17 Forest plot demonstrating the relative change from baseline in FEV_1 at 24 and 48 weeks in the adult phase 3 G551D study



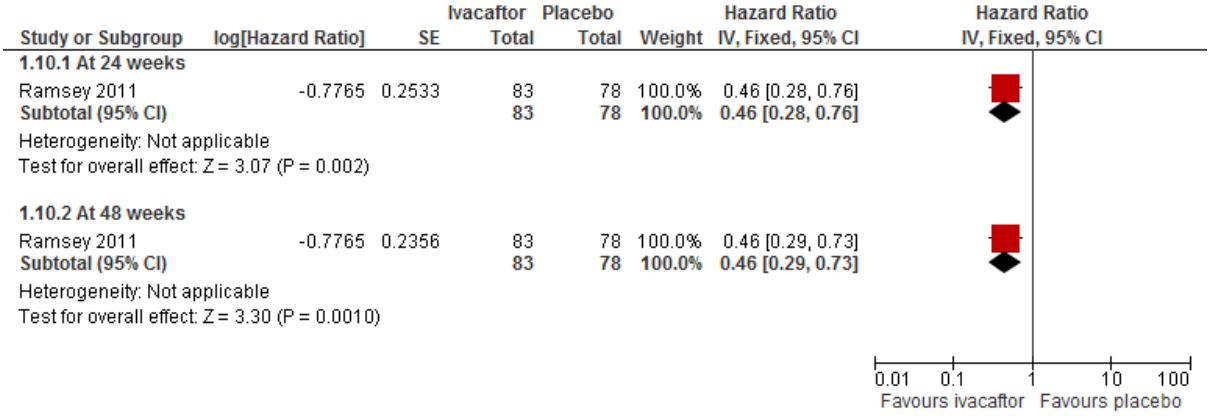
The plot in Figure 17 shows the advantage for treatment with Ivacaftor over placebo in relative change from baseline in FEV_1, in adults with the G551D mutation. The point estimates at 24 weeks and 48 weeks indicate that improvements in FEV_1 were reported in participants treated with Ivacaftor. The advantage can also be seen in the mean difference values and the 95%CI s suggest statistically significant advantage of Ivacaftor over placebo.

Significant improvements were also seen at 24 weeks in the paediatric trial, MD 17.4% (P <0.0001), but results at 48 weeks were not published.[200] No significant improvements in relative change in FEV_1 were reported in the ΔF508 trial.[199]

Combined data from the two G551D phase 3 trials demonstrated a reduced reporting of cough, OR 0.57 (95% CI 0.33 to 1.00) and reduced episodes of decreased pulmonary function, OR 0.29 (95% CI 0.10 to 0.82) in the Ivacaftor group. Increased reports of dizziness were recorded in patients

receiving Ivacaftor in the adult G551D trial,[198] but this adverse effect was not reported by any other trial.[199-201] No trial reported a significant increase in adverse effects leading to study drug interruption or study drug termination.[198-201] Combined data from the phase 3 G551D trials demonstrate significantly fewer 'serious' cases of pulmonary exacerbation in the Ivacaftor group, OR 0.34 (95% CI 0.17 to 0.70). When considering all data for exacerbations, fewer episodes were reported in adults in the Ivacaftor group by the phase 3 G551D trial, OR 0.54 (95% CI 0.29 to 1.01),[198] but this was not reported by the other trials.[199-201] Ramsey reported that more participants in the placebo group required hospitalisation and IV antibiotics for pulmonary exacerbations and a greater proportion of participants in the Ivacaftor group were exacerbation-free at the 24 and 48 week time-points (Figure 18).[198]

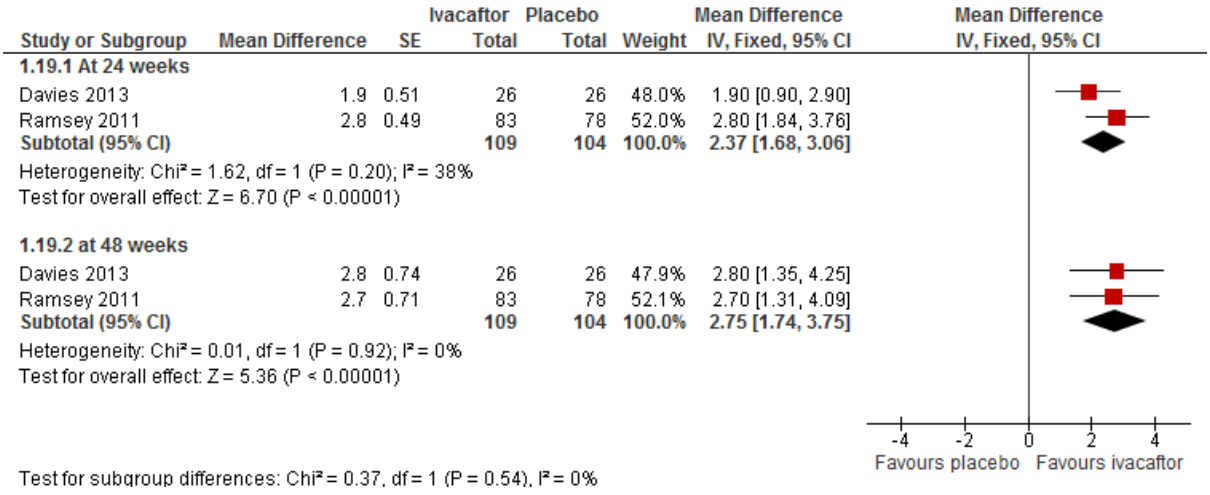
Figure 18 Forest plot demonstrating hazard ratios for the time to next pulmonary exacerbation in adults with the G551D mutation treated with Ivacaftor.



The plot in figure 18 shows the advantage for treatment with Ivacaftor over placebo in exacerbation-free time, in adults with the G551D mutation. The point estimates at 24 weeks and 48 weeks indicate that improvements in exacerbation free time were seen in participants treated with Ivacaftor. The advantage can also be seen in the hazard ratio values and the 95%CIs suggest a statistically significant advantage of Ivacaftor over placebo.

Both children and adults in the phase 3 G551D trials demonstrated significant weight gain on Ivacaftor at 24 weeks, MD 2.37kg (95% CI 1.68 to 3.06) and 48 weeks, MD 2.75 kg (95% CI 1.74 to 3.75) (Figure 19).

Figure 19 Forest plot demonstrating the change from baseline in weight in participants with G551D treated with Ivacaftor compared to placebo.

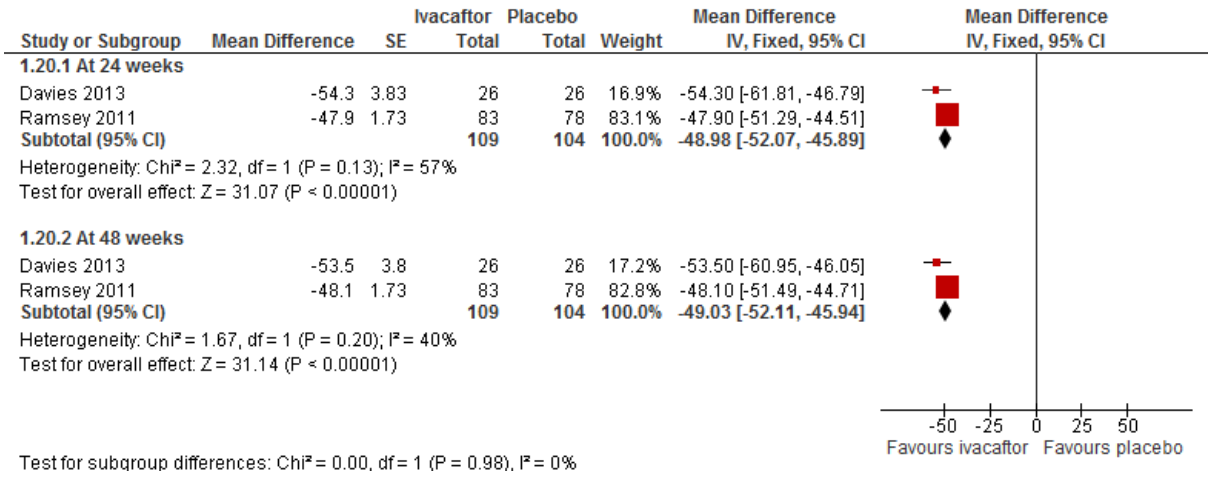


The plot in Figure 19 shows the advantage for treatment with Ivacaftor over placebo in the change from baseline in weight, in adults and children with the G551D mutation. The mean differences and point estimates at 24 weeks and 48 weeks both indicate that improvements in weight gain were reported in participants treated with Ivacaftor. The pooled mean difference values reinforce this and the 95% CIs suggest statistically significant advantage of Ivacaftor over placebo. There was no heterogeneity between trials.

These two trials also reported that significantly higher BMI scores were achieved in the Ivacaftor group; for children, MD 1.09 kg/m2 (P = 0.0003) and for adults, MD 0.93 kg/m2 (P < 0.0001). Significantly higher BMI-for-age z scores were reported in the phase 3 G551D paediatric trial in the Ivacaftor group at 24 weeks, MD 0.34 (P ≤ 0.001) and at 48 weeks, MD 0.45 (P < 0.001).[200] In a subset of participants aged 12 to 20 years old from the phase 3 G551D adult trial, a higher BMI-for-age Z score was reported at 48 weeks, MD 0.33 (P = 0.0490).[198] No significant weight gain or change in BMI scores were reported in ΔF508 trial.[199]

In the phase 2 G551D trial, Accurso reported significant reductions in sweat chloride concentration at 28 days in the 150 mg Ivacaftor group (median -64.5 mmol/L; P = 0.02) and 250 mg Ivacaftor group (median -43.0 mmol/L; P = 0.03)[201]. Combined data from the phase 3 G551D trials demonstrated significant reductions in sweat chloride concentration in participants on Ivacaftor at 24 weeks, MD -48.98 mmol/L (95% CI -52.07 to -45.89) and 48 weeks, MD -49.03 mmol/L (95% CI -52.11 to -45.94) (Figure 20).[198, 200]

Figure 20 Forest plot demonstrating the change from baseline in in sweat chloride concentration in participants with G551D treated with Ivacaftor compared to placebo.



The plot in Figure 20 shows the advantage for treatment with Ivacaftor over placebo in the change from baseline in sweat chloride concentration, in adults and children with the G551D mutation. The point estimates at 24 weeks and 48 weeks indicate reductions in sweat chloride concentration in participants treated with Ivacaftor. The advantage can also be seen in the mean difference values. The pooled mean difference values reinforce this and the 95%CIs suggest statistically significant advantage of Ivacaftor over placebo. There was no heterogeneity between trials.

The ΔF508 study, also reported a significant reduction in sweat chloride concentration, MD -2.9 mmol/L (95% CI -5.6 to -0.2).

3.1.5 Conclusion

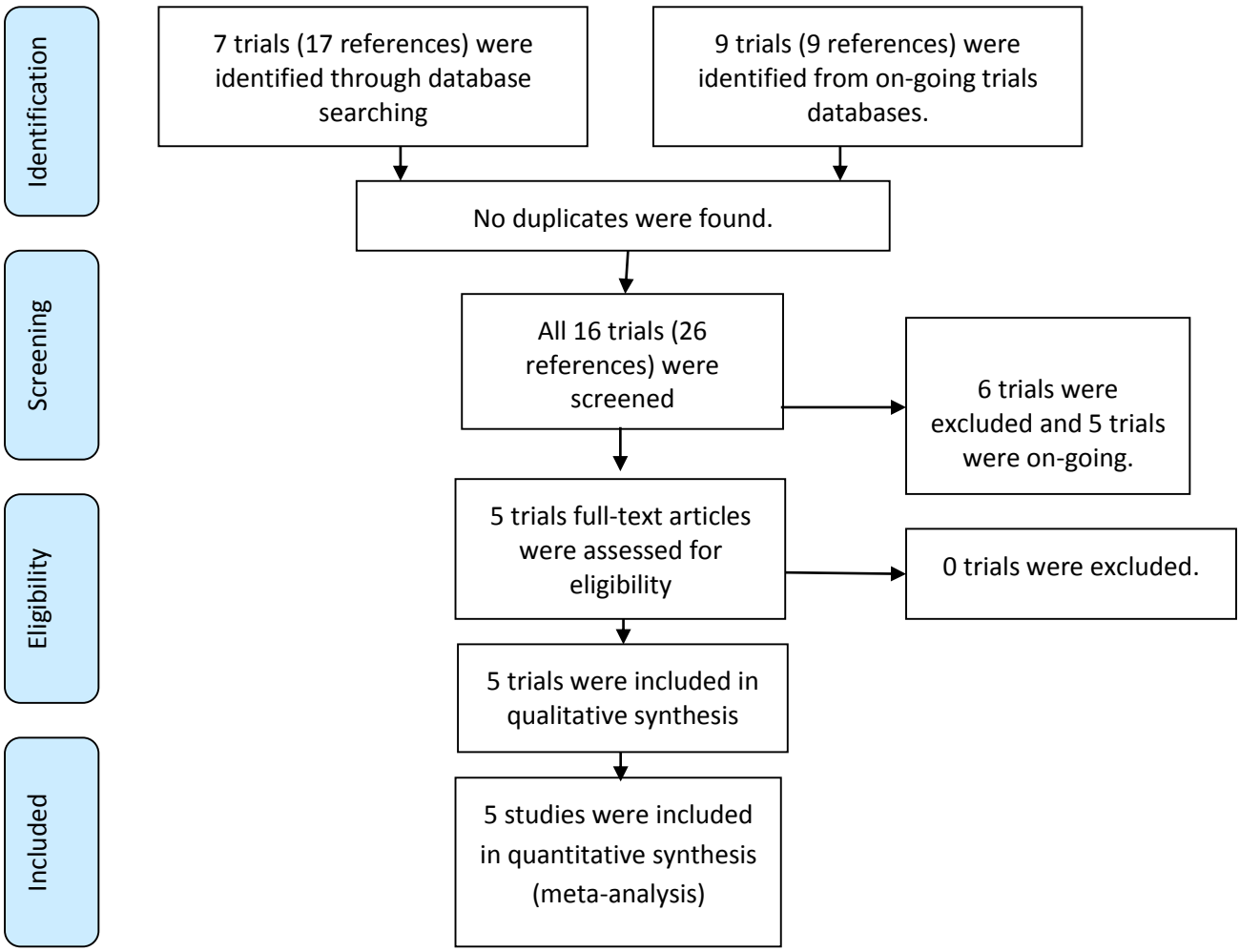
The G551D phase 3 trials demonstrated a clinically relevant impact of Ivacaftor on outcomes at 24 and 48 weeks, providing evidence for the use of Ivacaftor in children (over six years of age) and adults with CF and the G551D mutation. There is no evidence to support the use of Ivacaftor in ΔF508 patients. This evidence is based on trials of moderate methodological quality and readers should be aware of the high risks of attrition and selective outcome reporting bias found across studies.

3.2: Summary of findings from the CFTR correctors review

3.2.1 Results of the literature search

We identified a total of 25 publications representing 16 trials were identified from searches of electronic databases and hand searching of conference abstracts.[117-120, 123-125, 127-130, 192, 202-205] One additional reference (full report), for a study already identified, was found whilst scanning recently published literature.[127] The literature search was assessed as up-to-date on the 14th July 2014. No additional studies were identified by contacting primary authors or screening relevant references. After application of the eligibility criteria to the 16 trials, five trials (15 references) were included,[118-120, 125, 127], 6 trials were excluded[117, 192, 202-205] and five trials were on-going[123, 124, 128-130]. Results of the search are displayed in a PRISMA flow diagram (Figure 21). Relevant data were extracted from each of the five eligible trials by two authors (SP and IS) independently. When comparing the two data exertion forms, no disagreements arose. The data extraction forms for each of the 5 studies are included in the appendix.

Figure 21 PRISMA flow diagram demonstrating the results of the literature search



3.2.2 Characteristics of included studies

Five trials with 225 participants met the inclusion criteria for this review; and compared 4-Phenylbutyrate (4PBA)[118, 119], 8-cyclopentyl-1, 3-dipropylxanthine (CPX),[120] Lumacaftor monotherapy [125, 127] and Lumacaftor and Ivacaftor combination therapy to placebo.[127] All studies examined the impact of CFTR correctors in patients homozygous for the ΔF508 mutation (class II mutation on both alleles).[118-120, 125, 127]

The first trial by Rubenstein was a pilot trial (n=18) comparing 19g of 4PBA daily to placebo in patients aged 14 years and older.[118] We called this trial the pilot 4PBA study. This was followed by a phase 2 trial by Zeitlin (n=19) comparing escalating doses of 4PBA (20g, 30g and 40g daily) to placebo in patients 18 years and older (mean age 28.5 years).[119] We called this study the phase 2 4PBA study. Both 4PBA studies reported on safety and change in sweat chloride concentration at 1 week.[118, 119] The CPX study by McCarty was a phase 1 study (n=37) that examined escalating doses of CPX (1 mg, 3 mg, 10 mg, 30 mg, 100 mg, 300mg, 1000mg) in patients aged 18 years of age and older. Sweat chloride concentration values were reported at day 1 and adverse effects were monitored for 1 week.[120] Two trials compared escalating doses of Lumacaftor to placebo.[125, 127] The first of these studies by Clancy (n = 89) examined escalating doses of Lumacaftor (25mg, 50mg, 100mg and 200mg) in participants 18 years of age and older (median age 26 years) and reported on QoL, FEV_1, adverse effects and sweat chloride concentration at 28 days.[125] We called this study the Lumacaftor study. The remaining study by Boyle compared 1) Lumacaftor monotherapy and 2) Lumacaftor and Ivacaftor combination therapy to placebo; we called it the Lumacaftor-Ivacaftor study.[127] This study was composed of 3 cohorts, but we only included data for participants randomised in cohort 1 (n = 62). This is because data for placebo participants in cohorts 2 and 3 were combined in the analysis although randomisations in these cohorts were conducted separately. This invalidated the effects of randomisation and the review authors judged it inappropriate to include this data. In this review, the Lumacaftor-Ivacaftor study refers to cohort 1 of the study only. This study recruited patients 18 years and older (mean 29.1 years of age) and reported on FEV_1, adverse effects and sweat chloride concentration at day 14 (Lumacaftor monotherapy) and day 21 (Lumacaftor and Ivacaftor combination therapy)[125]. A summary of the characteristics of the included studies on-going studies and reasons for exclusions are provided (Tables 13, 14 and 15)

We approached all the trial authors of the included studies for additional data on outcomes reported in our systematic review, but not reported in the published trials (E-mail 12-17). We also contacted the primary author of the Lumacaftor-Ivacaftor study for separate data for placebo participants in these cohorts 2 and 3 (E-mail 17). Four out five authors responded; two authors had no additional data to contribute (12 and 14) and the remaining two authors have not yet provided any additional

data (E-mail 13 and 15). Therefore, all data included data in this review is from published reports. We also approached the primary author of one of the on-going studies to illicit any preliminary data, but we did not receive a response (E-mail 18). We could not request additional data for the remaining on-going trials because contact information was not provided.

Table 13 Characteristics of studies included in the CFTR correctors review

	Study design				Participants			Interventions		Outcomes	
Study	**Study design**	**Commercial research support**	**Treatment period (weeks)**	**Number of sites**	**Number of participants**	**Age group (years of age)**	**Genotype**	**Intervention**	**Control**	**Primary outcome**	**Secondary outcomes**
Rubenstein 1998[118] (pilot 4PBA study)	RCT of parallel design	NIH, CF Foundation	7 days	Single site in USA	18	> 14	Homozygous ΔF508	19g 4PBA	Placebo	NPD	Safety, Sweat cl values
Zeitlin 2002[119] (phase 2 4PBA study)	RCT of parallel design	CF Foundation	7 days	Single site in USA	22	> 18	Homozygous ΔF508	20g 30g and 40g 4PBA	Placebo	NPD	Safety, sweat cl values, FEV_1, sputum microbiology
McCarty 2002[120] (CPX study)	RCT of parallel design	FDA office of orphan products development	1 day (single dose assessment)	4 sites in the USA	37	> 18	Homozygous ΔF508	1 mg CPX 3 mg, 10 mg, 30 mg 100 mg, 300 mg, 1000 mg CPX	Placebo	Safety	NPD, sweat cl values
Clancy[125] (Lumacaftor study)	RCT of parallel design	Vertex Pharmaceuticals Incorporated	28 days	25 sites N. America & Europe	89	> 18	Homozygous ΔF508	25mg, 50mg 100mg & 200mg of Lumacaftor	Placebo	Safety	Δ BL sweat cl, NPD, lung function, CFQ-R score
Boyle 2014[127] (Lumacaftor-Ivacaftor study) Cohort 1 only	RCT of parallel design	Vertex Pharmaceuticals Incorporated	21 days	90 sites in N.America, Europe & Australia	62	> 18	Homozygous ΔF508	200mg Lumacaftor (D 1-21) and 150mg/250mg Ivacaftor (D 14-21)	Placebo	Δ BL sweat cl(Lumacaftor and Ivacaftor therapy)& safety	Δ BL FEV_1, sweat cl (Lumacaftor only)

Table 14 Characteristics on-going of studies examining CFTR correctors

Study and title	Sponsor	Methods	Participants. Confirmed diagnosis of CF +:	Interventions	Outcomes (Δ BL - change from baseline)*	Status at literature search
Donaldson 2013[123] *(* NCT01531673*)*	Vertex	Phase 2, double-blind, placebo-controlled, 3-part RCT of parallel design. Expected to enrol 130. Duration: 28 days.	1. Homozygous or heterozygous for the ΔF508 mutation. 2. ≥ 18 years	10mg, 30mg 100mg and 150mg VX-661 OD +/- 150mg Ivacaftor BD	Primary: Safety + : Δ BL in sweat cl Secondary: Absol + rel Δ BL FEV_1,	Study recently complete, no data published to date.
NCT02070744 2014[124]	Vertex	Phase 2, double-blind, placebo controlled RCT of parallel design with outcomes measured at 12 weeks	1. homozygous for the ΔF508 mutation. 3. ≥ 18 years old	VX-661 + Ivacaftor (doses unknown) compared to placebo	Primary: Safety Secondary: Absol Δ BL FEV_1, Rel Δ BL FEV_1, Δ BL weight,	Recruiting participants
NCT01807923 2013 or TRAFFIC or study VX12-809-103.[128]	Vertex	Phase 3 double-blind, placebo-controlled study RCT of Parallel design. 90 sites Worldwide Est to enrol 559 for 24 weeks.	1. homozygous for the ΔF508 mutation 2. 12-65 years old	600mg Lumacaftor OD or 400mg Lumacaftor BD + 250mg Ivacaftor BD compared to placebo	Primary: Absol Δ BL FEV_1 (Secondary: Rel Δ BL FEV_1, No of PEx, Δ BL BMI, Δ BL weight,	On-going, no data available
NCT01807949 or TRANSPORT or study VX12-809-104.[129]	Vertex	Phase 3 double-blind, placebo-controlled study RCT of Parallel design. 82 sites Worldwide Est to enrol 563 for 24 weeks.	1. homozygous for the ΔF508 mutation 2. 12-65 years old	600mg Lumacaftor OD or 400mg Lumacaftor BD + 250mg Ivacaftor BD	Primary: Absol Δ BL FEV_1 (Secondary: Rel Δ BL FEV_1, No of PEx, Δ BL BMI	On-going, no data available
NCT01931839 (Rollover study from TRAFFIC and TRANSPORT)[130]	Vertex	Phase 3, double-blinded rollover study of parallel design. 211 Worldwide. Estimated to enrol: 1122 for 100 weeks.	Participants from TRAFFIC and TRANSPORT	600mg Lumacaftor OD or 400mg Lumacaftor BD + 250mg Ivacaftor BD	Absol Δ BL FEV_1, Rel Δ BL FEV_1, Safety, Δ BL BMI, Δ BL weight,	On-going

*not all outcomes presented, refer to characteristics of included studies in the full review for more detail

Table 15 Studies excluded from the CFTR potentiators review

Study ID	Reason for exclusion
NCT01897233 2013[203]	Single group assignment
NCT01899105 2013[202]	Cross-over design
Leonard 2012[205]	Cross-over design
Lebecque 2011[204]	Cross-over design
Rubenstein 2006[192]	Cross-over design
Chadwick 1998[117]	No relevant outcome measures

3.2.3 Risk of bias in included studies

Two authors (myself and IS) individually assessed each of the four included trials for risk of bias. The Lumacaftor-Ivacaftor study by Boyle sufficiently described methods to account for selection bias, detection bias and performance bias, however in the remaining studies, it was less clear; none reported on random sequence generation, allocation concealment or blinding. These studies were therefore judged to have an unclear risk of selection bias, detection bias and performance bias except the phase 2 4PBA study which was judged to have a high risk of performance bias because intervention assigned participants, assigned to different doses of 4PBA (20g, 30g and 40g), followed different daily schedules and were given different quantities of tablets.

We assessed for attrition bias in the same way we did for the studies included in the CFTR potentiators review. With regards to withdrawals; all randomised participants completed the final study visit or the percentages of withdrawals were low (all less than 15%). With regards to missing participant data, in the Lumacaftor study by Clancy, data was excluded from the analysis for no apparent reason and in the phase 2 4PBA study by Zeitlin, the number of participants included in the analysis was unclear.[119, 125] We approached the primary authors of these trials for clarification, but no additional information was provided (E-mail 13 and 15). In Lumacaftor-Ivacaftor study (cohort 1), participants were excluded from the analysis due to insufficient data from the patient. For example, data were excluded for the analysis of change from baseline in sweat chloride concentration because insufficient sweat was gathered for analysis. Although this reason does not specifically lead to participants with unfavourable characteristics being excluded from the analysis (unlike for example withdrawals due to adverse events), the review authors judged attrition bias in this review to be unclear because we could not determine the effects of these exclusions on the balance

between groups in baseline characteristics.[127] In the remaining two trials, all participant data was included in the analysis.[118, 120]

Zero trial protocols were available online or were provided after approaching the trialists. We assessed selective outcome reporting by comparing outcomes reported on online trials registries or in the methods section to the outcomes reported in the results. Both review authors identified a high risk of selective outcome reporting in the phase 2 4PBA study and the Lumacaftor study because trialists failed to report data for pre-defined outcomes.[119, 125] We approached the primary authors of both trials for missing outcome data. The remaining three trials reported all pre-defined outcomes.[118, 120, 127]

We did not find any other sources of bias amongst the included trials and no disagreements arose whilst comparing the risk of bias assessments.

Overall, we judged the methodological quality (risks of bias) in the included studies to be moderate, because most of the data in this trial was judged to be of unclear risk of bias. A summary of the risk of bias judgements is demonstrated in the Figures 22 and 23. For more detail on the individual risk of bias assessments for each domain for each study, refer to the characteristics of studies section of the full review.

Figure 22 Summary of risk of bias for each included trial using Cochrane Risk of Bias Tool in the CFTR correctors review

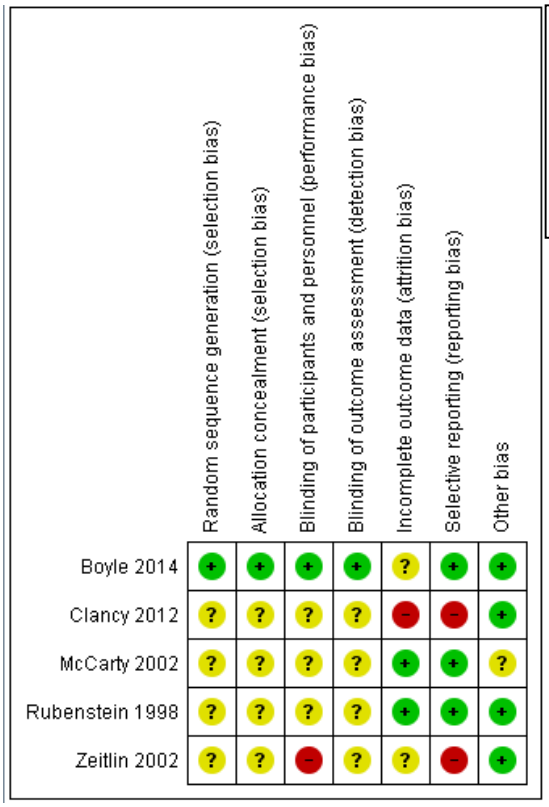


Figure 22 provides a summary of the individual risks of bias assessments for each study included in the CFTR potentiators review.

Risk of bias was based on author's judgements. The symbols represent different risk of bias: + represents low risk of bias, − represents a high risk of bias and ? indicates an unclear risk of bias.

Figure 23 Summary of risk of bias for each included trial using Cochrane Risk of Bias Tool in the CFTR correctors review.

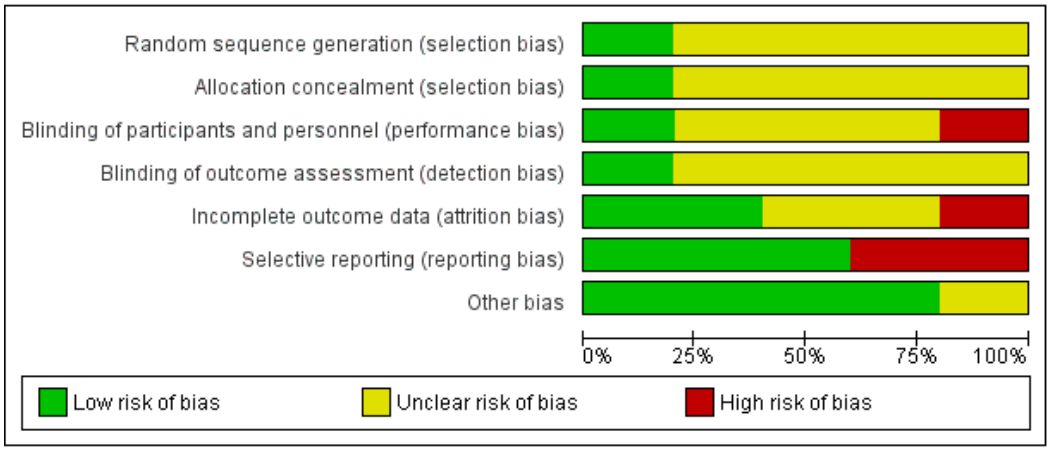


Figure 23 shows a large proportion of domains were judged to have an unclear risk of bias in the CFTR correctors review.

3.2.4 Effects of the intervention

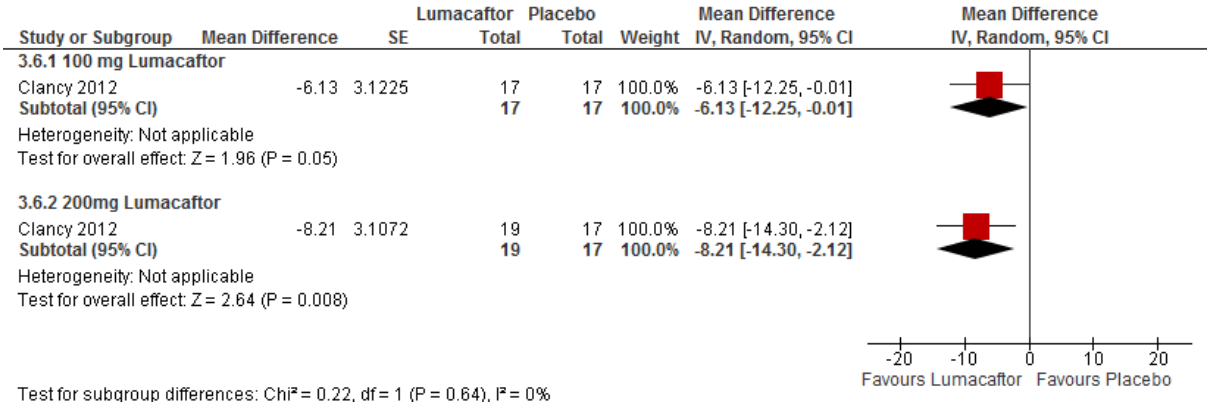
This summary of the effect of the Ivacaftor has been extracted from the discussion section of the CFTR correctors review and highlights all pertinent results.

With respect to the primary outcomes for this review (survival, QoL, relative and absolute change in FEV_1 from baseline and the change from baseline in FVC) the evidence was limited. No deaths reported in any of the trials and size and length of trials precluded valid assessment of CFTR correctors on survival. The Lumacaftor study by Clancy measured QoL; where lower QoL (demonstrated by lower CFQ-R domain scores) was reported by participants taking 25 mg, 50 mg and 200 mg of Lumacaftor at 28 days.[125] With regards to lung function, patients treated with Lumacaftor monotherapy and Lumacaftor and Ivacaftor combination therapy did not demonstrate short term improvements in FEV_1.[125, 127]

There was no difference between the CPX treatment groups and the placebo group in the frequency of mild adverse effects.[120] Participants assigned to 20 g of 4PBA in the phase 2 4PBA study by Zeitlin reported episodes of transient nausea, headache, sleepiness after the initial dose (relieved by an acetaminophen) and body odour, but this was not reported by participants taking 19 g 4PBA in the pilot trial by Rubenstein.[119] We considered 30 g and 40 g doses of 4PBA, examined in the phase 2 4PBA study by Zeitlin, to be unsafe. All participants in the 30 g 4PBA group reported episodes of transient nausea, headache, sleepiness and transient visual disturbances after the initial dose and two out of six participants in the 30 mg group either discontinued the study drug or reduced the dose. All three participants receiving 40 g 4PBA developed episodes of nausea, headache and visual disturbances and one participant reported cramp in hands and fingers. One participant withdrew from the study and one participant was assigned to a reduced study dose. All participants were eventually terminated from the 40 g group after it was considered unsafe by the safety monitoring committee.[119] In participants assigned 100 mg and 200 mg of Lumacaftor (combined data), in the Lumacaftor study by Clancy, there was no significant difference in the frequency of mild adverse effects when compared to participants randomised to placebo.[125] This was consistent with findings in the Lumacaftor-Ivacaftor study, which demonstrated no difference between patients treated with 1) Lumacaftor monotherapy and placebo and 2) Lumacaftor and Ivacaftor combination therapy and placebo (combined data), in the number of participants experiencing mild adverse effects. Four participants, one from each of the Lumacaftor dosage groups, withdrew from the Lumacaftor study due to respiratory adverse effects,[125] which was also in line Lumacaftor-Ivacaftor study where one participant withdrew during the 200mg Lumacaftor monotherapy period due to chest tightness.[127]

Treatment with CPX or 4PBA did not improve CFTR function in sweat glands.[118-120] Data at 28 days in the Lumacaftor study demonstrated significant reductions in sweat chloride concentration in participants assigned to 100 mg of Lumacaftor once daily, MD -6.13 mmol/L (95% CI -12.25 to -0.01) and 200 mg Lumacaftor once daily, MD -8.21mmol/L (95% CI -14.33 to -2.09) (Figure 24).[125]

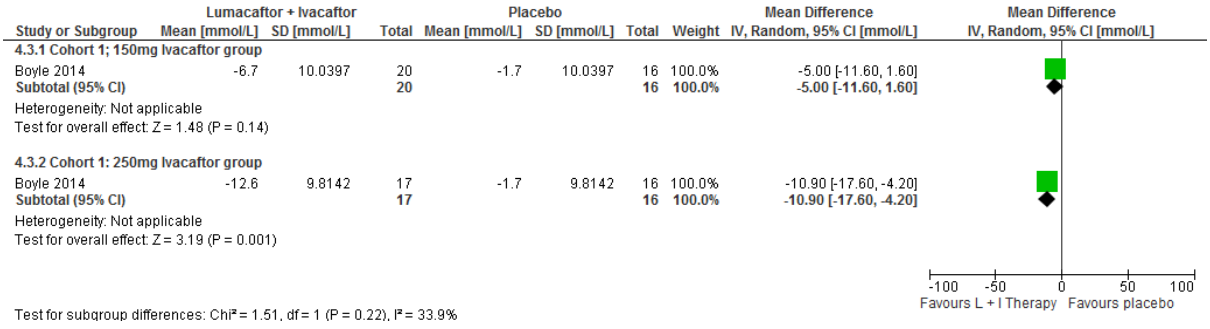
Figure 24 Forest plot demonstrating the change from baseline in sweat chloride concentration in homozygous ΔF508 participants treated with Lumacaftor at 28 day.



The plot in figure 24 shows the advantage for treatment with Lumacaftor over placebo in the change from baseline in sweat chloride concentration, in adults homozygous for the ΔF508 mutation. The point estimates at 28 days indicate reductions in sweat chloride concentration in participants treated with 100mg and 200mg of Lumacaftor. The advantage can also be seen in the mean difference values and the 95%CIs suggest statistically significant advantage of Lumacaftor over placebo for both dose groups.

Data at 21 days, for patients treated with 200mg Lumacaftor once daily (day 1-21) and 250mg of Ivacaftor twice daily (day 15-21) in the Lumacaftor-Ivacaftor study, demonstrated the greatest reduction in sweat chloride concentration compared to placebo in patients with the ΔF508 mutation, MD -10.90 mmol/L (95% CI -17.60 to -4.20) (figure 25).[127]

Figure 25 Forest plot demonstrating the change from baseline in sweat chloride concentration in homozygous ΔF508 participants treated with Lumacaftor and Ivacaftor at day 21.



The plot in figure 25 shows the advantage for treatment with Lumacaftor + Ivacaftor over placebo in the change from baseline in sweat chloride concentration, in adults homozygous for the ΔF508 mutation. The point estimates and mean differences at 21 days indicate reductions in sweat chloride concentration in participants treated with Lumacaftor and Ivacaftor. Only the 95%CI for the participants treated with 200mg Lumacaftor and 250mg Ivacaftor suggest a statistically significant advantage of Lumacaftor and Ivacaftor combination therapy over placebo.

3.2.5 Conclusion

The CFTR correctors review examined the impact of 4PBA, CPX and Lumacaftor (monotherapy and combination therapy with Ivacaftor). Short-term treatment with Lumacaftor demonstrated statistically significant but clinically minimal reduction in sweat chloride concentration when used as monotherapy (28 days) and when used as combination therapy with Ivacaftor (21 days) in homozygous ΔF508 patients. Lumacaftor monotherapy and combination therapy with Ivacaftor did not demonstrate a statistically significant impact on any other clinically relevant outcomes in patients with ΔF508. Neither 4PBA nor CPX demonstrated a clinically relevant impact on outcomes in patients with ΔF508. In light of the results presented in this review, there is no current evidence to support the short-term use of CFTR correctors (as monotherapy or combination therapy) for patients with class II mutations. This evidence is based on studies with an overall moderate methodological quality. It was downgraded from high to moderate because of the large proportion of domains with an unclear risk of bias (Figure 23).

Chapter 4

Discussion

The protein products of Class II mutations are mainly degraded within the cell, but minimal amounts of CFTR reach the cell membrane where they demonstrate defective function. The protein products of Class III mutations are transported normally to the cell membrane but display defective function. In both cases, inadequate or compromised CFTR at the cell membrane leads to compromised ion transport and a severe CF phenotype characterised by chronic infection and inflammation and lung damage. New therapies that target the underling defect of class II (CFTR correctors) and III mutations (CFTR potentiators) aim to restore normal ion conductance and are likely to represent a significant healthcare resource in patients with Cystic Fibrosis.

We conducted two systematic reviews to examine the impact of 1) CFTR potentiators and 2) CFTR correctors on clinically important outcomes in patients with Cystic Fibrosis. The reviews included 9 RCTS with 603 CF patients and lasted between 1 day (single dose phase 1 trial) and 48 weeks.

4.1 CFTR potentiators

4.1.1 Findings of the review

Appraisal and synthesis of the evidence on CFTR potentiators demonstrated that Ivacaftor had a clinically relevant impact on outcomes at 24 and 48 weeks in adults and children with the G551D mutation (class III mutation) but not in patients with the ΔF508 mutation (class II mutation). These results were in accordance with increased chloride conductance seen in cell studies, recently published systematic review on Ivacaftor by Whiting, and the current recommendations for the use of Ivacaftor for patients with the G551D mutation in the UK.[43, 109, 206]

4.1.2 Strengths and limitations of the included studies

The included studies in this review had a number of strengths. All included studies were RCTs of parallel design representing the best form of clinical trial design for establishing the evidence for an intervention. These trials also reported on a number of key clinical outcomes included in our review; quality of life, lung function, adverse effects of therapy, hospitalisations, extra courses of antibiotics, weight gain, BMI and sweat chloride concentration. Reporting of these outcomes are important to both clinicians and patients to inform clinical decision making.

In addition to this, all trials in this review were conducted at multiple sites demonstrating a more precise effect of Ivacaftor on outcomes. Two studies in this review (phase 3 G551D studies)

demonstrated homogeneity with regards to patient characteristics (i.e. genotype but not age), trial settings, treatment regimens and end-points and also demonstrated consistency in the effect of Ivacaftor on lung function (FEV_1), weight gain and sweat chloride reduction. These trials also reported data in a form that permitted inclusion into a meta-analysis and by combining the data; we were able to demonstrate a more precise estimate of the benefits and harms of Ivacaftor on patients with G551D.

Trials in this review also had number of limitations. With regards to methodological quality, the included studies in this review demonstrated adequate random sequence generation, allocation concealment (low selection bias) and blinding of study personnel (low performance bias). The trials however, were limited by high risks of attrition bias and selective outcome reporting bias. High risks of these biases are associated with over-estimation of the treatment effect through; 1) exclusion of unfavourable participant outcome data (attrition bias) 2) disruption of matching between groups in baseline characteristics (attrition bias) and 3) having bias towards reporting of significant results over non-significant results (selective outcome reporting bias).[177, 182] Attrition and selective outcome reporting bias therefore limit the overall methodological quality of the included studies and the internal validity of the evidence. To demonstrate the impact of these biases on the overall treatment effect estimate we planned to conduct a sensitivity analysis but this was not possible with the small number of included trials.

This assessment of methodological quality was not in line with the assessment of quality in the systematic review by Whiting.[206] Both reviews employed the Cochrane risk of bias tool but in the Whiting review, the judgements of risks of biases gave the trials a higher overall methodological quality. For more details regarding the differences, refer to the discussion section of full review. In both reviews, the review authors have provided a written description to support their risk of bias judgements, and differences between reviews highlight the subjective nature of risk of bias assessments when using the Cochrane risk of bias tool.

The trials were further limited by length, which precluded proper assessment of the long-term impact of Ivacaftor on survival and other outcomes (beyond 48 weeks), and missing data for other outcomes we considered clinically important. There was insufficient data for the outcomes school or work attendance; radiological measures of lung disease; acquisition of respiratory pathogens; and cost of treatment.

4.1.3 Applicability of the evidence

In this review, three trials recruited 238 patients with the G551D mutation representing a significant proportion of patients with this mutation in the countries they were recruited. Also, 140 patients

with the ΔF508 mutations were recruited also representing a significant proportion of patients with this mutation above the age of 12. We can therefore assume that the results of this review are applicable to patients with these mutations not included in these studies, although CF patients less than 6 years of age or in pregnancy were not enrolled. In addition, all trials were conducted at multiple sites in a number of continents accounting for the differences on healthcare systems and environment on clinical outcomes, providing greater generalizability of the results.

4.2 CFTR correctors

4.2.1 Findings of the review

The review on CFTR correctors demonstrated that there is no evidence for the use of CFTR correctors as monotherapy or combination therapy, in patients with ΔF508 (class II mutations) in the short term. As we are aware, this is the first systematic review to be conducted on CFTR correctors. This is not surprising given the modernity of RCTs included in this review.

4.2.2 Strengths and limitations of the included studies

The included studies in this review had a number of strengths and limitations. All included studies were RCTs of parallel design representing the best form of clinical trial design for establishing the evidence for an intervention. One study (Lumacaftor-Ivacaftor study by Boyle) examined the impact of Lumacaftor and Ivacaftor combination therapy. This study was of high methodological quality, demonstrating low risks of biases for all but one domain (incomplete outcome data domain), which was judged to be unclear. Therefore the quality of the evidence for combination therapy was high. The Lumacaftor-Ivacaftor study also examined Lumacaftor monotherapy in addition to the Lumacaftor study by Clancy. The overall methodological quality of the evidence for Lumacaftor however was limited by high risks of attrition bias and selective outcome reporting bias found in the Lumacaftor study. These studies reported data for Lumacaftor monotherapy at different time-points so we were unable to combine data in a meta-analysis despite the studies demonstrating homogeneity with regards to patients (age and genotype), trial settings, treatment regimens and end-points.

Two studies examined the impact of 4PBA and one trial examined the impact of CPX. These trials were of moderate methodological quality, because risks of bias for the majority of domains were unclear. All three studies stated they were double-blinded RCTs, but did not describe the method of randomisation, allocation concealment or blinding. The phase 2 4PBA study by Zeitlin was judged to have a high risk of performance bias and attrition bias, which further limited the quality of the evidence for 4PBA. With regards to consistency, adverse events demonstrated by the 4PBA studies were variable, with more participants in the 20g group of the phase 2 4PBA study reporting mild adverse events than participants given 19g 4PBA in the pilot study. Both 4PBA trials demonstrated no significant impact on sweat chloride concentration on patients treated with 4PBA, but the precision of the overall treatment effect were limited by unstandardized and inconsistent reporting of data precluding combination of data in a meta-analysis.

All trials included in this review were further limited by length (range 1 day – 28 days), which precluded proper assessment of 1) the longer-term impact of CFTR correctors on outcomes (e.g. survival) and 2) the longer term safety profile of CFTR correctors. In addition to this, there were missing data for key clinical outcomes; FVC, hospitalisations, school or work attendance, extra courses of antibiotics, radiological measures of lung disease, acquisition and eradication of respiratory pathogens, weight gain, BMI and growth. Only one trial reported on QoL,[125] relative change from baseline in $FEV_{1,}$[125] and absolute change from baseline in FEV_1.[127]

4.2.3 Applicability of the evidence

In this review, five studies recruited 225 patients homozygous for the ΔF508 mutation (class II) and examined the impact of four different CFTR corrector therapies. A total of 151 patients were recruited to examine Lumacaftor monotherapy and 62 patients were recruited to examine Lumacaftor in combination with Ivacaftor; representing significant portions of patients aged 18 years upwards with this mutation. We can therefore assume that the limited results with regards to Lumacaftor monotherapy and combination therapy with Ivacaftor can be applied to CF patients not included in these trials. 37 patients were recruited and to assess the impact of CPX and a further 37 patients were recruited to examine 4PBA; again demonstrating representative proportions of patients with this mutation. In light of the adverse safety profile of 4PBA, further clinical trials examining this therapy may not be appropriate.

4.3 Limitations in research on CFTR potentiators and CFTR correctors for Cystic Fibrosis

The research of the effects of CFTR potentiators and CFTR correctors are further limited by several factors; the lack of uniform reporting of adverse events, the lack of shared definitions for pulmonary exacerbations, ambiguity about why participants were excluded from the analysis and inappropriate selection of design and choice of analysis.

4.3.1 Reporting of adverse effects

Trialists are recommended to adhere to the CONSORT statement for harms when reporting adverse events in clinical trials.[207] This statement encourages trialists to list adverse effects, to provide a definition of the adverse effect and to provide definitions for headings used when grading severity. For example, if trialists labelled adverse effects as mild, they should provide a definition of mild. In the included studies, trialists failed to report definitions for headings used in grading severity and this limited the ability of the reviewer to interpret these adverse effects and to potentially combine these adverse effects for a more precise estimate of harms of the interventions.

In addition, studies included in this review reported on withdrawals, discontinuations and dose reductions but often failed to report all clinically relevant data associate with this. In a number of trials it was unclear what adverse effect lead to withdrawal/discontinuations/dose reduction, whose decision it was, what group the participant was assigned to and whether blinding was maintained during the process .

4.3.2 Lack of definition for pulmonary exacerbations

Pulmonary exacerbations are an important clinical outcome and are being used as primary and secondary outcomes in CF clinical trials.[98] However to date, there is no established definition for a pulmonary exacerbation meaning trialists can identify events using either pre-defined criteria (protocol defined) or their own clinical judgement (physician defined). As physician defined exacerbations can include exacerbations identified using different criteria, it is inappropriate to combine data for protocol and physician defined exacerbation in the same meta-analysis. The review author can identify studies that share similar definitions (only if protocol-defined) to include in a meta-analysis, but without a well-established definition, data from studies will continue to be excluded, limiting the precision of the treatment effect estimate for this important outcome.

4.3.3 Exclusion of participants from the analysis

In order to assess attrition bias, the review authors need to know 1) the number of withdrawals from the study and reasons for withdrawals and 2) the number of participants excluded from the analysis

and reasons for exclusions. Review authors require information on why participants were excluded from the analysis to judge whether intervention assigned participants who had unfavourable results, were more likely to be excluded from the analysis leading to an over-estimation of the treatment effect. In 6 out of the 9 included studies in this review, participant data were excluded from the analysis. Only in 2 studies was information provided on the reasons for exclusions. In one study, insufficient data were acquired for analysis. This was judged to have an unclear risk of bias because it was unknown how incomplete participant data would affect the balance between randomised groups in baseline characteristics. In the other study a modified ITT approach had been employed, whereby participant data were excluded for participants who withdrew, so was judged to have a high risk of attrition bias. In the remaining 4 studies, participants appeared to have been excluded from the analysis for no apparent reason and these trials were judged to have a high risk of bias. Attrition bias in these studies limited the internal validity of the evidence of the reviews they were included in.

4.3.4 Inappropriate selection of design and choice of analysis

Trials of cross over design did not seem appropriate for examining CFTR potentiators and CFTR correctors given that these interventions target the underlying defect of CF. However, from our literature searches we found a number of trials that were of cross over design which would have otherwise been eligible for inclusion in our reviews.[187-189, 202, 204, 205] This limited the amount of data for the examined therapies.

In addition to this, the evidence on Lumacaftor monotherapy and combination therapy was limited because in the Lumacaftor-Ivacaftor study, data for placebo participants in cohorts 2 and 3 were inappropriately combined, undoing the effects of randomisation.[127] This is a shame given the large number of participants enrolled in these cohorts and the number of clinically relevant outcomes reported. The primary author was approached for separate placebo assigned participant data.

4.4 Strengths and limitations of the review process

4.4.1 The protocol stage

The systematic review process has a number of strengths starting with review protocol. Both systematic reviews in this thesis were conducted in accordance with peer reviewed and published protocol.[141, 142] This is in line with Cochrane systematic review guidelines, and offers advantages over reviews conducted without protocol. The protocol stage allows authors to pre-determine the review methodology before the review stage is started so the review authors have a clear idea of how it is to be conducted and can use it as guidance. Also, the protocol stage helps reduce introducing bias during the review stage; pre-planned search strategy helps reduce bias from being introduced during the selection of studies and pre-defined outcome measures ensure selective outcome reporting bias, which limits the validity of many systematic reviews, is minimised.[208] The main advantage of the protocol stage, however, is the improvement in methodological transparency it offers to the review process. It allows readers of the review to determine how results and conclusions in the review stage were formed, so they can use this information to form their own judgements on the evidence.

4.4.2 The review stage

There are also many advantages to the review process. For each review, we conducted a thorough literature search. This included searching relevant online databases and registries, hand searching conference abstracts and contacting relevant leaders and pharmaceutical companies investigating mutation-specific therapies. This comprehensive approach to literature searching ensured that all relevant data (published and unpublished) contributed to the evidence base. In addition to this, by conducting regular searches of databases and journals and indexing the relevant studies with appropriate keywords in the group's Cystic Fibrosis registry, the review authors could easily ensure the most up-to-date evidence was provided.

Two individual assessors applied the pre-defined inclusion criteria to the results of the literature search to identify studies eligible for inclusion. 4 studies were included in the CFTR potentiators review and 5 studies were included in the CFTR correctors review. Given the modernity of the examined interventions assessed in these reviews, in particular Ivacaftor and Lumacaftor, we were not surprised with the small number of identified trials and the large number of on-going studies. Two authors extracted relevant data from included studies, reducing the risk of inaccurate extraction.

The methodological quality (risk of bias) of the included studies was assessed using the Cochrane risk of bias tool. This tool permitted the review authors to provide both a written description and an

overall judgement on the risk of bias for each domain (high risk, low risk or unclear risk). Given this is a subjective assessment, it was important that review authors were transparent about how judgements were derived so that readers of the reviews could form their own verdicts on the internal validity of the evidence. As per Cochrane protocol, two authors were recruited to reduce the risk of inaccurate methodological assessments.

In these systematic reviews, we only combined data for studies that demonstrated homogeneity, increasing the precision of the treatment effect estimate.[198, 200] Data were combined with the appropriate method for synthesis (random effects or fixed effect model) and checked for appropriateness by the review statistician.

This methodological and transparent approach to the review process ensured bias was kept low and meant the highest quality of evidence was provided. It also meant that methods can be easily replicated for future updates of this review. This is particularly important for the included reviews given then that the assessed therapies were new, and that there are a number of on-going studies.

There are also a number of limitations to the review process. It is well known that studies with statistically significant results are more likely to be published with faster time to publication and when studies are published, there is selective reporting towards significant results.[182] This omission of data can deprive review authors of essential data for meta-analyses and can lead to over-estimation of the treatment effect; whereby patients may be recommended interventions that have serious adverse events or be deprived of cheaper or more effective medication.[178] In our systematic reviews, we approached trialists and relevant pharmaceutical companies for unpublished data but these were not made available. Therefore these systematic reviews are at risk of unavoidable publication bias. We have highlighted selective reporting of outcomes in our studies.

The issue of over-estimation of treatment effect due to selective outcome reporting can be further emphasised by selective outcome reporting in systematic reviews.[208, 209] However, in these reviews the review authors ensured that all data for pre-defined outcomes were included.

In these reviews, we reported on adverse effects of therapy as mild (therapy does not need to be discontinued), moderate (therapy is discontinued and the adverse effect deceases) and severe (life threatening or debilitating or persists after treatment is discontinued). This method of reporting adverse effects is clinically relevant and is recommended by the Cochrane Handbook of systematic reviews.[159] It is also advocated by the CONSORT statement for harms for the reporting of adverse effects by RCTs, as it reflects the participant's and/or clinician's ultimate decision to withdraw or discontinue study medication.[207] Despite this, there were a number of limitations in employing this

approach to reporting adverse effects. We first extracted the number of participants from each trial who required study drug interruption due to an to adverse effect (moderate adverse effect) and the number of participants experiencing life threatening or debilitating adverse events or adverse events that require the patient to discontinue the allocated treatment (severe adverse effect) as per protocol. With this classification however, all other adverse effects were considered under the heading mild, even though they may have reflected more severe adverse effects. In addition to this the reasons for study drug interruption or withdrawals in the included studies varied. This meant that participants who required study drug interruption or discontinuation due to mild adverse effects, for example a mild case of cough, were considered moderate or severe adverse effects in the review.

4.5 Conclusion

In conclusion, the two systematic reviews demonstrate that there is evidence for the use of Ivacaftor in patients with G551D mutation but no evidence to support the short-term use of CFTR correctors or CFTR potentiators, or both as combination therapy in patients with the ΔF508 mutation. Readers of these systematic reviews should be aware of the limitations in methodological quality in the included studies of both reviews.

4.6 Priorities for future research

Ivacaftor is the first intervention that corrects the underlying molecular defect in CF. It has demonstrated effectiveness in patients with a class III mutation (G551D) and has the potential to be used for other class III/IV mutations because both mutation classes are characterised by ion gating defects. This has been demonstrated by cell studies.[210] Trials examining the use of Ivacaftor in class III – IV mutations (e.g. R117H) are on-going, and we will update the findings of these reviews when they are available.[193-196]

As patients with class II mutations comprise a significant proportion of all CF patients, identifying an efficacious CFTR corrector (or CFTR corrector and CFTR potentiator combination) will have a profound impact on the field. Researchers have been investigating drugs that can correct the underlying intracellular processing defect of class II mutations (mainly ΔF508 mutation) for many years. These therapies have come under increased limelight after Lumacaftor and Ivacaftor combination therapy partially restored chloride ion conductance in ΔF508 cell studies, and the progression of these therapies into human clinical trials. This review however demonstrated that short term therapy with Lumacaftor monotherapy or combination therapy with Ivacaftor did not demonstrate a clinically relevant impact on patients with class II mutations. There is a need for larger, well-designed clinical trials to assess the long term benefits and harms of CFTR corrector monotherapy and combination therapy with CFTR potentiators in people with class II mutations. Given the on-going clinical trials on Lumacaftor[128-130] and VX-661,[123, 124] both in combination with Ivacaftor, we expect this data to be available soon, and to update the findings of this review when this data are available.

4.6.1 Study design

As new clinical trials on mutation-specific-therapies emerge, it is important that the lessons learnt from these reviews are taken on board, in particular with regards to study design. Future studies should be well powered and of sufficient length to demonstrate 1) the long term impact of therapies on key outcomes and 2) the long term safety profile of this intervention and its effect on survival. This is particularly relevant for Ivacaftor given its association with the formation of cataracts in

juvenile rats treated with 10mg/kg/day of Ivacaftor.[211] The mechanism of how this occurs is currently unknown. Post-market surveillance of this intervention will be equally as important.

In addition, trialists should ensure the appropriate RCT study design is employed (parallel and not cross-over) and that all participants are properly randomised.

4.6.2 Bias within included studies

It is also important that lessons are learnt about minimising risk of bias in particular with regards to selective outcome reporting and incomplete outcome data (attrition bias). To reduce selective outcome reporting, there must be greater emphasis on improving the methodological transparency of these trials, starting with publically available published protocol containing pre-defined outcomes. Trialists should then report on all data for pre-defined outcomes, regardless of whether or not they demonstrated statistical significance or were consistent with other findings. Data that cannot be included in the full report due to limitation in the word count can be reported in the supplementary appendix or on online trials registries (for example ClinicalTrials.gov).

In the USA and Europe, initiatives have been set up to reduce the burden of publication bias and selective outcome reporting. In the USA, trialists are required to publish all study protocols on online trials registries (www.clinicaltrials.gov).[212] In Europe, the 'ALLTRIALS' campaign (www.alltrials.net), which is supported by the Cochrane Collaboration, aims to make the reporting of data more transparent. One recommendation is for all results of all trials to be publically available within 1 year of completion.[213]

With regards to incomplete outcome data, future studies need to report on the number of participants included or excluded from the analysis and why participants were excluded. This will enable review authors to properly assess the risk of attrition bias.

4.6.3 Outcomes

With novel therapies and approaches, such as Ivacaftor, the reporting of adverse events is critical and this should be undertaken in a robust and consistent manner. Reporting the number of cases of temporary discontinuations and withdrawals is clinically relevant way of demonstrating the safety profile of an intervention. In order for adverse effect to be reported in this way, trialists must ensure that these data are reported with the following information 1) why participants were excluded 2) whose decision it was to discontinue or withdraw the patient and 3) whether blinding was maintained during this process. Only with this amount of detail can systematic reviews properly report on the safety profile of an intervention. This is particularly crucial to systematic reviews of novel therapies where the long-term safety profile of the intervention is mainly unknown. In

addition to this, there should be a more uniform and consistent reporting of pulmonary exacerbations by trialists, so that data from all relevant trials can be pooled.

It is also important that these trials examine all valid outcomes that are relevant to patients with CF and their families. At present, these outcomes can be found in systematic reviews of Cystic Fibrosis, but both review authors and trialists would benefit from a core outcome set for systematic review of CF.[208] Trialists would be aware of key outcomes expected to be reported during trials and this would increase the amount of data for synthesis in systematic reviews.

Furthermore, there should be continued research into end-points in clinical trials that demonstrate improvements in clinical status of patients with different disease severities (discriminant validity). This is particularly relevant given the existing limitations of current end-points, such as survival rates and FEV_1 in patients with mild lung disease, as patients with CF live longer and display better respiratory function.

4.6.4 Precision of the treatment effect

As more clinical trials are conducted on these interventions, more data will be available for inclusion in a meta-analysis providing a more precise estimate of the treatment effect. However, this is dependent on trials being homogenous and trialists reporting data in a standardised and consistent manner for pooling of the results. Therefore, before conducting new trials, trialists should also incorporate features of previously conducted trials, so that when data from these trials are combined they express minimal heterogeneity. Furthermore, trialists should continue to conduct multi-centre studies to demonstrate increased precision of the effect of mutation-specific-therapies on outcomes.

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Appendix

I. Interaction with primary authors of studies included in the CFTR potentiators review

A. Evidence of interaction with primary author of the ΔF508 study

E-mail 1: Request for additional outcome data for the ΔF508 study by Flume and response from Vertex.

From: Flume, Patrick A. [mailto:flumepa@musc.edu]
Sent: 07 January 2014 17:00
To: Patel, Sanjay [md0u92cd]
Cc: Southern, Kevin
Subject: RE: Cochrane systematic review of a mutation specific therapy in Cystic Fibrosis

Forgive the lengthy delay. I finally heard back from Vertex. The message is copied below.

pf

Patrick

Sorry this email got lost in my system (probably reflects a lack of system!).

I am not sure that there is much value to a Cochrane systematic review of the Kalydeco data since there is really only data from STRIVE and ENVISION.

Many of the data points that he is requesting are not available or were not collected in the study.

My suggestion is that you let him know that we are unable to supply these data at this time.

Thanks and a Happy New Year.

Best

CJ

From: Patel, Sanjay [md0u92cd] [mailto:S.Patel7@liverpool.ac.uk]
Sent: Thursday, December 05, 2013 11:40 AM
To: Flume, Patrick A.
Cc: Southern, Kevin
Subject: Cochrane systematic review of a mutation specific therapy in Cystic Fibrosis

Dear Professor Flume,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel Cystic Fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis Group to conduct a Cochrane systematic review on CFTR potentiators.

We would like to include the study "*Ivacaftor in Subjects With Cystic Fibrosis Who Are Homozygous for the F508del-CFTR Mutation*" (ClinicalTrials.gov Identifier: NCT00953706) in our review. Many of

the endpoints reported in this study match the outcomes of interest we would like to investigate in the review. However for some of the endpoints no numerical data is presented and for others no confidence Interval or standard deviation has been given, so we are unable to include them in the meta analysis.

The Mean Absolute change in CFQ-R respiratory domain scores are presented for both the placebo and the intervention group, however, no standard deviation or confidence intervals have been reported. Results have been reported in a similar way for absolute change in weight, absolute change in BMI, absolute change in weight-for-age-z-score and absolute change in BMI-for-age Z score. We would appreciate any standard deviation or confidence interval values for these means.

In addition to this, the full paper reported hospitalisations and extra courses of antibiotics as clinically relevant events. We understand that the results obtained for these outcomes were not clinically significant; however we would like to view any numerical data on these outcomes.

Finally, did you measure and would you be able to provide any information regarding

- cost of treatment,
- radiological measures of lung disease,
- acquisition of respiratory pathogens or eradication of respiratory pathogens.

These are other outcomes in our study that would like to comment on.

Thank you for taking the time to read this e-mail. Any information you can provide will help shape the results of the systematic review and will be highly appreciated.

Regards,

Sanjay Patel.

E-mail 2: Request for information on missing data in the ΔF508 study by Flume

From: Patel, Sanjay [md0u92cd]
Sent: 23 January 2014 11:05
To: flumepa@musc.edu
Cc: Southern, Kevin
Subject: FW: Cochrane systematic review of a mutation specific therapy in Cystic Fibrosis

Dear Professor Flume,

Whilst assessing whether an intention to treat analysis was employed, I noticed that in table 3 of the full paper, the number of participants in the ivacaftor group is displayed as 112. However, In the results section on clinicaltrials.gov, the number of participants in the ivacaftor group is shown to be 111. Could you provide an explanation for this discrepancy? I have attached both the full paper and a screenshot from clinical trials.gov. The following web address is a link to the results of the study on clincaltrials.gov:

http://clinicaltrials.gov/ct2/show/results/NCT00953706?term=Ivacaftor+AND+cystic+Fibrosis&rank=7§=X6015

In addition to this, one of the participants from the ivacaftor group was removed from the trial due to 'early termination per sponsor decision' as shown on clinicaltrials.gov. Could you provide further information as to why this participant was discontinued from the study.

I appreciate the time you have taken to read this e-mail and look forward to hearing from you.

Regards,

Sanjay Patel.

E-mail 3 : Request for trial protocol and key outcome data measured in the trial but not reported in the published text.

From: Patel, Sanjay [md0u92cd]
Sent: 02 February 2014 12:10
To: flumepa@musc.edu
Cc: Southern, Kevin
Subject: FW: Cochrane systematic review of a mutation specific therapy in Cystic Fibrosis

Dear Professor Flume,

Thank you for taking the time to contact Vertex regarding outcomes of interest. We appreciate the time taken to do so.

There are however, a couple more points I would like to ask about. I apologise for not requesting this information, and the information regarding intention to treat analysis, in the initial e-mail.

I would like to request the study protocol for the study "*Ivacaftor in Subjects With Cystic Fibrosis Who Are Homozygous for the F508del-CFTR Mutation.*" Currently, only the full paper and the supplementary appendix are available online. In addition, would you be able to provide any numerical data for the outcome change from baseline in Forced Vital Capacity (FVC). In the full paper, you have mentioned that no statistically significant differences were seen in this outcome; however no numerical data has been reported.

Thank you again, for your time and we look forward to hearing from you,

Sanjay Patel.

B. Evidence of Interaction with primary author of the child phase 3 G551D study

E-mail 4: Request for additional outcome data for the child phase 3 G551D study by Davies

From: Davies, Jane C [j.c.davies@imperial.ac.uk]
Sent: 06 December 2013 11:49
To: Patel, Sanjay [md0u92cd]
Cc: Southern, Kevin
Subject: RE: Cochrane systematic review of mutation specific therapy in Cystic Fibrosis

Dear Sanjay

None of those items were included in that study, which I agree is a shame.

Good luck with your review

Warm regards, Jane

From: Sanjay Patel [mailto:s.patel7@liverpool.ac.uk]
Sent: 05 December 2013 16:07
To: Davies, Jane C
Cc: Kevin Southern
Subject: Cochrane systematic review of mutation specific therapy in Cystic Fibrosis

Dear Dr Jane Davies,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel Cystic Fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis Group to conduct a Cochrane systematic review on CFTR potentiators.

We would like to include *"Study of Ivacaftor in Cystic Fibrosis Subjects Aged 6 to 11 Years With the G551D Mutation"* (ClinicalTrials.gov Identifier: NCT00909727) in our systematic review. Many of the outcomes reported on in this study match the outcomes of interest that we would like to investigate in the review. However I am contacting you to ask whether you measured or have data on any of the following outcomes.

- cost of treatment,
- hospitalisation,
- school work attendance,
- radiological measures of lung function,
- Acquisition of respiratory pathogens and
- Eradication of respiratory pathogens and height

Any additional information would go towards shaping the results of the review and would be highly appreciated.

Regards,

Sanjay Patel

E-mail 5: Interaction between review author, Professor Jane Davies and Vertex regarding incomplete outcome data in the child phase 3 G551D study.

Hi Jane,

Please find Haihong's response below. Essentially, the requester is correct in that not all patients had measurements available at every timepoint. This is addressed by the footnote in the table that reads, "*Least squares mean and mixed-effects model for repeated measures. Adjusted for all available".

Let me know if any additional information would be helpful.

Best,

Elizabeth

From: Haihong Li
Sent: Friday, May 17, 2013 10:22 AM
To: Elizabeth Dorn
Subject: RE: Cochrane review about Ivacaftor - request for information please

Hi Elizabeth,

In the supplement, the "N" represents number of subjects in the Full Analysis Set in respective arms, defined as all randomized subjects who took at one dose of study drug. (To address the last question, this represents modified ITT, and is not per-protocol.) For Study 770-103 N=26 for both ivacaftor and placebo.

Among these, not all subjects completed all assessments. Therefore, for a particular endpoint, the available subjects could be less than 26 per arm.

I went to clinicaltrial.gov and found that the number of subjects reported:

	Placebo	Ivacaftor
Percent Predicted FEV_1	25	26
CFQ-R	25	26
Sweat Chloride	23	23
Weight	26	26

Since the numbers in the supplement and at clinicaltrials.gov carry different interpretations, there is no real discrepancy, especially since the results are identical between these 2 places. However, if the editors insist, we can add the numbers of subjects with available assessments (i.e., numbers from CT.gov) to the table in the supplement.

Thanks,

Haihong

From: Elizabeth Dorn
Sent: Friday, May 17, 2013 8:46 AM
To: Haihong Li
Subject: FW: Cochrane review about Ivacaftor - request for information please

Hi Haihong,

Request from Jane Davies to clarify the following questions. Can you help?

iii) I noticed a few differences between the number of patients included in analyses for some outcomes when your data were presented on clinicaltrials.gov, and the number in the Supplementary file in your paper. In the paper, it is stated that n=52 (26/group) for all outcomes at 24 and 48 weeks, but on clinicaltrials.gov, this is different (although the results are identical) - in particular, there is a discrepancy for sweat chloride (46 on clinical trials.gov), FEV_1 (1 excluded from placebo group), and CFQR (1 excluded from placebo group). Weight appears to have included all patients in the analysis, both on clinicaltrials.gov, and in the paper. I would be grateful if you may clarify the discrepancies here, and whether the analysis was ITT or per-protocol.

E-mail 6: Request for trial protocol and key outcome data measured in the trial but not reported in the published text for the child phase 3 G551D study.

Dear Sanjay and Kevin

I've heard back from Vertex and I'm sorry to say that they are not willing to share these data; I'm disappointed in that decision and will discuss it with senior members of staff whom I'll be seeing soon at a forthcoming meeting but apologise that I can't help further at the moment

Warm regards, Jane

From: Patel, Sanjay [md0u92cd] [mailto:S.Patel7@liverpool.ac.uk]
Sent: 02 February 2014 12:56
To: Davies, Jane C
Cc: Southern, Kevin
Subject: RE: Cochrane systematic review of mutation specific therapy in Cystic Fibrosis

Dear Dr Jane Davies ,

Thank you for taking the time to respond to us regarding outcomes of interest.

There are however, a couple more points I would like to ask about. I apologise for not requesting this information, and the information regarding intention to treat analysis, in the initial e-mail.

Would I be able to request the study protocol for the study: *"Study of Ivacaftor in Cystic Fibrosis Subjects Aged 6 to 11 Years With the G551D Mutation (ENVISION)."* Currently, only the full paper and the supplementary appendix are available online. In addition, the supplementary appendix states that FVC was measured at visits scheduled on day 1, day 15 and week 8, then every week

through week 48, however results have not been reported. Would you be able to provide any numerical data for the outcome change from baseline in Forced Vital Capacity (FVC).

Thank you for your time and we look forward to hearing from you,

Sanjay Patel.

C. Evidence of Interaction with primary author of the adult phase 3 G551D study

E-mail 7: Request for additional outcome data for the adult phase 3 G551D study by Ramsey

From: Sanjay Patel [s.patel7@liv.ac.uk]
Sent: 05 December 2013 16:23
To: bonnie.ramsey@seattlechildrens.org
Cc: Southern, Kevin
Subject: Cochrane systematic review of a mutation specific therapy in Cystic Fibrosis

Dear Professor Ramsey,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel Cystic Fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis Group to conduct a Cochrane systematic review on CFTR potentiators.

We would like to include the study *"A CFTR Potentiator in Patients with Cystic Fibrosis and the G551D Mutation"* (ClinicalTrials.gov number NCT00909532). I am writing to request additional results you may have of the study.

Firstly, confidence intervals or the standard deviation have not been given for the mean difference between groups in the Cystic Fibrosis Questionnaire Revised domain score (CFQ-R). A treatment difference of +8.1 with a P value of P<0.0001 is given, however a standard deviation or confidence interval is required to include this result into the meta-analysis. Also, this is the result given for the pooled result from the adult and child questionnaires. Would it be possible to obtain the non-pooled results?

In addition to this, we would like to request any addition data you may have on the following endpoints of interest;

- Cost of treatment,
- school work attendance,
- radiological measures of lung function,
- Acquisition of respiratory pathogens,
- Eradication of respiratory pathogens and
- Height.

These are endpoints we have included in our review that we hope to comment on.

Any information that you are able to provide will help shape this review and would be highly appreciated.

Regards,

Sanjay Patel

E-mail 8: Request for information on incomplete outcome data in the adult phase 3 G551D study by Ramsey

From: Patel, Sanjay [md0u92cd]
Sent: 27 January 2014 13:14
To: bonnie.ramsey@seattlechildrens.org
Cc: Southern, Kevin
Subject: Cochrane systematic review of mutation specific therapy in Cystic Fibrosis

Dear Dr Ramsey,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel Cystic Fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis Group to conduct a Cochrane systematic review on CFTR potentiators. I have attached the protocol.

We would like to include the study *"Study of Ivacaftor in Cystic Fibrosis Subjects Aged 12 Years and Older with the G551D Mutation (STRIVE)"* (ClinicalTrials.gov number NCT00909532) in our review.

When assessing whether an intention to treat analysis was employed, according to the supplementary appendix table 1, the number of participants analysed in the placebo group is 78 and in the Ivacaftor group is 83. However, according to the results presented on clinicaltrials.gov, 71 participants in the placebo group and 80 in the Ivacaftor group were analysed for the CFQ-R respiratory domain scores. In addition to this, 74 participants in the placebo group and 78 in the Ivacaftor group were analysed for the outcome; change from baseline in sweat chloride concentration. Could you provide an explanation for this discrepancy? I have attached both the supplementary appendix and a screenshots from clinical trials.gov.

I appreciate the time you have taken to read this e-mail and look forward to hearing from you.

Regards,

Sanjay Patel.

E-mail 9: Request for data on outcomes pre-specified in the protocol but not reported in published text in the adult phase 3 G551D study by Ramsey.

This e-mail was sent but cannot be located. It was sent by a different review author.

E-mail 10: Request for clarification on discrepancy between full text and supplementary data in the relative change from baseline in FEV_1 at 24 weeks in the adult phase 3 G551D study by Ramsey.

From: Sanjay Patel [s.patel7@liv.ac.uk]
Sent: 20 January 2014 16:08
To: bonnie.ramsey@seattlechildrens.org
Cc: Southern, Kevin
Subject: Cochrane Systematic Review of CFTR Potentiators for Cystic Fibrosis

Dear Dr Ramsey

I am a Masters student at the University Of Liverpool, UK. I am currently conducting a Cochrane Systematic Review of CFTR potentiators and the study titled "A CFTR potentiator in Patients with Cystic Fibrosis and the G551D Mutation" is suitable for inclusion.

When reading the paper, I noticed a discrepancy. Under the subheading *"Clinical Efficacy"* in the Results section of the paper, you have reported a relative change from baseline of 17.2% in the Ivacaftor group and 0.1% in the placebo group at 24 weeks. This gives a treatment effect of 17.1%. In supplementary table 1 of the supplementary appendix however, you have reported a relative change from baseline of 17.6% in the Ivacaftor group and 0.7% in the placebo group at the same 24 week time point. These values give a treatment effect of 16.9%. Could you clarify this discrepancy.

I appreciate the time taken to read this e-mail and any information you are able to provide.

Regards,

Sanjay Patel.

D. Evidence of Interaction with primary author of the phase 2 G551D study

E-mail 11: Request for additional outcome data and key data that were measured during the trial but not reported in the full text in the phase 2 G551D study by Accurso.

From: Patel, Sanjay [md0u92cd]
Sent: 27 December 2013 13:38
To: accurso.frank@tchden.org
Cc: Southern, Kevin
Subject: Cochrane systematic review of mutation specific therapy in Cystic Fibrosis

Dear Dr Accurso,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel Cystic Fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis Group to conduct a Cochrane systematic review on CFTR potentiators. The following web address is a link to the protocol:

http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD009841/abstract

We would like to include Part 2 of the study *"Effect of VX-770 in Persons with Cystic Fibrosis and the G551D-CFTR Mutation"* (ClinicalTrials.gov number, NCT00457821) in our review. Many of the outcomes reported on in this study match the outcomes of interest that we would like to investigate in the review. However I am contacting you to ask whether you measured or have data on any of the following outcomes:

- survival,
- cost of treatment,
- hospitalisation,
- extra courses of antibiotics,
- radiological measures of lung function,
- Acquisition of respiratory pathogens,
- Eradication of respiratory pathogens,
- nutrition and growth (weight, height and BMI).

For any continuous data outcomes (e.g. measures of lung function) we would appreciate it if you were able to send us values for the mean and standard deviation for each group (treatment and control). For binary data, we need the number of patients experiencing an event (not the actual number of events) per group (treatment and control) in order to be able to analyse these correctly.

Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review

Regards,

Sanjay Patel.

2. Data extraction forms for studies included in the CFTR potentiators review.

Data Extraction Table 1 Phase 2 G551D study by Accurso (2010)

TRIAL ID	ACCURSO 2010				
MAIN REFERENCE	N Engl J Med 2010;363:1991-2003.				
OTHER REFERENCES					
FUNDING (delete)	Pharma	Non-pharma		**DETAILS: VERTEX, NIH, CFF, OTHER NON-PHARMA**	
POPULATION	AGE: >18 YO Median (Range): 21 (18-42) 10 MALES (53%) 9 FEMALES (47%)		GENETICS: AT LEST ONE G551D-CFTR allele		OTHER (EG FEV_1): FEV_1>40% PRED
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM (N RANDOMISED)					
DRUG	VX 770				
DRUG DOSE ARM 1	150 MG BD (N=8)				
DRUG DOSE ARM 2	250 MG BD (N=7)				
LENGTH OF TREATMENT	28 DAYS				
DESCRIPTION OF CONTROL	MATCHED PLACEBO (N=4)				
PRIMARY OUTCOME					
PRIMARY OUTCOME 1	SAFETY				
SAMPLE SIZE	REQUIRED: NOT CALCULATED. ASSUMED 38 SUFFICIENT			RANDOMISED: 19 IN PART 2 (AND 19 IN PART 1)	
PRIMARY TIMEPOINT	CONTINUOUS THROUGHOUT 1 MONTH PERIOD AND 1 WEEK FOLLOW UP				
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)					
MORTALITY		NO			
REL FEV_1		Days 1, 3, 14, 21, and 28			
QOL		Days 1, 14, and 28 ; CFQ-R			
OTHER PFT (SPECIFY)		FVC, FEV_1, FEV_1/FVC, and FEF25-75 ; Days 1, 3, 14, 21, and 28			
HOSPITAL ADMISSION		NO			
SCHOOL/WORK DAYS MISSED		**INCLUDED UNDER 'ROLE' DOMAIN OF CFQ-R**			
EXTRA ANTIBIOTICS		NO			
RADIOLOGICAL OUTCOMES		NO			
NUTRITIONAL OUTCOMES		WEIGHT : Days 1, 3, 14, 21, 28, and at follow-up = **MEASURED BUT NOT REPORTED AS AN OUTCOME**			
SWEAT CHLORIDE		Days 1, 3, 14, 21 and 28			
MICROBIOLOGICAL OUTCOME		NO			
ERADICATION OF RESPIRATORY PATHOGENS		No			
SAFETY		YES			

OUTCOME	TIME	COMPARISON		HOW EXPRESSED	RESULTS						NOTES EG TYPE OF OUTCOME ETC
		ARM 1 (N)	ARM 2 (N)	EG Δ BL, TIME TO	RESULT ARM 1	RESULT ARM 2	DIFF	VARIANCE (SPECIFY)	P	CI	
SWEAT CHLORIDE	D14	150 (8)	PLAC (4)	MEDIAN Δ BL					0.03		1. SWEAT CHLORIDE ALSO MEASURED ON D3 AND D21, BUT RESULTS NOT REPORTED 2. UNITS = MMOL/L
SWEAT CHLORIDE	D14	250 (7)	PLAC (4)	MEDIAN Δ BL					0.05		
SWEAT CHLORIDE	D28	150 (8)	PLAC (4)	MEDIAN Δ BL	-59.5	+5.0			0.02		
SWEAT CHLORIDE	D28	250 (7)	PLAC (4)	MEDIAN Δ BL	-38.0	+5.0			0.03		
FEV_1 (% predicted relative to baseline)	D14	150 (8)	PLAC (4)	MEDIAN REL Δ BL					0.46		1. FEV_1 ALSO MEASURED ON D3 AND D21, BUT RESULTS SHOWN ON FIGURE 3 – NOT STATISTICALLY SIGNIFICANT
		250 (7)	PLAC (4)	MEDIAN REL Δ BL					1.0		
FEV_1 (L)	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL					0.46		
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL					0.07		
FEV_1 (% predicted relative to baseline)	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	+ 8.7% (2.3 TO 31.3)	+7.3% (5.2 TO 8.2)			0.56		
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	+4.4% (0 TO 18.3)	+7.3% (5.2 TO 8.2)			0.78		
FEV_1 (L)	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	+0.25 L (0.05 TO 0.75)	+0.20 L (0.12 TO 0.33)			1.0		
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	+.017 L (0 TO 0.37)	+ 0.20 L (0.12 TO 0.33)			0.65		
FVC	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL	+0.18	+0.10					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL	+ 0.09 L	+0.10					
FEF25-75	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL	+0.06 L/SEC	+0.09			0.56		
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL	+0.44	+0.09			0.05		
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL	+0.05	+0.27					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL	+0.17	+0.27					
QOL – RESP DOMAIN OF CFQR	D14	150 (8)	PLAC (4)	MEDIAN	+5.6 (0	+2.8 (-			0.61		

				VALUE Δ BL (RANGE)	TO 16.7)	5.6 TO 11.1)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	+5.6 (-11.1 TO 11.1)	+2.8 (-5.6 TO 11.1)			0.71		
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	+8.3 (0 TO 16.7)	+2.8 (-5.6 TO 11.1)			0.45		
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	+11.1 (-5.6 TO 33.3)	+2.8 (-5.6 TO 11.1)			0.47		
QOL – BODY IMAGE	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-22.2 TO 0)	0 (-11.1 TO 22.2)					
		250 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-11.1 TO 22.2)	0 (-11.1 TO 22.2)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-22.2 TO 11.1)	-5.6 (-11.1 TO 22.2)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-11.1 TO 44.4)	-5.6 (-11.1 TO 22.2)					
QOL – DIGESTIVE SYMPTOMS	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 TO 22.2)	-5.6 (-22.2 TO 0)					
		250 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-11.1 TO 22.2)	-5.6 (-22.2 TO 0)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	5.6 (0 TO 22.2)	0 (-22.0 TO 0)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-11.1 TO 33.3)	0 (-22.0 TO 0)					
QOL – EATING DISTURBANCES	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 TO 11.1)	0 (-11.1 TO 11.1)					
		250 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-11.1 TO 0)	0 (-11.1 TO 11.1)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 TO 0)	-5.6 (-11.1 TO 0)					
		250 (7)	PLAC (4)	MEDIAN	0 (-11.1	-5.6 (-					

				VALUE Δ BL (RANGE)	TO 0)	11.1 TO 0)					
QOL – EMOTIONAL FUNCTIONING	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-26.7 TO 0)	13.3 (-6.7 TO 20.0)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-20.0 TO 26.7)	13.3 (-6.7 TO 20.0)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-6.7, 6.7)	3.3 (0, 20.0)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	6.7 (-6.7, 20.0)	3.3 (0, 20.0)					
QOL – HEALTH PERCEPTIONS	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-22.2 to 22.2)	5.6 (-11.1 to 11.1)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	(-33.3 to 11.1)	5.6 (-11.1 to 11.1)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-22.2 to 22.2)	0 (-11.1 to 11.1)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-11.1 to 11.1)	0 (-11.1 to 11.1)					
QOL – PHYSICAL FUNCTIONING	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	2.1 (-4.2 to 16.7)	0 (0 to 4.2)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	4.2 (0 to 8.3)	0 (0 to 4.2)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	4.2 (-8.3 to 25.0)	2.1 (0 to 4.2)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 to 12.5)	2.1 (0 to 4.2)					
QOL – ROLE	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-8.3 to 8.3)	0 (0 to 8.3)					
		150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 to 8.3)	0 (0 to 8.3)					
	D28	150 (8)	PLAC (4)	MEDIAN	0 (-8.3 to	0 (0 to 0)					

				VALUE Δ BL (RANGE)	8.3)						
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-8.3 to 8.3)	0 (0 to 0)					
QOL – SOCIAL	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-44.4 to 11.1)	2.8 (-5.6 to 5.6)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	-5.6 (-16.7 to 5.6)	2.8 (-5.6 to 5.6)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-11.1 to 5.6)	2.8 (-11.1 to 5.6)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-16.7 to 11.1)	2.8 (-11.1 to 5.6)					
QOL – TREATMENT BURDEN	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	-5.6 (-22.2 to 11.1)	0 (-11.1 to 11.1)					
		150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-22.2 to 22.2)	0 (-11.1 to 11.1)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	-5.6 (-22.2 to 11.1)	0 (0 to 0)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-22.2 to 11.1)	0 (0 to 0)					
QOL – VITALITY	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	4.2 (-8.3 to 16.7)	4.2 (-16.7 to 8.3)					
		150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-8.3 to 16.7)	4.2 (-16.7 to 8.3)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-16.7 to 25.0)	-8.3 (-16.7 to 0)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 to 16.7)	-8.3 (-16.7 to 0)					
QOL – WEIGHT	D14	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 to 33.3)	0 (0 to 33.3)					
		150 (8)	PLAC (4)	MEDIAN	0 (-33.3	0 (0 to					

				VALUE Δ BL (RANGE)	to 33.3)	33.3)					
	D28	150 (8)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (0 to 33.3)	0 (0 to 33.3)					
		250 (7)	PLAC (4)	MEDIAN VALUE Δ BL (RANGE)	0 (-33.3 to 33.3)	0 (0 to 33.3)					

OUTCOME	**TIME**	**COMPARISON**		**HOW EXPRESSED**	**RESULTS**						**NOTES EG TYPE OF OUTCOME ETC**		
		ARM 1 (N)	**ARM 2 (N)**	**EG Δ BL, TIME TO**	**RESULT ARM 1**	**RESULT ARM 2**	**DIFF**	**VARIANCE (SPECIFY)**	**P**	**CI**			
WEIGHT											MEASURED BUT NOT REPORTED IN PUBLICATION		
SWEAT CHLORIDE (MMOL/L)	D28	150	PLAC	MEDIAN VALUE Δ BL (RANGE)	-59.5 (-66.0 TO -19.0)	+5.0 (-2.0 TO +11.0)			0.02		D1, D3 , D14, D21 REPORTED BUT NOT EXTRACTED AS TOO SHORT TERM FOR THIS REVIEW?		
		250	PLAC	MEAN CHANGE BL	-38.0 (-47.0 TO -10.5)	+5.0 (-2.0 TO +11.0)			0.03				

SEVERITY OF ADVERSE EVENTS, AS GRADED BY TRIAL AUTHORS

	PLACEBO N=4	VX770 ANY DOSE N=15	VX770 150MG N=8	VX770 250 MG N=7
MILD	3 (75)	10	5 (63)	5 (71)
MODERATE	1 (25)	2	1 (13)	1 (14)
SEVERE	0	0	0	0

DISCONTINUATION OF STUDY DRUG : NONE IN ANY GROUP

ADVERSE EVENT/ EFFECT REQUIRING STUDY DRUG INTERRUPTION : NOT REPORTED

Data Extraction Table 2 Adult Phase 3 G551D study by Ramsey (2011)

TRIAL ID	RAMSEY 2011 – "STRIVE"			
MAIN REFERENCE	N Engl J Med 2011;365:1663-72.			
FUNDING (delete)	Pharma	Non-pharma	Unclear	**DETAILS: VERTEX AND OTHERS (NON-PROFIT EG NIH)**
POPULATION	AGE: >12 YO GENETICS: AT LEAST ONE G551D ALLELE OTHER (EG FEV_1): FEV_1>40%PRED			
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM				
DRUG	Ivacaftor			
DRUG DOSE ARM	150 MG BD			
LENGTH OF TREATMENT	48 WEEKS			
DESCRIPTION OF CONTROL	MATCHED PLACEBO			
PRIMARY OUTCOME				
PRIMARY OUTCOME 1	ABSOLUTE CHANGE FROM BASELINE IN FEV_1			
PRIMARY OUTCOME 2				
SAMPLE SIZE	REQUIRED: 80		RANDOMISED: 160	
PRIMARY TIMEPOINT	48 WEEKS			
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)				
MORTALITY				
FEV_1	Y	Spirometry (FEV_1,) measured D1, D15, W4, W8, W12, W16, W20 W24, W28, W32, W36, W44, W48		
QOL	Y	CFQ-R resp domain only measured D1, D15, W4, W8, W12, W16, W20 W24, W28, W32, W36, W44, W48 OTHER CFQ-R DOMAIN presented in abstract form - **significant results only** EQ-5D Measured D1, D15, W8, W16, W24, W32, W40, W48 – **NOT REPORTED IN PUBLICATION**		
OTHER PFT (SPECIFY)		FVC, FEV_1/FVC/FEF MEASURED ACCORDING TO PROTOCOL measured D1, D15, W4, W8, W12, W16, W20 W24, W28, W32, W36, W44, W48		
HOSPITAL ADMISSION	Y			
SCHOOL/WORK DAYS MISSED	N			
EXTRA ANTIBIOTICS	Y			
RADIOLOGICAL OUTCOMES	N			
NUTRITIONAL OUTCOMES	Y	WEIGHT Measured D1, D15, W8, W16, W24, W32, W40, W48 HEIGHT Measured D1, D15, W8, W16, W24, W32, W40, W48 – **NOT REPORTED IN PUBLICATION**		
SWEAT CHLORIDE	Y	Measured D1, D15, W8, W16, W24, W32, W40, W48		
MICROBIOLOGICAL OUTCOME	N			
SAFETY	Y	Adverse effects of therapy – measured D1, D15, W4, W8, W12, W16, W20 W24, W28, W32, W36, W44, W48		

OUTCOME	TIME (wks)	COMPARISON		HOW EXPRESSED	RESULTS						NOTES EG TYPE OF OUTCOME ETC
		ARM 1 (N)	ARM 2 (N)	EG Δ BL, TIME TO	RESULT ARM 1	RESULT ARM 2	MEAN DIFF	VARIANCE (SPECIFY)	P	CI	
FEV_1 %PRED	24	83	78	MEAN CHANGE BL %PRED +/- SE	10.4 +/- 0.7	-0.2 +/- 0.7	10.6	SEM +/-1.0	<0.0001	8.6-12.6	
FEV_1 %PRED	48	83	78	MEAN CHANGE BL %PRED	10.1+/- 0.7	-0.4 +/- 0.7	10.5	SEM +/-1.0	<0.0001	8.5-12.5	
FEV_1 LITRES	24	83	78	MEAN CHANGE BL, LITRES	0.4	0.0	0.4		<0.0001	0.3-0.4	
FEV_1 LITRES	48	83	78	MEAN CHANGE BL LITRES	0.4	0.0	0.4		<0.0001	0.3-0.4	
FEV_1 RELATIVE	24	83	78	MEAN CHANGE BL RELATIVE	17.6	0.7	16.9		<0.0001	13.6-20.2	
FEV_1 RELATIVE	48	83	78	MEAN CHANGE BL RELATIVE	17.5	0.8	16.8		<0.0001	13.5-20.1	
QOL – pooled child and adult respiratory domain	24	80	71	MEAN CHANGE BL +/- SE	6.0 +/- 1.2	-2.1 +/- 1.3	8.1	SEM +/-1.7	<0.0001	4.7-11.4	
QOL – pooled child and adult respiratory domain	48	80	71	MEAN CHANGE BL +/- SE	6.0 +/- 1.1	-2.7 +/- 1.2	8.6	SEM +/-1.7	<0.0001	5.3-11.9	
HOSPITALISATION	48/52	83	78	NUMBER OF EXACERBATIONS REQUIRING	21	33 *			0.1948		*NOTE – 33 REPORTED IN SUPPLEMENTARY DATA, BUT 31 REPORTED IN PUBLICATION
HOSPITALISATION	48/52	83	78	DAYS HOSPITALISED FOR EXACERBATIONS	3.92	4.15			0.0275		

PULMONARY EXACERBATION – REQUIRING IV ANTIBIOTICS	48	83	78	RATE OF EVENT	47	99			0.0003		NOTE, THIS IS NOT PRESPECIFIED AS AN OUTCOME IN OUR PROTOCOL		
EXTRA IV ANTIBIOTIC (PULMONARY EXACERBATION)	48	83	78	RATE OF EVENT	28	47			0.0776				
DAYS WITH IV ABX ADMINISTERED FOR PULMONARY EXACERBATIONS	48	83	78	DAYS WITH IV ABX FOR PULMONARY EXACERBATIONS	6.68 (19.43)	11.03 (20.36)			0.0183				
EXTRA IV ANTIBIOTIC (PULMONARY EXACERBATION)				TIME TO – REPORTED AS PROPORTION-FREE									
WEIGHT	24	83	78	MEAN CHANGE FROM BL, IN KG +/- SEM	3.0 +/- 0.4	0.2 +/- 0.2	2.8	SEM +/- 0.5	<0.0001	1.8 TO 3.7	IN SUPPLEMENTARY APPENDIX P VALUE FOR DIFFERENCE IS <0.0001 BUT IN BOROWITZ ABSTRACT P=0.0001 AT 48 WKS.		
WEIGHT	48	83	78	MEAN CHANGE FROM BL, IN KG +/- SEM	3.1 +/- 0.5	0.4 +/- 0.5	2.7	SEM +/- 0.7	<0.0001	1.3-4.1			
BMI	24/52			MEASURED BUT NOT REPORTED									
	48/52			MEASURED BUT NOT REPORTED									
NOTE – BMI AND HEIGHT, REPORTED FOR 12-20 YO WERE PRESENTED IN BOROWITZ ABSTRACT BUT NOT IN STUDY REPORT													
SWEAT CHLORIDE	24	78	74	MEAN CHANGE BL MMOL/L +/- SEM	-48.7 +/- 1.2	-0.8 +/- 1.3	-47.9	SEM +/- 1.7	<0.0001	-51.3 TO -44.5			
SWEAT CHLORIDE	48	78	74	MEAN CHANGE BL MMOL/L +/- SEM	-48.7 +/- 1.2	-0.6 +/- 1.3	-48.1	SEM +/- 1.7	<0.0001	-51.5 TO -44.7			

WEIGHT Z SCORE	48			MEAN CHANGE BL			0.33		0.0260		ONLY 47 PARTICIPANTS OF 161 WERE INCLUDED IN Z SCORE ANALYSIS. (BORROWITZ ABSTRACT)
BMI (KG/M^2)	48	83	78	MEAN CHANGE BL			0.93		<0.0001		(BORROWITZ ABSTRACT)
BMI Z SCORE (AGED 12-20)	48			MEAN CHANGE BL			0.33		0.0490		ONLY 47 PARTICIPANTS OF 161 WERE INCLUDED IN Z SCORE ANALYSIS. (BORROWITZ ABSTRACT)
HEIGHT Z SCORE (AGED 12-20)	24			MEAN CHANGE BL			+0.05				ONLY 47 PARTICIPANTS OF 161 WERE INCLUDED IN Z SCORE ANALYSIS. (BORROWITZ ABSTRACT) "NOT SIGNIFICANT' NO P VALUE PUBLISHED
HEIGHT Z SCORE (AGED 12-20)	48			MEAN CHANGE BL			+0.06				ONLY 47 PARTICIPANTS OF 161 WERE INCLUDED IN Z SCORE ANALYSIS. (BORROWITZ ABSTRACT) "NOT SIGNIFICANT' NO P VALUE PUBLISHED
QOL – pooled child and adult physical functioning scale domain (points)	48	?	?	MEAN CHANGE BL			4.4		0.0055		Quittner abstract 2012 European CF conference
QOL – pooled child and adult social functioning scale domain (points)	48	?	?	MEAN CHANGE BL			4.3		0.0026		
QOL – pooled child and adult eating disturbances scale domain (points)	48	?	?	MEAN CHANGE BL			3.3		0.0021		
QOL – pooled child and adult treatment burden scale domain (points)	48	?	?	MEAN CHANGE BL			3.3		0.0419		
% PRED FEV_1 in subgroup: Mean change in FEV_1 % PRED <5%	48	22	64	MEAN CHANGE BL			4.2		<0.0001		B.J Plant abstract only not in full text
Sweat chloride (mMol/L) in subgroup: Mean change in FEV_1 % PRED <5%	48	20	64	MEAN CHANGE BL			-46.1		<0.0001		
Body weight (kg) in subgroup: Mean change in FEV_1 % PRED <5%	48	22	64	MEAN CHANGE BL			3.3		<0.0001		

% PRED FEV_1 in subgroup: Mean change in FEV_1 % PRED >5%	48	61	12	MEAN CHANGE BL			6.2		0.0023		B.J Plant abstract only not in full text		
Sweat chloride (mMol/L) in subgroup: Mean change in FEV_1 % PRED >5%	48	58	11	MEAN CHANGE BL			-49.7		<0.0001				
Body weight (kg) in subgroup: Mean change in FEV_1 % PRED >5%	48	61	12	MEAN CHANGE BL			1.7		0.3313				

SAFETY PROFILE

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS IN WHOM STUDY DRUG WAS INTERRUPTED OR DISCONTINUED

	PLACEBO N= 78	VX770 150 MG N=83
INTERRUPTED	5 (6%)	11 (13%)
DISCONTINUED	4 (5%)	1 (1%)

Data Extraction Table 3 Child Phase 3 G551D study by Davies (2013)				
TRIAL ID	**DAVIES 2013 – 'ENVISION'-**			
MAIN REFERENCE	AM J RESPIR CRIT CARE MED. 2013 JUN 1;187(11):1219-25. DOI: 10.1164/RCCM.201301-0153OC CONFERENCE ABSTRACTS, ALSO INFORMATION TAKEN FROM CLINICALTRIALS.GOV			
FUNDING (delete)	Pharma			**DETAILS: SUPPORTED BY VERTEX**
POPULATION	AGE: 6-11 GENETICS: At least one G551D-CFTR allele OTHER (EG FEV_1): FEV_1 40-105% PRED (AGE, SEX, HEIGHT), WEIGHT => 15KG.			
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM				
DRUG				
DRUG DOSE ARM 1	150 MG VX 700 BD			
DRUG DOSE ARM 2				
DRUG DOSE ARM 3				
DRUG DOSE ARM 4				
LENGTH OF TREATMENT	48 WEEKS			
DESCRIPTION OF CONTROL	PLACEBO			
PRIMARY OUTCOME				
PRIMARY OUTCOME 1	Absolute change from baseline in percent predicted forced expiratory volume in 1 second (% predicted FEV_1)			
PRIMARY OUTCOME 2				
SAMPLE SIZE	REQUIRED: Minimum of 30 based on anticipated available population NOT power calculations			RANDOMISED: 52 (26 EACH ARM) – NOTE 1 PLACEBO WITHDRAWN AS WRONG GENETICS
PRIMARY TIMEPOINT	WEEK 24			
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)				
MORTALITY	Y	IN TEXT		
REL FEV_1	Y			
QOL (Total/domain SPECIFY)	Y	RESPIRATORY DOMAIN ONLY - Measured at days 1, 15 and weeks 8, 16, 24, 32, 40 and 48 DATA WEEK 24 AND 48, Other data plotted on graph		
OTHER PFT (SPECIFY)	Y	ABS FEV_1, FVC, FEV_1/FVC, $FEF_{25\%-75\%}$ Measured at days 1, 15 and weeks 8, 16, 24, 32, 40 and 48 ABS FEV_1 – DATA FOR W24, W48, other data plotted on graph		
HOSPITAL ADMISSION	N			
SCHOOL/WORK DAYS MISSED	N			
EXTRA ANTIBIOTICS	N			
RADIOLOGICAL OUTCOMES	N			

NUTRITIONAL OUTCOMES (SPECIFY)		WEIGHT Measured at days 1, 15 and weeks 8, 16, 24, 32, 40 and 48 DATA WEEK 24 AND 48 other data plotted on graph HEIGHT – **measured BMI but no data for height published except in Borrowitz abstact which reported height z scores** (protocol not available for schedule of assessments)
SWEAT CHLORIDE	Y	Measured at days 1, 15 and weeks 8, 16, 24, 32, 40 and 48 DATA WEEK 24 AND 48 - other data plotted on graph
MICROBIOLOGICAL OUTCOME	N	
ADVERSE EVENTS	Y	Measured at days 1, 15 and weeks 8, 16, 24, 32, 40 and 48 PULMONARY EXACERBATION Measured at days 1, 15 and weeks 8, 16, 24, 32, 40 and 48

OUTCOME	**TIME**	**COMPARISON**		**HOW EXPRESSED**	**RESULTS**						**NOTES EG TYPE OF OUTCOME ETC**
		ARM 1 (N)	**ARM 2 (N)**	**EG Δ BL, TIME TO**	**RESULT ARM 1**	**RESULT ARM 2**	**MEAN DIFF**	**VARIANCE (SPECIFY)**	**P**	**95%CI**	
FEV_1 (% PRED)	WEEK 24	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL %PRED +/-**SE	12.6 +/- 2.1	0.1 +/= 2.1	12.5	SEM 2.9	<0.0001	6.6 to 18.3	
FEV_1 (% PRED)	WEEK 48	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL %PRED +/-**SE	10.7 +/- 1.9	0.7 +/- 2.0	10.0	2.7	0.0006	4.4 to 15.5	
FEV_1 (L)	WEEK 24	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL (L)**	0.303	0.067	0.0236		0.0001	0.123 to 0.349	
FEV_1 (L)	WEEK 48	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL (L)**	0.325	0.125	0.200		0.0007	0.089 to 0.311	
FEV_1 (% PRED) – BASELINE FEV_1 < 90%	WEEK 24	150 GM (16)	PLAC (14)	MEAN ABSOLUTE **Δ BL (L)**			14.9			7.3 TO 22.5	Subgroup analysis
FEV_1 (% PRED) – BASELINE FEV_1 > 90%	WEEK 24	150 GM (10)	PLAC (11)	MEAN ABSOLUTE **Δ BL (L)**			6.9			-3.8 TO 17.6	
FEV_1 (% PRED) – N. AMERICA	WEEK 24	150 GM (12)	PLAC (14)	MEAN ABSOLUTE **Δ BL (L)**			5.8			-2.6 TO 14.1	
FEV_1 (% PRED) – EUROPE	WEEK 24	150 GM (6)	PLAC (5)	MEAN ABSOLUTE **Δ BL (L)**			24.6			6.4 TO 42.9	
FEV_1 (% PRED) – AUSTRALIA	WEEK 24	150 GM (6)	PLAC (8)	MEAN ABSOLUTE **Δ**			4.2			-3.7 TO 12.0	

				BL (L)							
FEV_1 (% PRED) – MALE	WEEK 24	150 GM (9)	PLAC (15)	MEAN ABSOLUTE **Δ BL (L)**			5.2			-2.2 TO 12.5	
FEV_1 (% PRED) – FEMALE	WEEK 24	150 GM (17)	PLAC (10)	MEAN ABSOLUTE **Δ BL (L)**			13.8			4.2 TO 23.4	
QOL – CFQ-R Respiratory domain CHILD VERSION (<=12)	WEEK 24	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL +/-**SE	6.3 **+/- 2.5**	0.3 **+/-** 2.6	6.1	+/- 2.7	0.1092	-1.4 to 13.5	
QOL – CFQ-R Respiratory domain CHILD VERSION (<=12)	WEEK 48	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL +/-**SE	6.1 +/- 2.2	1.0 +/- 2.3	5.1	3.3	0.1354	-1.6 TO 11.8	
QOL – CFQ-R Respiratory domain PARENT/CAREGIVER VERSION	WEEK 24	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL**	4.9	-1.1	5.9		0.0330	0.5 to 11.4	
QOL – CFQ-R Respiratory domain PARENT/CAREGIVER VERSION	WEEK 48	150 GM (26)	PLAC (25)	MEAN ABSOLUTE **Δ BL**	3.7	-1.2	4.9		0.1354	-0.4 TO 10.2	
SWEAT CHLORIDE (mmol/l)	WEEK 24	150 GM (23)	PLAC (23)	MEAN ABSOLUTE **Δ BL +/-**SE	-55.5 +/- 2.6	-1.2 +/- 2.6	-54.3	3.7	<0.0001	-61.8 TO -46.8	
	WEEK 48	150 GM (23)	PLAC (23)	MEAN ABSOLUTE **Δ BL +/-**SE	-56.0 +/- 2.5	-2.6 +/- 2.6	-53.5	3.7	<0.0001	-60.9 TO -46.0	
WEIGHT (KG)	WEEK 24	150 GM (26)	PLAC (26)	MEAN ABSOLUTE **Δ BL +/-**SE	3.7 +/- 0.4	1.8 +/- 0.4	1.9	0.5	0.0004	0.9 TO 2.9	
	WEEK 48	150 GM (26)	PLAC (26)	MEAN ABSOLUTE **Δ BL +/-**SE	5.9 +/- 0.5	3.1 +/- 0.5	2.8	0.7	0.0002	1.3 TO 4.2	
$FEF_{25\%-75\%}$	WEEK 24	150 GM (26)	PLAC (24)	MEAN ABSOLUTE **Δ BL**	20.7	-1.6	22.3		0.0002	(11.1 to 33.5)	
	WEEK 48	150 GM (26)	PLAC (24)	MEAN ABSOLUTE **Δ BL**	18.5	-0.7	19.2		0.0011	(8.1 to 30.2)	
BMI for age Z SCORES	WEEK 24						0.34		P<0.001		Limited information precluding inclusion into the meta-analysis.
	WEEK 48						0.45		P>0.001		

FEV_1 (RELATIVE)	WEEK 24	150 GM (26)	PLAC (24)	MEAN RELATIVE**Δ BL**	21.7%	4.3%	17.4%		P<0.0001		
WEIGHT Z SCORES	WEEK 24	150 GM (26)	PLAC (26)	MEAN **Δ BL**			0.39		<0.0001		
BMI (KG/M^2)	48	26	26	MEAN **Δ BL**			1.09		0.0003		(BORROWITZ ABSTRACT)
BMI Z SCORE	48	26	26	MEAN CHANGE BL			0.45		<0.0001		(BORROWITZ ABSTRACT)
HEIGHT Z SCORE	24			MEAN CHANGE BL			+0.06				(BORROWITZ ABSTRACT) "NOT SIGNIFICANT' NO P VALUE PUBLISHED
HEIGHT Z SCORE	48			MEAN CHANGE BL			+0.12				(BORROWITZ ABSTRACT) "NOT SIGNIFICANT' NO P VALUE PUBLISHED
QOL – POOLED SCORE	48	26	26	MEAN CHANGE BL			5.1		0.1354		Quittner abstract 2012 European CF conference
% PRED FEV1 in subgroup: Mean change in FEV_1% PRED <5%	48	10	18	MEAN CHANGE BL			1.6		0.5093		B.J Plant abstract only not in full text
Sweat chloride (mMol/L) in subgroup: Mean change in FEV_1% PRED <5%	48	9	17	MEAN CHANGE BL			-55.8		<0.0001		
Body weight (kg) in subgroup: Mean change in FEV_1% PRED <5%	48	10	18	MEAN CHANGE BL			2.0		0.0582		
% PRED $_{FEV1}$ in subgroup: Mean change in FEV_1% PRED >5%	48	16	6	MEAN CHANGE BL			9.8		0.0522		B.J Plant abstract only not in full text
Sweat chloride (mMol/L) in subgroup: Mean change in FEV_1% PRED >5%	48	16	6	MEAN CHANGE BL			-53.9		<0.0001		
Body weight (kg) in subgroup: Mean change in FEV_1% PRED >5%	48	16	6	MEAN CHANGE BL			3.4		0.0094		

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS

	PLACEBO N= 26	VX770 150 MG N=26
INTERRUPTED	3 (11%)	1 (4%)
DISCONTINUED	1 (4%)	0

Data Extraction Table 4 ΔF508 study by Flume (2011)						
TRIAL ID	FLUME 2011 – "DISCOVER"					
MAIN REFERENCE	FLUME 2011 CONFERENCE ABSTRACTS + METHODS AND RESULTS INFO FROM CLINICALTRIALS.GOV					
FUNDING (delete)	Pharma	Non-pharma	Unclear	**DETAILS:**		
POPULATION	AGE: >12 YEARS		GENETICS: HOMOZYGOUS FOR DF 508		OTHER (EG FEV_1): >40%	
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM						
DRUG	VX770					
DRUG DOSE ARM 1	150 MG VX770 BD					
DRUG DOSE ARM 2						
DRUG DOSE ARM 3						
DRUG DOSE ARM 4						
LENGTH OF TREATMENT	16 WEEKS					
DESCRIPTION OF CONTROL	PLACEBO					
PRIMARY OUTCOME						
PRIMARY OUTCOME 1	ABSOLUTE CHANGE IN %PRED FEV_1					
PRIMARY OUTCOME 2						
SAMPLE SIZE	REQUIRED:				RANDOMISED: 140 (112 VX770, 28 PLAC)	
PRIMARY TIMEPOINT	16 WEEKS					
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)						
MORTALITY						
FEV_1	Y	D15, W8, W16				
QOL	Y	CFQ-R respiratory domain score				
OTHER PFT (SPECIFY)		ABS Δ FEV_1 D15, W8, W16 – NO DATA FOR WEEK 8 FVC, FEF				
HOSPITAL ADMISSION						
SCHOOL/WORK DAYS MISSED						
EXTRA ANTIBIOTICS	Y	ABX Rx FOR SINOPULOMNARY SIGNS OR Sx				
RADIOLOGICAL OUTCOMES						
NUTRITIONAL OUTCOMES	Y	WEIGHT BMI WEIGHT FOR AGE SCORE				
SWEAT CHLORIDE	Y					
MICROBIOLOGICAL OUTCOME						
SAFETY	Y	ADVERSE EVENTS NO PULMONARY EXACERBATIONS				

OUTCOME	TIME	COMPARISON		HOW EXPRESSED	RESULTS						NOTES EG TYPE OF OUTCOME ETC		
		ARM 1 (N)	ARM 2 (N)	EG Δ BL, TIME TO	RESULT ARM 1	RESULT ARM 2	MEAN DIFF	VARIANCE (SEM)	P	CI			
FEV_1	16 WEEK	VX770 (111)	PLAC (28)	ABSOLUTE CHANGE BL % PRED	1.5 +/- 0.5	-0.2 +/- 1.1	1.7	1.2	0.15	-0.6 TO 4.1			
QOL – CFQ-R RESP DOMAIN	16 WEEK	VX770 (111)	PLAC (28)	ABSOLUTE CHANGE BL	-0.1 +/- 1.0	-1.4 +/- 1.9	1.3	2.1	0.54	-2.9 TO 5.6			
SWEAT CHLORIDE	16 WEEK	VX770 (111)	PLAC (28)	ABSOLUTE CHANGE BL	-2.7 +/- 0.6	0.1 +/- 1.2	-2.9	1.4	0.04	-5.6 TO 0.2			
WEIGHT	16 WEEK	VX770 (111)	PLAC (28)	ABSOLUTE CHANGE BL	0.8 +/- 0.2	0.9 +/- 0.4	-0.2	0.5	0.727	-1.1 TO 0.7			
BMI (kg/m^2)	16 WEEK	VX770 (112)	PLAC (28)	ABSOLUTE CHANGE BL	0.21	00.25	0.04						
WEIGHT-FOR-AGE-Z-SCORE	16 WEEK	VX770 (56)	PLAC (7)	ABSOLUTE CHANGE BL	0.43	0.0007							
BMI-FOR-AGE-Z-SCORE	16 WEEK	VX770 (56)	PLAC (7)	ABSOLUTE CHANGE BL	0.73	-0.002							
PULMONARY EXACERBATION	16 WEEK	VX770 (112)	PLAC (28)	NO (%)	25 (21)	10 (32)							
ABX Rx FOR SINOPULOMNARY SIGNS OR Sx	16 WEEK	VX770 (112)	PLAC (28)	NO (%)	48 (43)	16 (56)							

SAFETY PROFILE

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS

	PLACEBO N= 28	VX770 150 MG N=112
INTERRUPTED	0	2 (1.8%)
DISCONTINUED	2 (7.1%)	3 (2.7%)

3. Interaction with primary authors of studies included in the CFTR correctors review

Evidence of interaction with primary author of the pilot 4PBA study

E-mail 12: Interaction with primary author of the pilot 4PBA study

From: Ron Rubenstein [mailto:rrubenst@mail.med.upenn.edu]
Sent: 21 April 2014 11:57
To: Patel, Sanjay [md0u92cd]
Cc: Southern, Kevin
Subject: Re: Cochrane Systematic Review of CFTR correctors - request for information
Hi Sanjay

In response to your queries:

I cannot find the trial protocol. Dr Pam Zeitlin may have it.

The change from baseline sweat Cl concentration was presented in a figure in the publication

As this was a one week trial, pulmonary function, QOL (for which there was no validated instrument at the time), admissions, work/school missed, extra antibiotics, radiology and respiratory pathogens were not assessed.

Good luck with your review,

Ron Rubenstein

Ron Rubenstein, MD, PhD

Associate Professor of Pediatrics

Perelman School of Medicine at the University of Pennsylvania

Director, Cystic Fibrosis Center

Richard B. Johnston, Jr. Endowed Chair in Pediatrics

Division of Pulmonary Medicine and Cystic Fibrosis Center

The Children's Hospital of Philadelphia

Email: rrubenst@mail.med.upenn.edu

From: "Patel, Sanjay [md0u92cd]" <S.Patel7@liverpool.ac.uk>
Date: Wednesday, April 16, 2014 5:52 AM
To: "'rrubenst@welchlink.welch.jhu.edu'" <rrubenst@welchlink.welch.jhu.edu>, Ronald Rubenstein <rrubenst@mail.med.upenn.edu>
Cc: "Southern, Kevin" <K.W.Southern@liverpool.ac.uk>
Subject: Cochrane Systematic Review of CFTR correctors - request for information

Dear Professor Rubenstein,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel cystic fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis & Genetic Disorders Group to conduct a Cochrane systematic review on CFTR correctors. I have attached a copy of the protocol for this systematic review for your information.

We would like to include the study *"***A Pilot Clinical Trial of Oral Sodium 4-Phenylbutyrate (Buphenyl) in *D*F508-Homozygous Cystic Fibrosis Patients***"*. I am contacting you to request
1) Additional data for the outcomes measured during the trial
2) Data for other outcomes important to our review
3) The trial protocol
4) Information on any additional clinical trials of CFTR corrects that you may be aware of.

1. Additional data required from outcomes measured during the trial:
    a. Change from baseline sweat chloride concentration

2. There are outcomes in our review, that do not correspond to outcomes measured in your trial however we feel it is important to request this information in case information is available. The additional outcomes are
    a. pulmonary function (relative change of FEV_1, FVC)
    b. quality of life
    c. hospital admissions,
    d. school/work days missed,
    e. extra antibiotics
    f. radiological outcomes
    g. acquisition and eradication of respiratory pathogens

For continuous data outcomes (e.g. measures of lung function) we would appreciate it if you were able to send us values for the mean and standard deviation for each group (all treatment groups and control).

3. We would also like to request the trial protocol.

4. So far we have retrieved the following additional studies through our electronic and manual literature searches.

1. Boyle 2011: *"Study of VX-809 Alone and in Combination With VX-770 in Cystic Fibrosis (CF) Patients Homozygous or Heterozygous for the F508del-CFTR Mutation"* (Clinical trial number NCT01225211)
2. Clancy 2012: *"Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation"* (Clinical trial number NCT00865904)
3. Donaldson 2013: *"VX-661, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the F508Del-CFTR mutation"* (Clinical trial number NCT01531673)
4. McCarty 2002: *"A Phase 1 Randomized, Multicenter Trial of CPX in Adult Subjects With Mild Cystic Fibrosis"* (Clinical trial number NCT00004428)

5. Zeitlin 2002: *"Evidence of CFTR Function in Cystic Fibrosis after Systemic Administration of 4-Phenylbutyrate"*

As a leader in the field of cystic fibrosis, I would also like to ask if you know about any additional clinical trials on CFTR Correctors (with published or unpublished results) that we may have missed out.

Thank you for taking the time out to read this e-mail. Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review.

Sanjay Patel

Evidence of interaction with primary author of the phase 2 4PBA study

E-mail 13: interaction with primary author of the phase 2 4PBA study

From: Pamela Zeitlin [mailto:pzeitlin@jhmi.edu]
Sent: 28 April 2014 15:45
To: Patel, Sanjay [md0u92cd]
Cc: Southern, Kevin
Subject: RE: Cochrane Systematic Review of CFTR correctors - request for information

Dear Sanjay

Thank you for your interest in your trial. I may not be able to get everything you request given how "ancient" our trials seem now,

But I will take a look. What sort of time frame are you working in?

Pam

Pamela L. Zeitlin, M.D. Ph.D.
Professor of Pediatrics
Director, Eudowood Division of Pediatric Respiratory Sciences
Deputy Director, Institute for Clinical and Translational Research
Phone: 410 955 2035
Fax: 410 955 103

From: Patel, Sanjay [md0u92cd] [mailto:S.Patel7@liverpool.ac.uk]
Sent: Wednesday, April 16, 2014 6:05 AM
To: Pamela Zeitlin
Cc: Southern, Kevin
Subject: Cochrane Systematic Review of CFTR correctors - request for information

Dear Professor Zeitlin,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel cystic fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis & Genetic Disorders Group to conduct a Cochrane systematic review on CFTR correctors. I have attached a copy of the protocol for this systematic review for your information.

We would like to include the study ***"Evidence of CFTR Function in Cystic Fibrosis after Systemic Administration of 4-Phenylbutyrate".*** I am contacting you to request for

1) additional data for the outcomes measured during the trial
2) data for other outcomes important to our review
3) information on missing data
4) information on any additional clinical trials of CFTR corrects that you may be aware of.

1. Additional data required from outcomes measured during the trial
    a. Change from baseline in sweat chloride concentration at day 2, day 3, day 4 and day 7
    b. Change from baseline in pulmonary function test results at day 3, day 4 and day 7
    c. respiratory pathogens scores at day 7

2. There are outcomes in our review, that do not correspond to outcomes measured in your trial however we feel it is important to request this information in case information is available. The additional outcomes are
    a. survival,
    b. pulmonary function (relative change of FEV_1, FVC)
    c. quality of life
    d. hospital admissions for adverse events
    e. extra antibiotics
    f. radiological outcomes
    g. nutritional outcomes (weight, height, BMI)

For continuous data outcomes (e.g. measures of lung function) we would appreciate it if you were able to send us values for the mean and standard deviation for each group (all treatment groups and control).

3. We would appreciate any clarification on the following issues:
    a. A sample size of 6 participants per group was planned, but only 4 participants were randomised to the placebo group. Why weren't six participants randomised to the placebo group?
    b. In Table 1 showing baseline NPD parameters, the total number of participants enrolled in the study appears to be 35.
    c. It is unclear how many participants were used in the analysis of each outcome measure, so it is unclear whether an ITT approach was taken.

4. So far we have retrieved the following additional studies through our electronic and manual literature searches.

1. Boyle 2011: *"Study of VX-809 Alone and in Combination With VX-770 in Cystic Fibrosis (CF) Patients Homozygous or Heterozygous for the F508del-CFTR Mutation"* (Clinical trial number NCT01225211)
2. Clancy 2012: *"Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation"* (Clinical trial number NCT00865904)
3. Donaldson 2013: *"VX-661, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the F508Del-CFTR mutation"* (Clinical trial number NCT01531673)
4. McCarty 2002: *"A Phase 1 Randomized, Multicenter Trial of CPX in Adult Subjects With Mild Cystic Fibrosis"* (Clinical trial number NCT00004428)
5. Rubenstein 1998 *"A Pilot Clinical Trial of Oral Sodium 4-Phenylbutyrate (Buphenyl) in DF508-Homozygous Cystic Fibrosis Patients"*

As a leader in the field of cystic fibrosis, I would also like to ask if you know about any additional clinical trials on CFTR correctors that we may have missed out during literature search.

Thank you for taking the time out to read this e-mail. Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review.

Sanjay Patel

Evidence of interaction with primary author of CPX study

E-mail 14: interaction with primary author of CPX study

From: McCarty, Nael [mailto:namccar@emory.edu]
Sent: 16 April 2014 13:46
To: Patel, Sanjay [md0u92cd]; 'nael.mccarty@biology.gatech.edu'
Cc: Southern, Kevin
Subject: RE: Cochrane Systematic Review of CFTR correctors - request for information

There are literally hundreds of clinical trials on correctors and potentiators that have taken place since the CPX trial. These are readily found with judicious searches in PubMed.

NM

From: Patel, Sanjay [md0u92cd] [mailto:S.Patel7@liverpool.ac.uk]
Sent: Wednesday, April 16, 2014 5:40 AM
To: McCarty, Nael; 'nael.mccarty@biology.gatech.edu'
Cc: Southern, Kevin
Subject: Cochrane Systematic Review of CFTR correctors - request for information

Dear Professor McCarty

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel cystic fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis & Genetic Disorders Group to conduct a Cochrane systematic review on CFTR correctors. I have attached a copy of the protocol for this systematic review for your information.

We would like to include the study *"***A Phase 1 Randomized, Multicenter Trial of CPX in Adult Subjects With Mild Cystic Fibrosis***"* (Clinical trial number NCT00004428). I am contacting you to request for
1) additional data for the outcomes measured during the trial
2) data for other outcomes important to our review
3) the trial protocol
4) information on any additional clinical trials of CFTR corrects that you may be aware of.

1. Additional data required from outcomes measured during the trial:
    a. Pulmonary function test results (measured at day 1 and day 2)

2. There are outcomes in our review, that do not correspond to outcomes measured in your trial however we feel it is important to request this information in case information is available. The additional outcomes are
    a. extra antibiotics
    b. radiological outcomes
    c. acquisition and eradication of respiratory pathogens

For continuous data outcomes (e.g. measures of lung function) we would appreciate it if you were able to send us values for the mean and standard deviation for each group (all treatment groups and control).

3. We would also like to request the trial protocol.

4. So far we have retrieved the following additional studies through our electronic and manual literature searches.

1. Boyle 2011: *"Study of VX-809 Alone and in Combination With VX-770 in Cystic Fibrosis (CF) Patients Homozygous or Heterozygous for the F508del-CFTR Mutation"* (Clinical trial number NCT01225211)
2. Clancy 2012: *"Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation"* (Clinical trial number NCT00865904)
3. Donaldson 2013: *"VX-661, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the F508Del-CFTR mutation"* (Clinical trial number NCT01531673)
4. Rubenstein 1998 *"A Pilot Clinical Trial of Oral Sodium 4-Phenylbutyrate (Buphenyl) in DF508-Homozygous Cystic Fibrosis Patients"*
5. Zeitlin 2002 *"Evidence of CFTR Function in Cystic Fibrosis after Systemic Administration of 4-Phenylbutyrate"*

As a leader in the field of cystic fibrosis, I would also like to ask if you know about any additional clinical trials on CFTR Correctors (with published or unpublished results) that we may have missed out.

Thank you for taking the time out to read this e-mail. Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review.

Sanjay Patel

Evidence of interaction with primary author of the Lumacaftor study

E-mail 15: Interaction with primary author of the Lumacaftor study

From: Clancy, John [mailto:John.Clancy@cchmc.org]
Sent: 18 April 2014 16:02
To: Patel, Sanjay [md0u92cd]
Cc: Southern, Kevin
Subject: RE: Cochrane Systematic Review of CFTR correctors - request for information

thank you for the note, Sanjay. I'll need to ask vertex for the data/information that you request. I do not have the data personally

best, jpc

From: Patel, Sanjay [md0u92cd] [S.Patel7@liverpool.ac.uk]
Sent: Wednesday, April 16, 2014 5:26 AM
To: Clancy, John
Cc: Southern, Kevin
Subject: Cochrane Systematic Review of CFTR correctors - request for information

Dear Professor Clancy,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel cystic fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis & Genetic Disorders Group to conduct a Cochrane systematic review on CFTR correctors. I have attached a copy of the protocol for this systematic review for your information.

We would like to include the study *"**Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation**"* (Clinical trial number NCT00865904). I am contacting you to request for:
1) additional data for the outcomes measured during the trial;
2) data for other outcomes important to our review;
3) information on missing data;
4) the trial protocol;
5) information on any additional clinical trials of CFTR corrects that you may be aware of.

1. For the following continuous data outcomes we would appreciate it if you were able to send us values for the mean and standard deviation for each group (all treatment groups and control).

a) Relative change from baseline in FEV_1
b) Change from baseline in FVC and FEF_{25-75}
c) Change from baseline in sweat chloride concentration

In addition we would like to know what respiratory adverse events caused study drug termination in the 4 discontinued participants and how many subjects required study interruption and the reason for this.

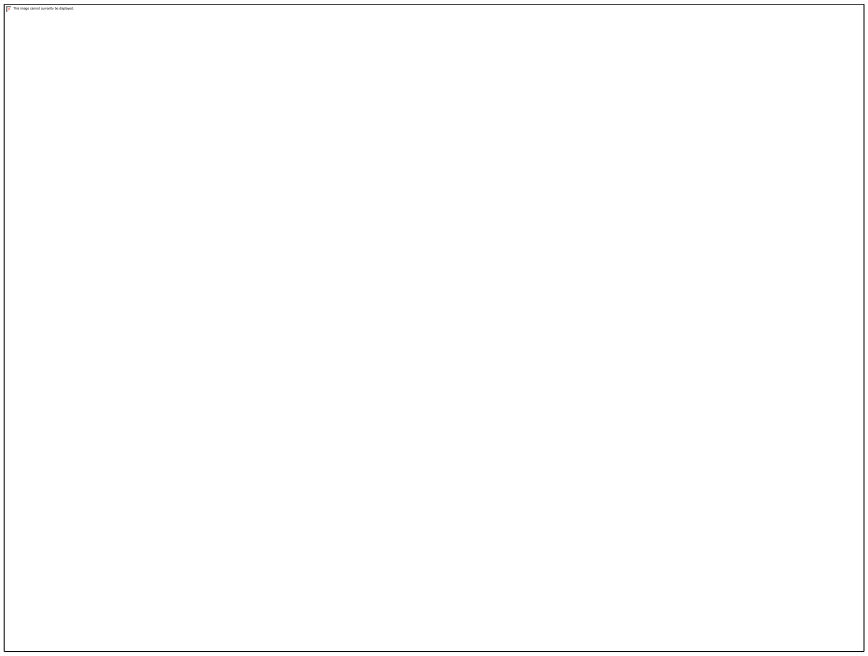
Some of this information has been provided in the publications. However the table below shows exactly what information is required.

Outcome measure	Data required
Relative change from baseline in FEV_1 through day 28	1. Standard deviation values for the mean change from baseline results in all treatment arms
Change from baseline in FVC and FEF25 75 through day 28	1. Mean change from baseline in each arm. 2. Standard deviation for the change from baseline results in all treatment arms
Change from baseline in sweat chloride concentration through day 28	1. Mean change from baseline in each arm. 2. Standard deviation for the change from baseline results in all treatment arms. NB: We understand that data has been plotted on a graph, but we cannot estimate the values from the graph with accuracy.
Safety	1. Number of participants who required study drug interruption and why 2. What respiratory adverse events caused study drug termination in the 4 discontinued participants?

2. There are outcomes in our review, that do not correspond to outcomes measured in your trial however we feel it is important to request this information, in case the information available. The additional outcomes we would like data for are
    a. Survival
    b. Hospital admissions
    c. School/work days missed
    d. Extra antibiotics
    e. Radiological outcomes
    f. Nutritional outcomes (weight, height, BMI)
    g. Acquisition and eradication of respiratory pathogens

3. Issues on missing data that require clarification:

a. In Table 2 (Frequency of occurrence of adverse events occurring in more than one subject in any VX-809 treatment group), the total number of subjects in the trial is shown to be 45, yet the total number of randomised participants is 89.
b. In Figure 1b (Sweat chloride change from baseline to day 28) the total number of participants in the treatment arms adds up to 63 (16+16+15+16). However, a total of 72 participants were randomised to the intervention arms.



c. In Supplementary appendix, the CFQ-R domain scores have been reported. The number of participants analysed (n = 85) excludes 4 recruited participants.

4. We would also like to request the trial protocol.

5. So far we have retrieved the following additional studies through our electronic and manual literature searches.

1. Boyle 2011: *"Study of VX-809 Alone and in Combination With VX-770 in Cystic Fibrosis (CF) Patients Homozygous or Heterozygous for the F508del-CFTR Mutation"* (Clinical trial number NCT01225211)
2. Donaldson 2013: *"VX-661, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the F508Del-CFTR mutation"* (Clinical trial number NCT01531673)
3. McCarty 2002: *"A Phase 1 Randomized, Multicenter Trial of CPX in Adult Subjects With Mild Cystic Fibrosis"* (Clinical trial number NCT00004428)
4. Rubenstein 1998 *"A Pilot Clinical Trial of Oral Sodium 4-Phenylbutyrate (Buphenyl) in DF508-Homozygous Cystic Fibrosis Patients"*
5. Zeitlin 2002 *"Evidence of CFTR Function in Cystic Fibrosis after Systemic Administration of 4-Phenylbutyrate"*

As a leader in the field of cystic fibrosis, I would also like to ask if you know about any additional clinical trials on CFTR correctors (with published or unpublished results) that we may have missed out.

Thank you for taking the time out to read this e-mail. Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review.

Sanjay Patel

Evidence of contact with primary author of the Lumacaftor-Ivacaftor study

E-mail 16: Interaction with primary author of the Lumacaftor-Ivacaftor study before publication of full report

From: Patel, Sanjay [md0u92cd]
Sent: 16 April 2014 10:16
To: 'mboyle@jhmi.edu'
Cc: Southern, Kevin
Subject: Cochrane Systematic Review of CFTR correctors - request for information

Dear Professor Boyle,

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel cystic fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis & Genetic Disorders Group to conduct a Cochrane systematic review on CFTR correctors. I have attached a copy of the protocol for this systematic review for your information.

We would like to include the study titled *"**Study of VX-809 Alone and in Combination With VX-770 in Cystic Fibrosis (CF) Patients Homozygous or Heterozygous for the F508del-CFTR Mutation**"* (Clinical trial number NCT01225211). Through our literature search we identified three abstracts (2011, 2012 and 2013 conference abstracts) for this study. I am contacting you to request for:
1) additional data for the outcomes measured during the trial;
2) data for cohorts 3 and 4;
3) data for other outcomes important to our review;
4) the trial protocol;
5) information on any additional clinical trials of CFTR corrects that you may be aware of.

1. Additional data required for outcomes measured during the trial

COHORT 1

1. How many participants have been randomised to each of the three arms?
2. For the following continuous data outcomes we would appreciate it if you were able to send us values for the mean and standard deviation for each group (all treatment groups and control);
    a. Change from baseline to day 14 (after VX-809 therapy alone), in sweat chloride concentration,
    b. Change from day 15 to day 21 (after VX-809 +VX-770 therapy) in sweat chloride concentration
    c. Change from baseline to day 14 (after VX-809 therapy alone) in FEV_1
    d. Change from day 15 to day 21 (after VX-809 +VX-770 therapy) in FEV_1
3. The number of participants included in the analysis of each outcomes measure
4. Steps taken to minimising risk in the trial

COHORT 2

1. For the following continuous data outcomes we would appreciate it if you were able to send us values for the mean and standard deviation for each group (all treatment groups and control);

a. Change from baseline to day 28 (after VX-809 therapy alone) in sweat chloride concentration,
b. Change from baseline to day 56 (after VX-809 + VX-770 therapy) in sweat chloride concentration,
c. Change from baseline to day 28 (after VX-809 therapy alone) in FEV_1
d. Change from baseline to day 56 (after VX-809 + VX-770 therapy) in FEV_1
e. Change from baseline in CFQ-R scores through day 56

2. According to our literature search conducted in February 2014, no results for Cohorts 3 and 4 have yet been published. We would like to request any available data for these cohorts.

3. There are outcomes in our review, that do not correspond to outcomes measured in your trial however we feel it is important to request this information, in case information is available. The additional outcomes are

a. Survival
b. Hospital admissions
c. School/work days missed
d. Extra antibiotics
e. Radiological outcomes
f. Nutritional outcomes (weight, height, BMI)
g. Acquisition and eradication of respiratory pathogens

4. We would also like to request the study protocol.

5. So far we have retrieved the following additional studies through our electronic and manual literature searches.

1. Clancy 2012: *"Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation"* (Clinical trial number NCT00865904)
2. Donaldson 2013: *"VX-661, an investigational CFTR corrector, in combination with ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the F508Del-CFTR mutation"* (Clinical trial number NCT01531673)
3. McCarty 2002: *"A Phase 1 Randomized, Multicenter Trial of CPX in Adult Subjects With Mild Cystic Fibrosis"* (Clinical trial number NCT00004428)
4. Rubenstein 1998 *"A Pilot Clinical Trial of Oral Sodium 4-Phenylbutyrate (Buphenyl) in DF508-Homozygous Cystic Fibrosis Patients"*
5. Zeitlin 2002 *"Evidence of CFTR Function in Cystic Fibrosis after Systemic Administration of 4-Phenylbutyrate"*

As a leader in the field of cystic fibrosis, I would also like to ask if you know about any additional clinical trials on CFTR correctors (with published or unpublished results) that we may have missed out.

Thank you for taking the time out to read this e-mail. Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review.

Regards,

Sanjay Patel

E-mail 17: Interaction with primary author of the Lumacaftor-Ivacaftor study after publication of full report

From: Patel, Sanjay [md0u92cd]
Sent: 24 July 2014 13:19
To: mboyle@jhmi.edu
Cc: Southern, Kevin
Subject: Cochrane Systematic Review of CFTR correctors - request for information

Dear Professor Boyle,

Further to my previous e-mail (see thread), we would like to include data from the recently published, full report of the study: *"**A CFTR corrector (Lumacaftor) and a CFTR potentiator (Ivacaftor) for treatment of patients with Cystic Fibrosis who have a phe508del CFTR mutation; a phase 2 randomised controlled trial**"* (Clinical trial number NCT01225211) into our Cochrane Systematic review on CFTR correctors. I am contacting you to request for:

1) Separate data for the placebo groups randomised in cohort 2 and 3

2) Data for pre-specified outcomes

3) Data for other outcomes important to our review;

4) The trial protocol

1. Separate data for the placebo groups randomised in cohort 2 and 3

In this study, data for placebo participants in cohorts 2 and 3 were combined. As randomisation for cohorts 2 and 3 were conducted separately, combining the data undoes the effects of randomisation. Therefore, in order to include the data for cohorts 2 and 3 into the meta-analysis, I would like to request the following separate data for placebo participants randomised in cohort 2 and cohort 3:

· Mean (SD) change from baseline in sweat chloride concentration at day 28 (Lumacaftor monotherapy) and day 56 (Lumacaftor and Ivacaftor combination therapy).

· Mean (SD) change from baseline in CFQ-R scores at day 14 and 28 (Lumacaftor monotherapy) and day 42 and 56 (Lumacaftor and Ivacaftor combination therapy).

· Mean (SD) relative change from baseline in FEV_1 at day 14 and 28 (Lumacaftor monotherapy) and day 42 and 56 (Lumacaftor and Ivacaftor combination therapy).Mean (SD) absolute change from baseline in FEV_1 at day 14 and 28 (Lumacaftor monotherapy) and day 42 and 56 (Lumacaftor and Ivacaftor combination therapy).

· Safety assessment of Lumacaftor monotherapy and Lumacaftor and Ivacaftor combination therapy, including adverse effects of therapy.

2. To assess for selective outcome reporting in this study, we compared outcomes presented on the US National Institute of Health trials registry (http://clinicaltrials.gov/), to the results in the full report. We identified that in the full report there were no data for the pre-specified outcomes; absolute change in BMI or absolute change in body weight for the patients heterozygous for the phe508del mutation. We would like to request this data if they are available.

3. There are outcomes in our review, that do not correspond to outcomes measured in your trial however we feel it is important to request this information, in case data are available. The additional outcomes are

a. Survival

b. Hospital admissions

c. School/work days missed

d. Extra antibiotics

e. Radiological outcomes

f. Nutritional outcomes (weight, height, BMI)

g. Acquisition and eradication of respiratory pathogens

4. We would also like to request the study protocol.

Thank you for taking the time out to read this e-mail. Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review.

Regards,

Sanjay Patel

Evidence of interaction with the primary author of the on-going study examining the impact of VX-661 on patients homozygous for the F508del-mutation.

E-mail 18: interaction with the primary author of the on-going study examining the impact of VX-661 on patients homozygous for the F508del-mutation.

From: Patel, Sanjay [md0u92cd]
Sent: 16 April 2014 10:33
To: 'scott_donaldson@med.unc.edu'
Cc: Southern, Kevin
Subject: Cochrane Systematic Review of CFTR correctors - request for information

Dear Professor Donaldson

I am a postgraduate researcher at the University of Liverpool, UK. My research topic is on the novel cystic fibrosis mutation specific therapies that target the underlying CFTR defect. As part of my research, I am working with the Cochrane Cystic Fibrosis & Genetic Disorders Group to conduct a Cochrane systematic review on CFTR correctors. I have attached a copy of the protocol for this systematic review for your information.

We would like to include the study *"**VX-661, an investigational CFTR corrector, in combination with Ivacaftor, a CFTR potentiator, in patients with CF and homozygous for the F508Del-CFTR mutation**"* (Clinical trial number NCT01531673). So far, we have access to one reference for this trial (2013 conference abstract taken from the Journal of Cystic Fibrosis). There are no results in the abstract and therefore we cannot yet include this trial in our review. I am writing to request any data that you are willing to make available for this trial.

For continuous data outcomes (e.g. measures of lung function), we would appreciate it if you were able to send us values for the mean and standard deviation for each group (all treatment groups and control). For binary data, we need the number of patients experiencing the event (not the actual number of events) per group (treatment and control) in order to be able to analyse these correctly.

Outcomes that we would like to report on in our trial are

a. survival
b. pulmonary function (Relative change of FEV_1, FVC)
c. quality of life
d. adverse effects of therapy
e. hospital admissions,
f. school/work days missed,
g. extra antibiotics
h. radiological outcomes
i. nutritional outcomes (weight, height, BMI)
j. acquisition and eradication of respiratory pathogens
k. sweat chloride concentration

In addition, so far we have retrieved the following additional studies through our electronic and manual literature searches.

1. Boyle 2011: *"Study of VX-809 Alone and in Combination With VX-770 in Cystic Fibrosis (CF) Patients Homozygous or Heterozygous for the F508del-CFTR Mutation"* (Clinical trial number NCT01225211)
2. Clancy 2012: *"Results of a phase IIa study of VX-809, an investigational CFTR corrector compound, in subjects with cystic fibrosis homozygous for the F508del-CFTR mutation"* (Clinical trial number NCT00865904)
3. McCarty 2002: *"A Phase 1 Randomized, Multicenter Trial of CPX in Adult Subjects With Mild Cystic Fibrosis"* (Clinical trial number NCT00004428)
4. Rubenstein 1998 *"A Pilot Clinical Trial of Oral Sodium 4-Phenylbutyrate (Buphenyl) in DF508-Homozygous Cystic Fibrosis Patients"*
5. Zeitlin 2002 *"Evidence of CFTR Function in Cystic Fibrosis after Systemic Administration of 4-Phenylbutyrate"*

As a leader in the field of cystic fibrosis, I would also like to ask if you know about any additional clinical trials on CFTR Correctors (with published or unpublished results) that we may have missed out.

Thank you for taking the time out to read this e-mail. Any additional information would go towards shaping the results of the review and would be highly appreciated. We will, of course, acknowledge your assistance in the published version of the review.

Sanjay Pate

4. Data extraction forms for studies included in the CFTR potentiators review.

Data Extraction Table 5 Pilot 4PBA study by Rubenstein (1998)						
TRIAL ID	Rubenstein 1998					
MAIN REFERENCE	**AM J RESPIR CRIT CARE MED 1998;157:484–490.**					
FUNDING (delete)	Pharma	Non-pharma	Unclear	**DETAILS: ??**		
POPULATION	AGE: >=14 years Placebo: 24.8 (4.9) 4PBA: 22.3 (5.9)		GENETICS: Homozygous ΔF508 -CFTR		OTHER (EG FEV_1): Mean FVC, % PRED (SD): Placebo: 65.5 (18.6) 4PBA: 73.4 (20.3) Mean FEV_1, % PRED (SD): Placebo: 47.5 (22.1) 4PBA: 57.8 (27.2)	
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM						
DRUG	Sodium 4-phenylbutyrate (Buphenyl, 4BPA)					
DRUG DOSE ARM 1	19 grams daily PO divided into three doses of 6, 6 and 7 grams.					
DRUG DOSE ARM 2						
DRUG DOSE ARM 3						
DRUG DOSE ARM 4						
LENGTH OF TREATMENT	1 week					
DESCRIPTION OF CONTROL	Placebo					
PRIMARY OUTCOME						
PRIMARY OUTCOME 1						
PRIMARY OUTCOME 2						
SAMPLE SIZE	REQUIRED:			RANDOMISED: 18		
PRIMARY TIMEPOINT						
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)						
MORTALITY	N					
FEV_1	N					
QOL	N					
OTHER PFT (SPECIFY)	N					
HOSPITAL ADMISSION	N					
SCHOOL/WORK DAYS MISSED	N					
EXTRA ANTIBIOTICS	N					
RADIOLOGICAL OUTCOMES	N					

NUTRITIONAL OUTCOMES	N	
SWEAT CHLORIDE	Y	YES (18/18)
MICROBIOLOGICAL OUTCOME	N	
SAFE	Y	ADVERSE EVENTS

SWEAT CHLORIDE VALUES

Values for sweat chloride concentration were presented in a graph and could not be extracted with accuracy. Primary author reported that there was no significant difference in sweat chloride concentration between 4PBA and placebo assigned subjects (P = 0.387

ADVERSE EVENT PROFILE

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS IN WHOM STUDY DRUG WAS INTERRUPTED OR DISCONTINUED

	PLACEBO N=9	19G 4PBA N=9
INTERRUPTED	0	0
DISCONTINUED	0	0

Data Extraction Table 6 Phase 2 4PBA Study by Zeitlin (2002)					
TRIAL ID	Zeitlin 2002				
MAIN REFERENCE					
FUNDING (delete)	Pharma	Non-pharma	Unclear	**DETAILS:**	
POPULATION	AGE: Mean (SD) 28.5 (7.1)		GENETICS: Homozygous ΔF508 mutation		OTHER (EG FEV_1): Mean weight (SD) – 62.6 (17.0) Mean FEV_1(% predicted) (SD) – 63.7 (17.0)
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM					
DRUG	4-Phenylbutyrate (Buphenyl)				
DRUG DOSE ARM 1	20G (n = 6)				
DRUG DOSE ARM 2	30G (n = 6)				
DRUG DOSE ARM 3	40G (n = 6)				
DRUG DOSE ARM 4					
LENGTH OF TREATMENT					
DESCRIPTION OF CONTROL	PLACEBO				
PRIMARY OUTCOME					
PRIMARY OUTCOME 1	Change in NPD				
PRIMARY OUTCOME 2					
SAMPLE SIZE	REQUIRED: 6 PER GROUP = 24 based on NPD			RANDOMISED: 22	
PRIMARY TIMEPOINT					
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)					
MORTALITY					
FEV_1					
QOL					
OTHER PFT (SPECIFY)	Y	ABSOL **Δ BL** D0, D3, D4, D7 – NO RESULTS REPORTED IN TRIAL			
HOSPITAL ADMISSION					
SCHOOL/WORK DAYS MISSED					
EXTRA ANTIBIOTICS					
RADIOLOGICAL OUTCOMES					
NUTRITIONAL OUTCOMES					
SWEAT CHLORIDE	Y	ABSOLUTE CALUES NOT CHANGE FROM BASELINE BL, D2, D3, D4, D7 – CHANGE FROM BASELINE REQUESTED			

MICROBIOLOGICAL OUTCOME		
SAFETY	Y	ADVERSE EVENTS, HEPATIC ENZYMES

OUTCOME	TIME	COMPARISON		HOW EXPRESSED	RESULTS						NOTES EG TYPE OF OUTCOME ETC		
		ARM 1 (N)	ARM 2 (N)	EG Δ BL, TIME TO	RESULT ARM 1	RESULT ARM 2	MEAN DIFF	VARIANCE (SPECIFY)	P	CI			
Sweat chloride	BL	20g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	119.2 (12.7)	110.1 (16.6)							
		30g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	116.8 (16.8)	110.1 (16.6)							
	D2	20g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	119.8 (10.4)	119.2 (7.4)							
		30g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	96.2 (45.3)	119.2 (7.4)							
	D3	20g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	117.2 (9.0)	113.6 (13.6)							
		30g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	121.1 (13.5)	113.6 (13.6)							
	D4	20g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	116.2 (9.9)	111.0 (8.7)							
		30g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	110.3 (16.2)	111.0 (8.7)							
	D7	20g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	116.5 (10.7)	117.2 (10.3)							
		30g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	119.8 (4.4)	117.2 (10.3)							
P. aeurginosa (likert scale 0 (light)-6 (heavy))	BL	20g (6)	PLAC (4)	MEDIAN	2	4.5							
		30g (6)	PLAC (4)	MEDIAN	5	4.6							
	D7	20g (6)	PLAC (4)	MEDIAN	NS	NS							
		30g (6)	PLAC (4)	MEDIAN	NS	NS							

Sweat chloride	BL	20g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	119.2 (12.7)	110.1 (16.6)					Author reported 'great inter-subject variability'		
		30g (6)	PLAC (4)	ABSOL VALUE, MEAN (SD)	116.8 (16.8)	110.1 (16.6)							
	D2	20g (6)	PLAC (4)	CHANGE FROM BL	0.6	9.1	-8.5						
		30g (6)	PLAC (4)	CHANGE FROM BL	-20.6	9.1	-29.7						
	D3	20g (6)	PLAC (4)	CHANGE FROM BL	-2.0	3.5	-5.5						
		30g (6)	PLAC (4)	CHANGE FROM BL	4.3	3.5	0.8						
	D4	20g (6)	PLAC (4)	CHANGE FROM BL	-3.0	0.9	-3.9						
		30g (6)	PLAC (4)	CHANGE FROM BL	-6.5	0.9	-7.4						
	D7	20g (6)	PLAC (4)	CHANGE FROM BL	-2.7	7.1	-9.8						
		30g (6)	PLAC (4)	CHANGE FROM BL	3	7.1	-4.1						
	D2	20g (6)	PLAC (4)	REL CHANGE FROM BL	0.5%	8.3%	-7.8%						
		30g (6)	PLAC (4)	REL CHANGE FROM BL	-17.6%	8.3%	-25.9%						
	D3	20g (6)	PLAC (4)	REL CHANGE FROM BL	-1.7%	3.2%	-4.9%						
		30g (6)	PLAC (4)	REL CHANGE FROM BL	3.7%	3.2%	0.5%						
	D4	20g (6)	PLAC (4)	REL CHANGE FROM BL	-2.5%	0.8%	-3.3%						
		30g (6)	PLAC (4)	REL CHANGE FROM BL	-5.6%	0.8%	-6.4%						
	D7	20g (6)	PLAC (4)	REL CHANGE FROM BL	-2.3%	6.4%	-8.7%						
		30g (6)	PLAC (4)	REL CHANGE FROM BL	2.6%	6.4%	-3.9%						

ADVERSE EVENT PROFILE

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS IN WHOM STUDY DRUG WAS INTERRUPTED OR DISCONTINUED

	PLACEBO N=4	20G 4PBA N=6
INTERRUPTED	0	0
DISCONTINUED / DOSE REDUCTION	0	0

	PLACEBO N=4	30G 4PBA N=6
INTERRUPTED	0	0
DISCONTINUED / DOSE REDUCTION	0	2

	PLACEBO N=4	40G 4PBA N=3
INTERRUPTED	0	0
DISCONTINUED / DOSE REDUCTION	0	2

The 40 g cohort was terminated early following analysis of the data by the safety monitoring committee

Data Extraction Table 7 CPX study by McCarty (2002)						
TRIAL ID	McCarty 2002					
MAIN REFERENCE	Pediatric Pulmonology 33:90-98 (2002)					
FUNDING (delete)	Pharma	**Non-pharma**	Unclear	**DETAILS: Funded by NIH**		
POPULATION	AGE: >18 RANGE 18-38		GENETICS: HOMOZYGOUS ΔF508		OTHER (EG FEV_1): MILD CF (FEV_1>60%) 21 MALES AND 16 FEMALES	
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM						
DRUG	CPX (8-cyclopentyl-1,3-dipropylxanthine)					
DRUG DOSE ARM 1	1MG (n = 4)					
DRUG DOSE ARM 2	3MG (n = 4)					
DRUG DOSE ARM 3	10 MG (n = 4)					
DRUG DOSE ARM 4	30 MG (n = 4)					
DRUG DOSE ARM 5	100 MG (n = 5)					
DRUG DOSE ARM 6	300 MG (n = 4)					
DRUG DOSE ARM 7	1, 000 MG (n = 4)					
LENGTH OF TREATMENT	1 DOSE					
DESCRIPTION OF CONTROL	PLACEBO (n = 8)					
PRIMARY OUTCOME						
PRIMARY OUTCOME 1	SAFETY					
PRIMARY OUTCOME 2						
SAMPLE SIZE	REQUIRED: UNCLEAR			RANDOMISED: 37		
PRIMARY TIMEPOINT	D1 – SINGLE DOSE ASSESSMENT					
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)						
MORTALITY						
FEV_1						
QOL						
OTHER PFT (SPECIFY)						
HOSPITAL ADMISSION						
SCHOOL/WORK DAYS MISSED						
EXTRA ANTIBIOTICS						
RADIOLOGICAL OUTCOMES						

NUTRITIONAL OUTCOMES		
SWEAT CHLORIDE	Y	D0, D1
MICROBIOLOGICAL OUTCOME		
SAFETY	Y	ADVERSE EVENTS D1, D2, FOLOW UP

OUTCOME	TIME	COMPARISON		HOW EXPRESSED	RESULTS						NOTES EG TYPE OF OUTCOME ETC		
		ARM 1 (N)	ARM 2 (N)	EG Δ BL, TIME TO	RESULT ARM 1 (SD)	RESULT ARM 2 (SD)	MEAN DIFF	VARIANCE (SPECIFY)	P	CI			
SWEAT CHLORIDE CONCENTRATION (mEq/L)	D1	1MG (4)	PLAC (8)	MEAN (SD)	113.6 (19.8)	100.0 (18.2)							
SWEAT CHLORIDE CONCENTRATION	D1	3MG (4)	PLAC (8)	MEAN (SD)	107.9 (15.9)	100.0 (18.2)							
SWEAT CHLORIDE CONCENTRATION	D1	10MG (4)	PLAC (8)	MEAN (SD)	112.0 (8.0)	100.0 (18.2)							
SWEAT CHLORIDE CONCENTRATION	D1	30MG (4)	PLAC (8)	MEAN (SD)	105.7 (22.8)	100.0 (18.2)							
SWEAT CHLORIDE CONCENTRATION	D1	100MG (5)	PLAC (8)	MEAN (SD)	105.4 (16.0)	100.0 (18.2)							
SWEAT CHLORIDE CONCENTRATION	D1	300MG (4)	PLAC (8)	MEAN (SD)	115.6 (22.6)	100.0 (18.2)							
SWEAT CHLORIDE CONCENTRATION	D1	1000MG (4)	PLAC (8)	MEAN (SD)	91.3 (9.8)	100.0 (18.2)							
SWEAT CHLORIDE CONCENTRATION	D1	ALL CPX (29)	PLAC (8)	MEAN (SD)	107.9 (18.2)	100.0 (18.2)							
PRE-CPX/PLACEBO	D1			MEAN	106.0 (13.1)	106.0 (13.1)							
SWEAT CHLORIDE CONCENTRATION (mEq/L)	D1	1MG (4)	PLAC (8)	MEAN CHANGE FROM BL	7.6	-6.0	13.6						
SWEAT CHLORIDE CONCENTRATION	D1	3MG (4)	PLAC (8)	MEAN CHANGE FROM BL	1.9	-6.0	7.9						
SWEAT CHLORIDE CONCENTRATION	D1	10MG (4)	PLAC (8)	MEAN CHANGE FROM BL	6	-6.0	12						
SWEAT CHLORIDE CONCENTRATION	D1	30MG (4)	PLAC (8)	MEAN CHANGE FROM BL	-0.3	-6.0	5.7						
SWEAT CHLORIDE CONCENTRATION	D1	100MG (5)	PLAC (8)	MEAN CHANGE FROM BL	-0.6	-6.0	5.4						
SWEAT CHLORIDE CONCENTRATION	D1	300MG (4)	PLAC (8)	MEAN CHANGE FROM BL	9.6	-6.0	15.6						

SWEAT CHLORIDE CONCENTRATION	D1	1000MG (4)	PLAC (8)	MEAN CHANGE FROM BL	-14.7	-6.0	-8.7						
SWEAT CHLORIDE CONCENTRATION	D1	ALL CPX (29)	PLAC (8)	MEAN CHANGE FROM BL	1.9	-6.0	7.9						
SWEAT CHLORIDE CONCENTRATION (mEq/L)	D1	1MG (4)	PLAC (8)	REL CHANGE FROM BL	7.2%	-5.7%	12.8%						
SWEAT CHLORIDE CONCENTRATION	D1	3MG (4)	PLAC (8)	REL CHANGE FROM BL	1.8%	-5.7%	7.5%						
SWEAT CHLORIDE CONCENTRATION	D1	10MG (4)	PLAC (8)	REL CHANGE FROM BL	5.7%	-5.7%	11.3%						
SWEAT CHLORIDE CONCENTRATION	D1	30MG (4)	PLAC (8)	REL CHANGE FROM BL	-0.3%	-5.7%	5.4%						
SWEAT CHLORIDE CONCENTRATION	D1	100MG (5)	PLAC (8)	REL CHANGE FROM BL	-0.6%	-5.7%	5.1%						
SWEAT CHLORIDE CONCENTRATION	D1	300MG (4)	PLAC (8)	REL CHANGE FROM BL	9.1%	-5.7%	14.7%						
SWEAT CHLORIDE CONCENTRATION	D1	1000MG (4)	PLAC (8)	REL CHANGE FROM BL	-13.9%	-5.7%	-8.2%						
SWEAT CHLORIDE CONCENTRATION	D1	ALL CPX (29)	PLAC (8)	REL CHANGE FROM BL	1.8%	-5.7%	7.5%						

ADVERSE EVENT PROFILE

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS IN WHOM STUDY DRUG WAS INTERRUPTED OR DISCONTINUED

	PLACEBO N=8	CPX (combined) n=29
INTERRUPTED	0	0
DISCONTINUED / DOSE REDUCTION	0	0

Data Extraction Table 8 Lumacaftor study by Clancy (2012)					
TRIAL ID	CLANCY 2012				
MAIN REFERENCE	Thorax 2012;67:12**e**18				
FUNDING (delete)	Pharma	Non-pharma	Unclear	**DETAILS: SUPPORTED BY VERTEX, AND OTHER FUNDING EG NIH**	
POPULATION	AGE: >18 years		GENETICS: homozygous for df508	OTHER (EG FEV_1): **FEV_1 (% PRED)** Median: 71 Range: 34.2-128.3 **BMI, mg/m2** median: 22 Range: 16-34 **Sweat chloride (mmol/L)** median; 103.5 Range: 66.0 – 129.0	
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM					
DRUG	VX809				
DRUG DOSE ARM 1	25 MG OD (n = 18)				
DRUG DOSE ARM 2	50 MG OD (n = 18)				
DRUG DOSE ARM 3	100 MG OD (n = 17)				
DRUG DOSE ARM 4	200 MG 0D (n = 19)				
LENGTH OF TREATMENT	28 DAYS				
DESCRIPTION OF CONTROL	PLACEBO (n = 17)				
PRIMARY OUTCOME					
PRIMARY OUTCOME 1	SAFETY AND TOLERABILITY				
PRIMARY OUTCOME 2	SWEAT CL – NOTE THIS IS INFERRED FROM SAMPLE SIZE CALCULATION AS CO-PRIMARY. IT IS NOT STATED AS SUCH.				
SAMPLE SIZE	REQUIRED: 90			RANDOMISED: 89 (25 = 18, 50=18, 100=17, 200=19, PLAC = 19)	
PRIMARY TIMEPOINT	28 DAYS				
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)					
MORTALITY					
REL FEV_1	Y				
QOL	Y	CFQR			
OTHER PFT (SPECIFY)	Y	FEV_1, FVC, FEF25 75 – **FVC and FEF NOT REPORTED IN PUBLICATION**			

HOSPITAL ADMISSION		
SCHOOL/WORK DAYS MISSED		
EXTRA ANTIBIOTICS		
RADIOLOGICAL OUTCOMES		
NUTRITIONAL OUTCOMES		
SWEAT CHLORIDE	Y	MEAN CHANGE FROM BASELINE AT DAY 7 AND 28
MICROBIOLOGICAL OUTCOME		
SAFETY	Y	ADVERSE EFFECTS

OUTCOME	**TIME**	**COMPARISON**		**HOW EXPRESSED**	**RESULTS**						**NOTES EG TYPE OF OUTCOME ETC**
		ARM 1 (N)	**ARM 2 (N)**	**EG Δ BL, TIME TO**	**RESULT ARM 1**	**RESULT ARM 2**	**MEAN DIFF**	**VARIANCE (SPECIFY)**	**P**	**CI**	
SWEAT CHLORIDE	D28	25 mg	PLAC	MEAN CHANGE FROM BL			+0.1		NS		DATA ALSO PRESENTED AT EARLIER TIMEPOINTS – NOT INCLUDED IN REVIEW UNCLEAR OF NUMBERS IN EACH GROUP
SWEAT CHLORIDE	D28	50 mg	PLAC				-4.61		NS		
SWEAT CHLORIDE	D28	100 mg	PLAC				-6.13		<0.05	-12.25 TO -0.01	
SWEAT CHLORIDE	D28	200 mg	PLAC				-8.21		<0.01	-14.33 TO -2.10	
FEV_1	D28	25 mg	PLAC	MEAN REL CHANGE FROM BL %PRED	-2.46	+0.07			NS		DATA ALSO PRESENTED AT EARLIER TIMEPOINTS – NOT INCLUDED IN REVIEW UNCLEAR OF NUMBERS IN EACH GROUP
FEV_1	D28	50 mg	PLAC	MEAN REL CHANGE FROM BL %PRED	-2.15	+0.07			NS		
FEV_1	D28	100 mg	PLAC	MEAN REL CHANGE FROM BL %PRED	+0.32	+0.07			NS		
FEV_1	D28	200 mg	PLAC	MEAN REL CHANGE FROM BL	+0.47	+0.07			NS		

				%PRED									
FEF 25 – 75											**JUST REPORTED AS NS**		
FVC											**JUST REPORTED AS NS**		
CFQR	D28	25 (17)	PLAC (17)	MEAN CHANGE FROM BL	-5.2	+4.5			NS		THESE DATA RELATE TO RESPIRATORY DOMAIN. **TOTAL SCORES, AND SCORES FROM OTHER DOMAINS REPORTED IN THE SA.**		
	D28	50 (17)	PLAC (17)	MEAN CHANGE FROM BL	-6.3	+4.5			<0.05				
	D28	100 (16)	PLAC (17)	MEAN CHANGE FROM BL	-1.3	+4.5			NS				
	D28	200 (18)	PLAC (17)	MEAN CHANGE FROM BL	+2.2	+4.5			NS				
SWEAT CHLORIDE	D7	25 mg	PLAC	MEAN CHANGE FROM BL	-0.5	+2.2							
	D7	50 mg	PLAC	MEAN CHANGE FROM BL	-3.7				P = 0.03	-7.1 to -0.28			
	D7	100 mg	PLAC	MEAN CHANGE FROM BL	-2.3								
	D7	200 mg	PLAC	MEAN CHANGE FROM BL	-6.6				P = 0.0008	-10.27 to -2.83			

MEAN CHANGE FROM BASELINE IN CFQ-R DOMAIN SCORES AT DAY 28 IN PARTICIPANTS TREATED WITH LUMACAFTOR

	Lumacaftor				Placebo
Domain	25 mg (n = 17)	50 mg (n = 17)	100 mg (n = 16)	200 mg (n =18)	(n = 17)
Body	-0.21	-1.63	2.61	0.06	-1.34
Digestion	2.28	-0.72	0.25	2.58	4.62
Eating	-3.66	-7.27*	3.24	-2.58	2.11

Emotion	-3.22	-1.36	3.49	-2.62	4.86
Health Perceptions	-2.84	-6.97*	-0.44	-1.9	5.03
Physical	-5.97	-7.38*	-3.46	-0.98	1.23
Respiratory	-5.22	-6.32*	-1.29	2.22	4.53
Role	-5.94*	-4.6	1.1	-6.53*	2.21
Social	0	-1.01	0.47	-2.64	-0.55
Treatment Burden	4.19	-5.96*	1.42	-0.68	2.46
Vitality	-4.65	-7.23*	-1.52	0.73	-2.18
Weight	5.41	2.18	8.83	-4.19	0.3

* demonstrated significance when compared to placebo

ADVERSE EVENT PROFILE

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS IN WHOM STUDY DRUG WAS INTERRUPTED OR DISCONTINUED

	Placebo (n=17)	Lumacaftor (n=72)
INTERRUPTED	0	0
DISCONTINUED	0	4 (6%) participants withdrew due to respiratory adverse effects, one from each of the Lumacaftor groups.

Data Extraction Table 9 Lumacaftor-Ivacaftor study by Boyle (2014)						
TRIAL ID	BOYLE 2014 – COHORT 1					
MAIN REFERENCE	BOYLE 2014					
FUNDING (delete)	Pharma	Non-pharma	Unclear	**DETAILS: VERTEX**		
POPULATION	AGE: > 18 YEARS MEAN AGE 29.1 years		GENETICS: DF 508 HOMOZYGOTE ALL PARTICIPANTS DF 508 HOMOZYGOTE		OTHER (EG FEV_1): >40% MEAN FEV_1 PRED 66.9% (range 32.8-117.1) MEAN SWEAT CL 101.9 mmol/L (range 87.5-121.0)	
INTERVENTION (EXPERIMENTAL ARMS) AND CONTROL ARM						
DRUG	2 PERIODS : PART 1 = VX809 ALONE (D1 – D14) PART 2 = VX 809 + VX770 (D15-D28)					
DRUG DOSE ARM 1 (N=20)	VX 809 200MG OD D1 - D14, FOLLOWED BY VX 809 200 MG OD+ VX 770 150 MG BD D15-D21					
DRUG DOSE ARM 2 (N=21)	VX 809 200MG OD D 1 -14, FOLLOWED BY VX 809 200 MG OD+ VX 770 250 MG BD D15-21					
DRUG DOSE ARM 3						
DRUG DOSE ARM 4						
LENGTH OF TREATMENT	3 WEEKS					
DESCRIPTION OF CONTROL	MATCHED PLACEBO D 1 – D 21 (N=21)					
PRIMARY OUTCOME						
PRIMARY OUTCOME 1	CHANGE IN SWEAT CHLORIDE BETWEEN DAY 15 AND DAY 21					
PRIMARY OUTCOME 2	SAFETY AND TOLERABILITY OF STUDY THERAPY					
SAMPLE SIZE	REQUIRED: HOPED FOR 60 (20/GROUP)			RANDOMISED: 62		
PRIMARY TIMEPOINT	1) D 14-21 2) D35					
SECONDARY OUTCOMES (Y/N) , COMMENTS , TIMEPOINT(S) , WHO WAS BLINDED (IF REVLEVANT)						
MORTALITY						
FEV_1	Y	CHANGE IN ABSOLUTE % PRED FEV_1 AT D 7, 14 & 21				
QOL		CFQ-R RESP DOMAIN SCORES D1, 14, 28, 42 AND 56				
OTHER PFT (SPECIFY)						
HOSPITAL ADMISSION						
SCHOOL/WORK DAYS MISSED						
EXTRA ANTIBIOTICS						
RADIOLOGICAL OUTCOMES						
NUTRITIONAL OUTCOMES						
SWEAT CHLORIDE	Y	AFTER VX-809 ALONE D14				
MICROBIOLOGICAL OUTCOME						
SAFETY						

OUTCOME	TIME	COMPARISON		HOW EXPRESSED	RESULTS						NOTES EG TYPE OF OUTCOME ETC
		ARM 1 (N)	ARM 2 (N)	EG Δ BL, TIME TO	RESULT ARM 1	RESULT ARM 2	MEAN DIFF	VARIANCE (SPECIFY)	P	CI	
CHANGE IN FEV_1 D0 – D14	D14	VX 809 (?N)	PLACEBO (?N)	MEAN CHANGE	-0.2	+1.7					
CHANGE IN FEV_1 D14 – D21	D21	VX 809 + VX 770 150MG (?N)	PLACEBO (?N)	MEAN CHANGE	+3.5	-1.4					
CHANGE IN FEV_1 D14 – D21	D21	VX 809 + VX 770 250MG (17)	PLACEBO (?N)	MEAN CHANGE	+0.6	-1.4					
CHANGE IN FEV_1 D14 – D21	D21	VX 809 + VX 770 150MG (?N)	VX 809 + VX 770 250MG (17)	MEAN CHANGE	+3.5	+0.6					
CHANGE IN SWEAT CHLORIDE D0 – D14	D14	VX 809 (?N)	PLACEBO (?N)	MEAN CHANGE	-4.2	-2.9					
CHANGE IN SWEAT CHLORIDE D15 – D21	D21	VX 809 + VX 770 150MG (?N)	PLACEBO (?N)	MEAN CHANGE	-2.2	+1.3					
CHANGE IN SWEAT CHLORIDE D15 – D21	D21	VX 809 + VX 770 250MG (?N)	PLACEBO (?N)	MEAN CHANGE	-9.1	+1.3					
CHANGE IN SWEAT CHLORIDE D15 – D21	D21	VX 809 + VX 770 150MG (?N)	VX 809 + VX 770 250MG (17)	MEAN CHANGE	-2.2	-9.1					
CHANGE IN SWEAT CHLORIDE D0 – D21	D21	VX 809 + VX 770 150MG (?N)	PLACEBO (?N)	MEAN CHANGE	-6.4	1.6					
CHANGE IN SWEAT CHLORIDE D0 – D21	D21	VX 809 + VX 770 250MG (?N)	PLACEBO (?N)	MEAN CHANGE	-13.2	1.6					
DATA EXTRACTED FROM THE FULL TEXT											
CHANGE IN SWEAT CHLORIDE DURING **LUMACAFTOR MONOTHERAPY**	D14	200MG VX 809 OD(+150MG LUMACAFTOR)(19)	PLACEBO (21)	MEAN CHANGE	-4.8 (95% CI -8.6 TO -1.0)	-1.7 (95%CI -5.6 to 2.3)	-3.1	(95%CI -8.7, 2.4)	P=0.264		
CHANGE IN SWEAT CHLORIDE DURING **LUMACAFTOR**	D14	200MG VX 809 OD(+250MG	PLACEBO (21)	MEAN CHANGE	-4.1 (-8.1 to -	-1.7 (95%CI -	-2.4	(95% CI -8.0 TO 3.2)	P=0.393		

MONOTHERAPY		VX770)(21)			0.1)	5.6 to 2.3)					
CHANGE IN SWEAT CHLORIDE DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D14-D21	200MG VX 809 OD +150MG LUMACAFTOR BD(19)	PLACEBO (17)	MEAN CHANGE	-2.1 (95%CI -5.4, 0.9)	0.5 (95%CI -3.0, 4.1)	-2.7	(95%CI -7.5, 2.1)	P=0.267		
CHANGE IN SWEAT CHLORIDE DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D14-D21	200MG VX 809 OD +250MG VX770BD(14)	PLACEBO (17)	MEAN CHANGE	-9.1 (95%Ci -12.9, -5.4)	0.5 (95%CI -3.0, 4.1)	-9.7	(95% CI -14.8 TO -4.6)	**P=<0.001**		
CHANGE IN SWEAT CHLORIDE DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D1-D21	200MG VX 809 OD +150MG LUMACAFTOR BD(20)	PLACEBO (16)	MEAN CHANGE	-6.7 (95%CI -11.1, -2.4)	-1.7 (95%CI -6.5, 3.1)	-5.0	(-11.6, 1.5)	0.126		
CHANGE IN SWEAT CHLORIDE DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D1-D21	200MG VX 809 OD +250MG VX770BD(17)	PLACEBO (16)	MEAN CHANGE	-12.6 (95%CI -17.2, -7.9)	-1.7 (95%CI -6.5, 3.1)	-10.9	(95% CI -17.6 TO -4.2)	**P=0.02**		
CHANGE IN SWEAT DURING **LUMACAFTOR MONOTHERAPY**	D14	200MG VX 809 OD(+150MG LUMACAFTOR)(20)	PLACEBO (21)	MEAN CHANGE	-0.3 (95%CI -2.4, 1.7)	1.7 (95%CI -0.2, 3.6)	-2.1	95%CI -4.8, 1.7	**0.137**		
CHANGE IN SWEAT CHLORIDE DURING **LUMACAFTOR MONOTHERAPY**	D14	200MG VX 809 OD(+250MG VX770)(20)	PLACEBO (21)	MEAN CHANGE	-0.1 (95%CI -2.1, 2.0)	1.7 (95%CI -0.2, 3.6)	-2.2	95%CI -4.7, 1.1	0.123		
CHANGE IN FEV_1 DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D14-D21	200MG VX 809 OD +150MG LUMACAFTOR BD(20)	PLACEBO (21)	MEAN CHANGE	3.5 (95%CI 0.9, 6.1)	-1.4 (95%CI -3.9, 1.1)	4.9	95%CI 1.4, 8.4	0.007		
CHANGE IN FEV_1DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D14-D21	200MG VX 809 OD +250MG VX770BD(18)	PLACEBO (21)	MEAN CHANGE	0.6 (95%CI -2.2, 3.5)	-1.4 (95%CI -3.9, 1.1)	2.1	95%CI -1.8, 5.9	0.282		
CHANGE IN FEV_1DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D1-D21	200MG VX 809 OD +150MG LUMACAFTOR BD(20)	PLACEBO (21)	MEAN CHANGE	3.1 (95%CI 0.1, 6.1)	0.3 (95%CI -2.6, 3.1)	2.8	(95%CI -1.3, 7.0)	**0.176**		
CHANGE IN FEV_1DURING **LUMACAFTOR IVACAFTOR COMBINATION THERAPY**	D1-D21	200MG VX 809 OD +250MG VX770BD(18)	PLACEBO (21)	MEAN CHANGE	0.5 (95%CI -2.8, 3.8)	0.3 (95%CI -2.6, 3.1)	0.3	(-4.2, 4.7)	0.908		

ADVERSE EVENT PROFILE

SEVERITY OF ADVERSE EVENTS, WITH REGARDS WHETHER STUDY DRUG WAS INTERRUPTED OR WITHDRAWN – SHOWN AS NUMBER (%) OF PATIENTS IN WHOM STUDY DRUG WAS INTERRUPTED OR DISCONTINUED

	Placebo (n=21)	Lumacaftor (n=41)
INTERRUPTED	0	0
DISCONTINUED	0	1 (2.4%) (chest tightness) during the 200mg Lumacaftor once daily period.

	Placebo (n=21)	Lumacaftor + Ivacaftor (combined from both 150mg and 250mg Ivacaftor groups) (n=41)
INTERRUPTED	0	0
DISCONTINUED	0	0