Real World Clinical Outcomes with Novel Modulator Therapy Combinations in People with Cystic Fibrosis

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Context: RECOVER examines the impact of Elexacaftor/Tezacaftor/Ivacaftor in the treatment of people with cystic fibrosis. The RECOVER parent study commenced in 2020 in people aged 12 and over for whom the drug was prescribed clinically. In 2022, when the drug was prescribed for children aged 6 to 11, they were recruited into the study. The parent study duration is 2 years. The duration of the extension study is five years for each cohort. The extension study for the older cohort and the parent study for the younger cohort will run concurrently.

PROTOCOL SIGNATURE PAGE

Signature of Sponsor Representative: _____ Date: _____

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List of Abbreviations

Abbreviation	Term
ATS	American Thoracic Society
ADMA	Asymmetric Dimethylarginine
ArestCF	Australian Respiratory Early Surveillance Team for Cystic Fibrosis
BA-ratio	Bronchial Artery Ratio Score
CF:INK	CF: Irish Network for clinical trials in Kids
CFORMS	Children's Follow up Orkambi Real world MBW Study
CLPI	Cleaved SLPI
CIPSEA	Confidential Information Protection and Statistical Efficiency ACT
CMS	Content Management System
catS	Cysteine Protease Cathepsin S
CF	Cystic Fibrosis
CFLD	Cystic Fibrosis Liver Disease
CFQR	Cystic Fibrosis Questionnaire-Revised
CFRI	Cystic Fibrosis Registry of Ireland
CFRD	Cystic Fibrosis Related Diabetes
CFTR	Cystic Fibrosis Transmembrane Conductance Protein
DPA	Data Protection Act
DIOS	Distal Intestinal Obstruction Syndrome
ECFS-CTN	European CF Society Clinical Trials Network
ETI	Elexacaftor/Tezacaftor/Ivacaftor
EMA	European Medicines Agency
FDR	False Discovery Rate
FEV1	Forced Expiratory Volume in 1 second
FeNO	Fractional Exhaled Nitric Oxide
GLI	Global Lung Initiative
GCP	Good Clinical Practice
HITECH	Health Information Technology for Economic and Clinical Health
HBE	Human Bronchial Epithelial
iPSC	Induced Pluripotent Stem Cells
ILSCFLD	Irish Longitudinal Study on Cystic Fibrosis Liver Disease
KLIC	Kalydeco and Lung Inflammation in Children with CF
LC-MS/MS	Liquid Chromatography-Mass Spectrometry
LCI	Lung Clearance Index
LA	LungAnalysis
LFT	Liver Function Test
MTA	Material Transfer Agreement
MMPs	Matrix Metalloproteases
MEMS®	Medication Event Monitoring System
MPR	Medication Possession Ratio
MBW	Multiple Breath Washout
MPO	Myeloperoxidase
NCPE	National Centre of Pharmacoeconomics
NCRC	National Children's Research Centre
NE	Neutrophil Elastase
NO	Nitric Oxide
NSCFLD	Non-Specific Cystic Fibrosis Liver Disease
NACFC	North American Cystic Fibrosis Conference
OPS	Oropharyngeal Specimens
PI	Pancreatic Exocrine Insufficiency

PROM	Patient Reported Outcome Measure
PWCF	People with Cystic Fibrosis
PBMC	Peripheral Blood Monocytes
PIPA	Personal Information Protection Act
PIPEDA	Personal Information Protection and Electronic Documents Act
PIPITPA	Personal Information Protection and Identity Theft Prevention Act
PRAGMA-CF	Perth-Rotterdam Annotated Grid Morphometric Analysis for CF
PBS	Phosphate Buffered Saline
QALY	Quality-Adjusted Life-Years
RV	Residual Volume
GST	Guy's and St Thomas' NHS Foundation Trust
SHIELD CF	Study of Host Immunity and Early Lung Disease in CF
TLC	Total Lung Capacity
WOCBP	Women of Childbearing Potential

HYPOTHESIS AND SPECIFIC AIMS FOR PARENT STUDY

Hypothesis

a. Use of ETI in routine clinical practice is associated with significant and sustained improvements in airway, liver and gastrointestinal outcomes and quality of life in children and adults with cystic fibrosis.
b. Adherence to routine therapies will decrease after initiation of ETI

Specific Aims

1. To determine the effect of treatment with ETI on pulmonary function (FEV1 and LCI) in children and adults with CF over a period of 2 years.
2. To determine the effect of treatment with ETI on spirometry-controlled CT scores in children and adults with CF over a two-year period.
3. To determine the effect of treatment with ETI on airway infection and inflammation in children and adults with CF
4. To determine the effect of treatment with ETI on nutrition, gastrointestinal symptoms, gut inflammation and pancreatic function in children and adults with CF over a two-year period
5. To determine the effect of treatment with ETI on CF liver disease in children and adults with CF over a two-year period.
6. To determine the effect of treatment with ETI on antibiotic treatment of pulmonary disease in children and adults with CF over a two-year period
7. To assess the impact of the introduction of ETI on adherence with overall medical treatments for CF

HYPOTHESIS AND SPECIFIC AIMS FOR EXTENSION STUDY

Hypothesis

a. Use of ETI in routine clinical practice is associated with significant and sustained improvements in airway, liver and gastrointestinal outcomes and quality of life in children and adults with cystic fibrosis.

Specific Aims

1. To determine the longitudinal effect of ETI on pulmonary function (FEV1 and LCI) in children and adults with CF over an additional period of 5 years.
2. To determine the longitudinal effect of ETI on spirometry-controlled CT scores in children and adults with CF over an additional period of 5 years
3. To determine the effect of treatment with ETI on airway infection and inflammation in children and adults with CF.
4. To determine the longitudinal effect of ETI on nutrition, gastrointestinal symptoms, gut inflammation and pancreatic function in children and adults with CF over an additional period of 5 years.
5. To determine the longitudinal effect of treatment with ETI on CF liver disease in children and adults with CF over an additional period of 5 years.
6. To determine the longitudinal effect of treatment with ETI on antibiotic treatment of pulmonary disease in children and adults with CF over an additional period of 5 years.
7. To determine the longitudinal changes in mental health outcomes associated with the use of ETI in children and adults with CF over a period of 5 years.

BACKGROUND AND SIGNIFICANCE

Cystic Fibrosis

Cystic fibrosis (CF) is the most common progressive life threatening genetic disease in Caucasians(1). Approximately 80,000 people in the world and 1,200 people in Ireland have CF, almost half of whom are children (CFRI annual report 2016). CF is a multisystem disorder caused by a mutation in a gene that encodes cystic fibrosis transmembrane conductance (CFTR) protein. Lung disease is responsible for the majority of the morbidity and mortality in CF. Predicted survival for individuals with CF currently exceeds forty years, however the current mean age of death is still in the twenties(1). Medications that can modulate the natural history of CF and minimize, or even prevent, the development of life-threatening consequences of CF have been long sought after, particularly if these can be commenced in young children prior to the development of organ damage and irreversible disease.

CF Lung Disease

In healthy individuals, persistent or troublesome respiratory infection is prevented through the innate and adaptive immune function associated with normal airway epithelium and airway surface liquid, and the effectiveness of mucociliary clearance. In CF, defective CFTR activity leads to an abnormal airway surface liquid that is dehydrated (2) and has abnormal innate defence activity (3). Airway mucus is viscous and difficult to clear leading to mucus plugging. The physical and innate defence defects are associated with recurrent and/or persistent infection accompanied by significant airway inflammation, resulting ultimately in airway wall injury. Progressive inflammation over time is associated with the development of bronchiectasis (4), which is ultimately irreversible and leads to further impairment of airway clearance. The most common cause of death in people with CF is respiratory failure associated with progressive CF lung disease (5). CF lung disease starts in early infancy, and at school age more than 50% of children have bronchiectasis despite early diagnosis through screening and current standard treatments (6-9).

Markers of Airway Inflammation

Airway inflammation in CF is present from infancy, is disproportional to the infectious insult, persists and worsens with age, and leads to airway damage, a key driver of respiratory failure in CF (10). Although not used in the clinical management of individual patients, markers of airway inflammation provide very useful information in terms of airway disease activity and response to therapies. Several studies have described the utility of cytokines such as IL-8, IL-6, IL-1β, matrix metallproteases (MMPs) and neutrophil products such as neutrophil elastase (NE)(11, 12). In CF, disruption of the protease-antiprotease balance has been demonstrated to be established in some individuals within the first year of life(3, 8, 9). Work to date suggests an association between NE, MMP-9 and bronchiectasis and disease severity in children with CF(6, 11, 13, 14). In addition, elevated NE activity is associated with *Pseudomonas aeruginosa* infection and destruction of host defence mechanisms(14, 15).

The introduction of triple combination therapies presents an ideal opportunity to collect, catalogue and analyse sputum samples for inflammatory markers prior to and after introduction, coupled with other key outcome measures relating to lung function and structure.

Nasal Inflammation

Nasal and sinus disease is common in CF with 25 to 40% of people with CF experiencing chronic rhinosinusitis or nasal polyps(16), and almost 100% of people with CF having abnormal CT imaging of their upper airways(17). One of our collaborators (Jochen Mainz) has developed an expertise in collection, processing and analysis of nasal lavage in people with CF(18-22). Measures of nasal inflammation correspond to airway infection in both the upper and lower airways in people with CF and relate to responses to treatment of infection(23). As part of the GOAL study, the CFTR modulator Ivacaftor was associated with a reduction of nasal symptoms and improved quality of life, suggesting some impact on nasal infection/inflammation but this was not directly measured(24). Nasal inflammation will be tracked throughout RECOVER by measuring inflammatory mediators and microbial community structures in nasal airway lavage specimens.

CF Gastrointestinal Disease

Intestinal Disease

At birth, most new-borns will have pancreatic exocrine insufficiency (PI) and develop malabsorption, however, some will not, particularly those with mutations associated with residual CFTR function. Malabsorption, malnutrition and growth failure follow pancreatic insufficiency, particularly with advancing age and CF lung disease. Intestinal epithelial dysfunction, particularly in the setting of PI, can be associated with partial or complete bowel obstruction either in infancy (meconium ileus) or later in life (distal intestinal obstruction syndrome [DIOS]). The main features of Intestinal disease in CF are inflammation, dysbiosis and dysmotility, which in many can lead to a significant impairment in quality of life (25-27). Recent studies tracking abdominal complaints in CF using a structured questionnaire (CFAbd-Score, initially called JenAbdomen-CF score 1.0) confirmed that symptoms including reduced appetite and taste, pain, flatulence and abdominal distention are almost universal, and a relationship was observed between abdominal ultrasound findings and symptoms (28-30). The CFAbd-Score has undergone essential steps for validation of a patient reported outcome measure (PROM), as required by the FDA(31). The CFAbd-Score was developed specifically to measure abdominal symptoms in people with CF (32). The score was used by our group in the RECOVER parent study (see below). Recently a multicenter study demonstrated that, using the CFAbd score, abdominal symptoms improved with Elexacaftor/Tezacaftor/Ivacaftor (ETI) in people with CF aged 12 and above but did not reach the levels seen in healthy controls(33). Intestinal manifestations, although they can be very troublesome, can be hard to characterize, challenging to effectively treat and probably receive less attention overall than pulmonary manifestations in individuals with CF.

CF Liver Disease

CF Liver disease is an unusual and complex form of liver disease and is associated with a heterogeneous collection of abnormalities including impairments in transaminases, abnormal liver ultrasound appearances and progressive focal and multilobar biliary cirrhosis (35, 36). CFTR is expressed on the biliary epithelium but not on hepatocytes or cholangiocytes. CFLD is characterized by non-uniform portal tract abnormalities termed focal biliary cirrhosis.(37) The localization of the damage to the intrahepatic ducts leading to portal tract fibrosis with well-preserved hepatic architecture is characteristic of CFLD. The clinical significance of this abnormality, in contrast to other types of liver cirrhosis, is that portal hypertension and its sequelae such as gastric varices with or without gastrointestinal hemorrhage, can be prominent features of the disease without significant hepatocellular failure(37). Cirrhosis, previously considered a static, end-stage liver disease is now regarded as a dynamic condition which is not always progressive, and may on occasion be reversible(38, 39) This intriguing finding exemplifies the many unanswered questions on the complexity and heterogeneity of CFLD. Our group has shown that CFLD is associated with reduced life expectancy in CF(40-43). Three separate European studies have confirmed that the onset of CFLD is before the transition to adult care with no reports of new onset disease in adulthood(43). CFLD is associated with an increased risk of cystic fibrosis related diabetes (CFRD)(40). While CFLD is a male predominant complication of CF, females with CFLD have a worse outcome(40, 41). Liver failure is an infrequent cause of death in those with CFLD who usually die for other reasons(40, 42, 43). CFLD is associated with a greater risk of early mortality(40, 42, 43) and patients with CFLD have a 10 year lower median age of death compared a non-cirrhotic control group(42).

The prevalence of CFLD ranges from 10–30%, depending on the criteria used to define liver disease(41-43). Currently there are two very similar consensus approaches to define CFLD(35, 44) which classify PWCF into three groups (i) CFLD; includes those with clinical evidence of liver disease (with or without portal hypertension), histological evidence on liver biopsy or radiological evidence of portal hypertension (ii) NSCFLD includes those with non-specific ultrasound or biochemical evidence of liver disease who do not fulfill criteria for CFLD, and (iii) those with no evidence of liver disease. When a diagnosis is established there are no clinical indicators that predict disease progression. More importantly, there is no effective intervention for prevention or treatment of established disease until liver transplantation for end-stage liver failure(35, 37).

In clinical trials, CFTR modulator drugs have been shown to be associated with elevation of liver transaminases, at times leading to cessation of treatment, temporarily in most instances. Little is understood about this or the natural history of variations in transaminases in CF. The medium to long term implications of

alterations of transaminases with CFTR modulator therapy is also unclear. Weight gain in individuals on Ivacaftor is significant, and predominantly related to fat mass(45), raising concerns about the development of hepatic steatosis, although a single reported case has demonstrated the opposite, an improvement in steatosis on commencement of Ivacaftor(46). A structured approach to the reporting of ultrasound findings such as in this study will shed light on this.

CFTR Modulators
Ivacaftor, the first mainstream CFTR modulator (a potentiator) is licenced for use in children with CF aged one year and older with a gating mutation and leads to improvements in sweat chloride, pulmonary function, weight gain, quality of life and rate of decline in lung function (47-51). Interestingly, in children aged 1 to 5 years, Ivacaftor was associated with improvements in markers of pancreatic insufficiency on treatment (52). Ivacaftor is currently in clinical trials in infants from birth to one year, and if found to be safe, will be the first disease modifying treatment available to infants with CF from birth.

Ivacaftor is not effective when used alone in individuals with non-gating mutations such as Phe508del, the commonest CF mutation (53). Ivacaftor has been tested in combination with several CFTR correctors in an attempt to promote channel opening of CFTR that has been rescued and trafficked to the membrane. Lumacaftor, a CFTR corrector, in combination with Ivacaftor has been shown to reduce sweat chloride levels, improve pulmonary function, improve weight gain and reduce exacerbations in people with CF aged 12 years and older homozygous for the Phe508del mutation (54). Further to this, Lumacaftor/Ivacaftor was noted to reduce sweat chloride levels and improve pulmonary function, ventilation inhomogeneity (measured by lung clearance index), weight gain and quality of life in children six to eleven years of age homozygous for the Phe508del mutation (55, 56). Lumacaftor/Ivacaftor, marketed as Orkambi® is now licenced for use in children two years of age and older. Recently, another CFTR corrector, Tezacaftor, when used in combination with Ivacaftor has been shown to deliver similar clinical benefits with less adverse effects, both in Phe508del homozygotes and Phe508del heterozygotes with a second allele with residual CFTR function (57-60).

The triple combination modulator compound Elexacaftor/Tezacaftor/Ivacaftor (ETI), however, is associated with significant improvements in sweat chloride, nutrition and pulmonary disease in children aged 6 years and above with one or more Phe508del mutation(61-64) and is now approved for use in many jurisdictions. Ivacaftor and ETI are considered highly effective modulator therapy (HEMT) on the basis of their very significant impact on sweat chloride levels and end organ function. In longer term studies of Ivacaftor, the significant initial improvement in pulmonary function has returned to baseline after approximately 3-5 years of treatment(65,66), with data from the Irish registry suggesting that the pulmonary function trajectory after initial treatment gains is heavily influenced by age (worse in older subjects with more advanced disease)(67). Longer term data is not available yet for ETI. The availability of these medicines for a large proportion of our patients represents both a fantastic advance in our efforts to manage our patients effectively but also a truly ‘disruptive’ event in the history of clinical management of CF. It is vital that we seek to understand the impact of this across several different clinical and non-clinical spheres by collecting a wide range of outcome measures on a large group of individuals and following them over many years.

Clinical Outcome Measures in CF
Traditional outcome measures for clinical trials of new therapies in CF have focused on manifestations of more advanced disease such as FEV1, nutritional indices, survival and exacerbations(68). These outcome measures have been of use in individuals with advanced disease, low lung function and poor nutrition. In children (and many adults) who may have better preserved pulmonary function and nutrition more novel outcome measures are required, and increasingly used in the CF community.

Multiple Breath Washout
Most children in the 6-11 year old bracket, and many in the 12-16 year old group, have normal FEV1 values (CFRI annual report 2016) despite the finding that more than 50% of children with CF have evidence on CT scanning of structural lung damage in the form of bronchiectasis by school age(6) which is usually progressive and non-reversible(7). Multiple Breath Washout (MBW) is an effort independent non-invasive technique performed on spontaneously breathing individuals, which measures ventilation inhomogeneity. MBW involves

using an inert tracer gas, such as nitrogen, which is 'washed out' of the lungs by oxygen during quiet respiration(69, 70). Lung clearance index (LCI) is a measure of the amount of lung turnovers required to completely wash the tracer out and is one of the indices calculated from the MBW procedure. LCI reflects ventilation inhomogeneity (VI), which may be caused by airway narrowing due to structural damage, inflammation or infection(69, 71).

One of the main advantages of the MBW technique is that it only requires tidal breathing making it ideal for younger children, unlike conventional testing requiring forced expiratory maneuvers(69, 70, 72). LCI is repeatable and reproducible with very good feasibility(70-73). It has been shown to be more sensitive at identifying lung disease in young children with CF when compared to spirometry(69-73). LCI is mainly a research tool, although it is likely that it will become part of standard clinical practice in due course given the significant benefits it demonstrates(74). The European CF Society Clinical Trials Network (ECFS-CTN) Standardization Committee have approved the use of LCI as an outcome measure in clinical trials in children with CF(75).

LCI, while an effort-independent test, is operator dependent and significant efforts must be made to ensure that training, test performance and interpretation are standardized(75). A significant improvement in LCI was seen in subjects with CF on Ivacaftor treatment for four weeks (76). LCI was selected as an outcome measure for the recent trial of Orkambi in children 6-11 years of age and approved by regulators(56). This was the first study to use LCI as a primary end-point in a large multi-center randomized controlled trial. Despite the successful use of LCI as a clinical trials endpoint, there is little data on the use of LCI as a marker of changes in CF lung disease in clinical practice and it is not known how well LCI will translate into real world clinical practice. This study provides a unique opportunity to evaluate the use of LCI as an endpoint for CFTR modulators in a real-world setting.

Spirometry-Controlled CT
Chest CT is widely accepted to be the most sensitive imaging modality to detect and monitor CF structural lung disease(77). Chest CT has the ability to identify structural abnormalities before symptoms begin to occur in patients(8). In addition, chest CT is more sensitive than spirometry at detecting early CF lung disease(78). A number of studies in CF have validated Chest CT as a primary end point for clinical trials(77). Although CT is a well validated outcome measure, there are no published large-scale multi-centre studies to date using Chest CT as a primary end-point in CF clinical trials. This in part reflects difficulties in both standardizing imaging and coordinating imaging activity across multiple clinical sites. Furthermore, more clinical trial related data are needed comparing chest CT related outcome measures to established functional outcome measures. RECOVER presents an ideal opportunity to gain insight into the benefits of standardized chest CT scanning in terms of trial endpoints.

In order to obtain detailed and clinically relevant images of the lung parenchyma and airways in both inspiration and expiration, subjects are required to perform a standardized breath hold manoeuvre. Studies have shown that lung volumes obtained without this standardized method are often inconsistent in children and uncontrolled breath-hold imaging can lead to significant respiratory motion artefact and sub-optimal quality of CT scans(79, 80). Spirometry-controlled CT scans allow standardization of lung volumes and minimize motion artefact during the scanning process. This leads to improved images and allows for better comparison between baseline and follow up scans(79, 81, 82).

Exhaled Nitric Oxide (FeNO)
Nitric oxide (NO) is a well-established non-invasive airway biomarker. The metabolism of arginine, a substrate for NO synthases, and NO metabolism in the airways of individuals with cystic fibrosis are abnormal(83-86). Fractional exhaled NO (FeNO) has been shown to be abnormally low in infants with cystic fibrosis even prior to the advent of neutrophilic lung disease, and more evidently so in individuals with more impaired CFTR function(87). In older individuals, low levels of airway NO have been shown to be associated with lower lung function and infection with certain pathogens(88). The CFTR modulator Ivacaftor has been shown in two small studies to lead to a significant increase in levels of FeNO in treated CF patients (89, 90), suggesting that change in FeNO may have the potential to serve as biomarker of restored CFTR function in people with CF

treated with CFTR modulators. FeNO and nitric oxide downstream metabolites in sputum will be directly measured as part of RECOVER.

Health Related Quality of Life – Patient Reported Symptoms
Over the last few decades a growing recognition of the importance of patient reported health related quality of life indices has developed. These indices are now recognized by patient organizations, clinicians and regulators as a vital tool in the development and testing of new treatments in people with CF(91). The Cystic Fibrosis Questionnaire-Revised (CFQR) is a specifically designed quality of life questionnaire for people with CF. The respiratory domain of the CFQR has been extensively used in clinical trials of CFTR modulators(48, 54, 56, 59, 60, 92) to help to determine whether modulators lead to improvements in respiratory symptoms that may not be captured by changes in biological or other clinical outcome measures. We will utilize the CFQ-R treatment burden questionnaire in this study.

Mental Health
Although mental health is one of the top 3 research priorities of the CF community(93), major gaps remain regarding the epidemiology of mental health conditions in the CF population. The extension study will improve our knowledge within multiple Cystic Fibrosis Foundation (CFF) mental health areas of focus and areas of encouragement for CF clinical research, including anxiety, depression, neurocognitive functioning, sleep, relationships between mental health and physical health, relationships between comorbidities and mental health, and impact of CF disease management on mental health(94).

The International Depression Epidemiological Study (TIDES) revealed rates of depression and anxiety 2-3 times higher among PWCF ages ≥12 years than in the general community (95).Elevated depression and/or anxiety in pwCF are associated with poorer Health-Related Quality of Life, engagement in risky behaviors, more missed appointments, worse adherence to daily treatments, increased rates of pulmonary exacerbations, higher healthcare costs, and mortality (96-104). The findings of TIDES led to widely disseminated CFF/European CF Society (ECFS) consensus statements recommending standardized protocols for prevention, screening, and treatment of depression and anxiety in adolescents and adults with CF(105,106,107).

Their major impact notwithstanding, the utility of the TIDES data is limited by: 1) the cross-sectional study design; 2) collection more than a decade ago, in the pre-CFTR modulator era; 3) exclusion of children with CF under the age of 12 years (108) and mental health comorbidities beyond depression and anxiety (109) ; and 4) inclusion of very few physical health variables. A large, multicenter, longitudinal, prospective study of a cohort of children and adults with CF and is required to advance our fundamental understanding of CF mental health in children. Mental health outcomes were not initially included in the RECOVER parent study but will be in use in the ENHANCE study – a similar longitudinal study examining the natural history of CF in the new era of CFTR modulators, running in the same centres and others. In turn, this data will inform future research, clinical guidelines, and development and delivery of evidence-based mental health interventions.

Treatment Adherence
It is known that poor adherence to pharmacological treatment and medical advice is a global problem and is particularly important in chronic diseases(110). Historically the core treatment for CF was symptom management often involving complex pharmacological treatments that become extensive with increasing age. When viewed in combination with other required treatments such as physiotherapy, it is perhaps unsurprising that many people with CF report significant time burdens associated with daily treatments (111). Adherence is defined as “the extent to which a person’s behavior - taking medication, following a diet and/or executing lifestyle changes - corresponds with agreed recommendations from a healthcare provider”. Adherence in CF is suboptimal, and poor adherence has been linked to an increased burden of illness and greater healthcare costs(112, 113). Parents or guardians exhibit greater responsibility of treatment in younger children, whilst in adolescence parental influence reduces. By the time patients are transitioning to adult services, adherence may have reduced by 50%(112). Despite the well-known disease-modifying benefits of the already licensed CFTR modulator Ivacaftor, studies have found adherence to be suboptimal (114, 115).

Self-reporting is the most widely used method for measuring medication adherence due to ease of implementation and low cost. The limitations of self-reporting are that the patient can often over estimate adherence or fail to disclose non adherence due to recall bias (116).
A second method is medication pick-up rates which can identify patients at risk for treatment failure (117). This information is usually extracted from the computerized system in community pharmacies. A limitation is that it measures maximum possible adherence.
No single method fulfils the criteria for an optimal medication adherence monitoring and therefore multiple methods are often employed (118). A recent study examining this issue in individuals with CF taking Ivacaftor used multiple methods for measuring adherence, however had a small sample size (n=12). Adherence to Ivacaftor by Self-report was 100%, by pharmacy refill data (medication possession ratio)was 84% and using the gold standard of electronic monitoring MEMS® (medication event monitoring system) was 61%(115). The study was unable to measure the impact of the non-adherence. To our knowledge, there are no published longitudinal studies examining the effect of CFTR modulators on adherence to CF treatment regimens and associated clinical outcome measures.

Although the incidence of CF is low, the cost of illness can be substantial. In 2015, the National Centre of Pharmacoeconomics (NCPE) of Ireland compared CFTR modulator treatment with standard of care and estimated an incremental quality-adjusted life-years (QALY) gain of 2.45 at an incremental cost of €903,947.(119) With this significant costs comes a responsibility in the CF community to understand both modulator and overall medication use in the real world and determine what effect this might have on outcomes.

Genetic Modifiers of Treatment Response
CF is a disease area where considerable work has focused on the non-CFTR factors involved in disease phenotype heterogeneity. Modifier genes have been discovered relating to several different aspects of the CF phenotype including lung function, lung disease severity and abdominal and endocrine manifestations of CF(120). Our collaborator Prof. Felix Ratjen at SickKids Toronto has a specific interest in genes that modify the CF phenotype. His team have recently shown that certain modifier genes also affect the response to CFTR directed therapies such as Ivacaftor and lumacaftor. We are therefore very interested in studying these modifier genes in people with CF initiated on highly effective CFTR modulator therapies. **RECOVER represents an ideal opportunity to link the genotypic findings to in vivo drug response which will be monitored closely within the study.** Our collaborators at SickKids have the laboratory technologies in place and have secured funding for the genotyping which can occur as part of a Genome Canada funded project at any time when DNA samples will become available.

A New Era for CF Treatment
The availability of effective modulator treatment for a large proportion of individuals with CF represents a new era in the management of CF. This sea-change in the CF landscape brings enormous hope to people with CF and their families, but also brings uncertainty in terms of the potential change to treatment regimens and interactions with CF services. The CF medical community needs to be very aware of patient and parental attitudes to new therapies and their understanding of, and adherence to, both new and existing treatments. As a medical community we then need to assess the impact of these views, and associated treatment adherence, on clinical outcomes. If we can do this, it will enable us to optimize outcomes in the face of all of the changes and challenges occurring during this time.

Real World Studies
Most clinical trials are controlled with specific inclusion and exclusion criteria with carefully selected patient populations undergoing extensive monitoring and follow up. Real world studies are an important complementary source of knowledge on the impact of treatments and help to generate new hypotheses to be tested in future clinical trials. When clinical trials use outcome measures that are widely available clinically, real world studies can be very feasible. In the case of LCI and spirometry-controlled CT, these tests are not widely available but nonetheless of widely accepted importance and relevance. **RECOVER will be of vital importance as it will tie together both routine and novel endpoints, promising unique and exciting insights into the effect of these modulators.** Real world studies such as RECOVER are of vital importance to both regulators and bodies funding the provision of expensive medications such as these to obtain a

detailed understanding of the impact of the introduction of these medicines to the clinical environment and ensure that medium to long term clinical outcomes are in line with trial data.

Airway Inflammation

Our collaborative group (Paul McNally, Sinead Weldon, Cliff Taggart) have a specific interest in innate defence and inflammation in early CF lung disease. In collaboration with the Study of Host Immunity and Early Lung Disease in CF (SHIELD CF), we have identified the cysteine protease cathepsin S (cat S) as a novel biomarker for CF lung disease and inflammation(121). We have shown that levels and activity of cat S are elevated in lungs of patients with CF lung disease(121, 122) and that cat S correlates with a decline in lung function and increased pulmonary neutrophilic infiltration in the CF lung(121). With our collaborators at Randox Diagnostics, we (CT and SW) have developed a novel immunodiagnostic assay for cleaved SLPI (CLPI) which will be utilised in this study. CLPI is a unique NE-derived fragment(14) that can be easily detected in sputum (figure 1). This will be examined as part of RECOVER.

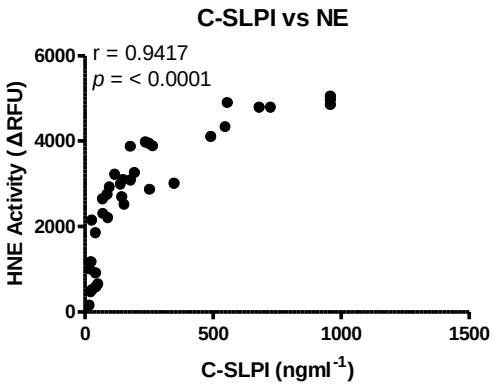


Figure 1. Correlation of cleaved SLPI to human neutrophil elastase activity in sputum from people with CF [unpublished data] (Spearman's Rank Correlation, n = 37).

A limited number of studies have sought to determine the effects of the first clinically available modulator compound, Ivacaftor, on airway inflammation in CF, with conflicting results (12, 123). Pilot data seen below in figure 2 (SHIELD CF data) from a collaborative study called KLIC (Kalydeko and Lung Inflammation in Children with CF, currently in data collection stage) between the ArestCF and SHIELD CF groups suggests that modulator therapy may have an impact on lower airway inflammation as measured in BAL.

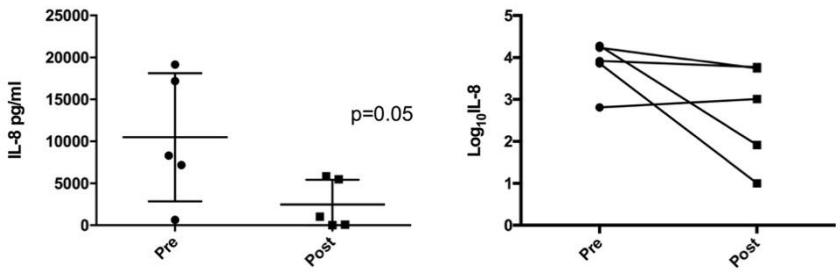


Figure 2: Pilot unpublished data (Irish sites) showing a reduction in BAL IL-8 levels in a sub-cohort of preschool children before and after starting on Ivacaftor therapy and enrolled in the KLIC study (Kalydeko and Lung Inflammation in Children with CF).

CF Liver Disease

In 2006 our collaborators (Marion Rowland and Billy Bourke) established the Irish Longitudinal Study on Cystic Fibrosis Liver Disease. The aim of this national cohort study was to determine the outcome and risk factors for liver disease in Cystic Fibrosis. All centres both adult and paediatric providing care for persons with CF (PWCF) are involved in the study. Between 2006-8 PWCF under 18 years of age attending any Irish Pediatric Centre were enrolled prospectively in a 20-year longitudinal study to examine the risk factors and outcome for CFLD. 522 (93% of eligible population) agreed to participate. Children so far have had nutritional, clinical, biochemical and radiological assessments in 2006/8; 2011/2 and 2016/17. In the intervening years data on height, weight, pulmonary function, laboratory parameters and mortality were obtained through our collaborators at the Cystic Fibrosis Registry of Ireland (CFRI) with the consent of our research participants.

In the first prospective study on CFLD we have confirmed, that despite significant investment in services for PWCF in Ireland the outcome for CFLD is poor for children who develop CFLD (Figure 3). Over a relatively short follow-up (median 10-year follow-up period the mortality rate for those classified as having CFLD in 2007 was 7.75 (95%CI 4.6-12.25) per 100 person years of follow-up compared to 2.3 (95% CI 1.4 – 3.6) in those with non-specific liver disease compared to 1.13 (95% CI 0.77- 1.59) per 100 person years of follow-up in those with no evidence of liver disease. The mortality rate difference between those with no evidence of liver

disease and those with CFLD was 6.63 (95% CI 3.0-10.2; p,0.001). There was a similar mortality rate difference between those with nonspecific liver disease and CFLD was 5.4 (95%CI 1.7-9.1 p<0.01). This longitudinal dataset will provide an ideal backdrop with which to understand the impact of CFTR modulation on liver disease parameters in CF.

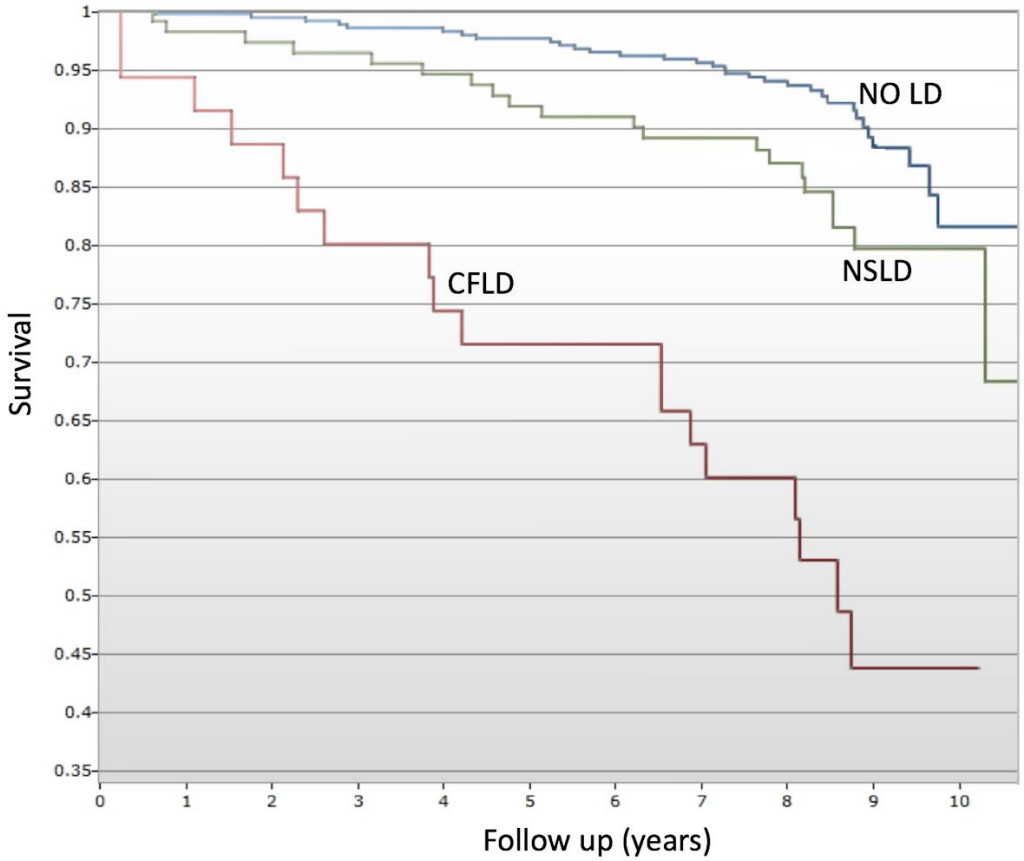


Figure 3. Survival in Irish PWCF between 2008-2018 with no liver disease (blue), non-specific liver disease (green) and CF liver disease (red).

Of 445 participants who were classified as either having no evidence of liver disease (n = 335) or nonspecific changes (n= 110) in 2007, 28 (6.29%) participants developed CFLD with portal hypertension by 2017. This comprised 15/110 (13.63%) participants who had NSCFLD and 13/335 (3.88%) participants who has NoLD at baseline in 2007 (risk difference 9.76% 95%C1 3.02-16.49 P<.001). The incidence of CFLD with portal hypertension was 7.51 per 1000 person years of follow-up (95% CI 4.99-10.86). The incidence rate for participants under 10 years of age in 2007 was 10.77 per 1000 person years of follow-up (95% CI 6.97-15.9) compared to 2.13 (95%CI 0.42-6.23) in participants over 10 years of age, incidence rate difference 8.63 (95% CI 3.77-13.55 P<.05). No participant older than 10 years of age in 2007 who was classified as NoLD progressed to CFLD by 2017 (median age at follow-up 22.86 years IQR 5.2). Among 355 participants with NoLD in 2007 13 (3.88%) progressed to CFLD with PH, while 35.5% (119/335) displayed nonspecific evidence of liver disease at follow-up in 2017.

By 2017 18/35 (51.43%) participants with CFLD had died compared to 21/110 (19.09%) with NSCFLD and 32/335 (9.55%) with NoLD (P< .001) Using patient years of follow-up as the denominator, the mortality rate for participants with CFLD was 7.75 (95%CI 4.59-12.25) per 100 person years of follow-up (PYRS), for those with NSCFLD 2.34/100PYRS (95%CI 1.48 – 3.57) and 1.13 /100PYRS (95%CI 0.77-1.59) for those with NoLD. The mortality rate difference between those with CFLD and the combined group of NoLD and NSCFLD was 6.33 (95%CI 2.73-9.3) per 100 PYRS (P<.05). Four participants who developed CFLD during follow-up died, two of whom were under 15 years of age. The median age at death of participants who progressed to CFLD was 6.83 (IQR 6.33) years while the median age of death of the 49 participants without liver disease was 13.25 (IQR 5.0) p = NS. Of those with CFLD who died during follow-up 9/18(50%) died from hepatic causes of whom

4/8 received a liver transplant while 8/18 (44.4%) died from pulmonary causes. By combining the liver-specific focus of the ILSCFLD with ENHANCE the power to elucidate the effects of the CFTR modulators on CFLD is greatly enhanced.
Clinical trials of CFTR modulators have specifically excluded people with CFLD, so we know very little about the effects of modulators in this group. **RECOVER will include a large number of children, adolescents and adults with CFLD (with a longitudinal dataset) in whom we will be able to test the hypothesis that CFTR modulation improves indices of CFLD**.

LCI/CT
CFORMS examined the real-world clinical impact of LUM/IVA in Irish children using LCI and spirometry-controlled CT scores as outcome measures. We built the study on strong relationships between the clinical sites the ECFS CTN centers for MBW (Jane Davies) and CT imaging (Harm Tiddens, LungAnalysis) to ensure accurate measurement of meaningful outcomes. CFORMS collected LCI data at 4 Irish Centers and CT data at one center in collaboration with the CF Registry of Ireland (CFRI). We recruited a group of controls from the Royal Brompton Hospital, UK, where, at the outset of the study LUM/IVA was not reimbursed for children aged 6-11 homozygous for the F508del mutation. We found no statistically significant change from baseline in LCI among cases, and no difference in rates of change between cases and controls. This was also the case for FEV1 (Table 1). These data are being prepared for publication.

Change from baseline	Cases			Controls		
	n	Mean (95%CI)	p-value	n	Mean (95%CI)	p-value
$LCI_{2.5}$	51	-0.34 (-0.95 to 0.26)	p=0.26	13	-0.71 (-2.1 to 0.68)	p=0.31
$ppFEV_1$	71	-2.45 (-4.44 to 2.54)	p=0.66	14	5.51 (-1.76 to 12.78)	p=0.14

Table 1. Change from baseline in LCI in a cohort of children homozygous for the F508del mutation, aged 6-11 on treatment with Lumacaftor/Ivacaftor (Cases) and on no treatment (controls).

We carried out spirometry-controlled CT scans at baseline, one year and two years after the introduction of LUM/IVA in 35 children aged 6-11, homozygous for the F508del mutation. Standardization of the scanner output, protocol and data transfer was managed through the LungAnalysis team. Images were scored using the Perth Rotterdam Annotated Grid Morphometric Analysis (PRAGMA) scoring system. Over the course of 2 years on LUM/IVA, while improvements were seen in the mean air trapping scores, bronchiectasis scores increased (Table 2). These data suggest that LUM/IVA can improve air trapping but does not prevent the progression of bronchiectasis in these young children with CF. The data were presented at NACFC 2021 and currently being submitted for publication. Ongoing work is examining the airway wall and artery dimensions in detail in these sans using specifically developed proprietary software in an automated fashion.

PRAGMA Score	Baseline		Year 1		Year 2		
	Mean (95% CI) n=32		Mean (95% CI) n=32		Mean (95% CI) n=31		p-value
% Bronchiectasis	0.82	(0.48 - 1.16)	0.85	(0.51 - 1.19)	1.24	(0.89 - 1.60)	**0.005**
% Disease	2.78	(2.12 - 3.45)	2.46	(1.79 - 3.14)	2.25	(1.55 - 2.94)	0.138
% Trapped Air	0.13	(0.09 – 0.16)	0.10	(0.07 – 0.14)	0.07	(0.03 – 0.11)	**0.016**
% Mucous plugging	0.57	(0.31 – 0.83)	0.28	(0.01 - 0.54)	0.33	(0.04 - 0.62)	0.221

Table 2. Change from baseline in PRAGMA CT scores in 35 children homozygous for the F508del mutation, aged 6-11 on treatment with Lumacaftor/Ivacaftor.

PRELIMINARY data from RECOVER Parent Study
Despite unexpectedly accelerated approval of ETI in Europe in 2020 and a global pandemic, we have reasonable levels of recruitment to date (85% target) and retention of subjects in the first phase (12+) of RECOVER. One-year outcomes were presented at ECFS 2022 (Table 3 below) and Phase 2 of RECOVER (6-11) is continuing to recruit subjects. Figure 4 below shows significant differences in baseline (pretreatment) values for FEV1 and LCI in the 12+ and 6-11 groups, underlining the 'normalization' of LCI in younger children

and highlighting the need for more sensitive outcome measures in this group. Baseline (n=78) and 1 year (n=65) spirometry-controlled CT scans have been analysed and will be presented at ECFS and published shortly. Table 4 below summarises the PRAGMA scores analysed to date (unpublished). Table 3 outlines the key available data currently from the first phase of RECOVER showing significant improvements in all measured indices over 6 and 12 months. Over 6 months of ETI use, global CF Abd scores improved significantly from 15.0±1.4 to 10.7±1.1; p<0.0001, with significant improvements in sub-scores for domains of 'pain', 'GERD', 'impairment of quality of life' and 'appetite' but not 'disorders of bowel movement'.

	Baseline (N=114)			6 m (N=103)			12 m (N=90)			BL v all FU	BL v 6m	6m v 12m
	N	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	p-value	p-value	p-value
Sweat Chloride	107	85.78	18.054	94	44.92	18.886	80	44.23	18.936	<.0001	<.0001	0.7358
LCI	82	12.21	3.829	83	10.14	3.689	49	9.85	4.274	<.0001	<.0001	0.3187
pp FEV1	114	83.65	15.399	82	91.73	16.032	87	92.11	16.319	<.0001	<.0001	0.7356
Weight z score	114	0.07	0.812	103	0.32	0.790	90	0.27	0.827	<.0001	<.0001	0.1253
BMI z score	114	0.12	0.819	103	0.36	0.792	90	0.28	0.863	<.0001	<.0001	0.0484
FeNO	112	13.59	10.407	103	16.30	12.397	90	15.96	12.397	0.0118	0.0192	0.7827
CFQR-RD	37	83.32	15.212	27	97.20	7.508	22	95.49	8.792	<.0001	<.0001	0.4386

Table 3. Available cleaned data from people with CF aged 12 and over and one or more copies of F508del from the RECOVER study.

All Participants							
	Baseline (N=85)			12 months (N=64)			Baseline v 12 mths
Variable	N	Mean	Std Error	N	Mean	Std Error	p-value
% Bronchiectasis	85	3.86	0.428	64	3.29	0.388	0.3256
% Disease	85	7.34	0.697	64	3.98	0.406	0.0001
% Trapped Air	85	20.61	1.606	63	11.48	1.749	0.0002
% Mucous plugging	85	2.22	0.351	64	0.11	0.042	<.0001
% Bronchial Wall Thickening	85	1.21	0.114	64	0.55	0.070	<.0001
F508del/F508del							
	Baseline (N=55)			12 months (N=41)			Baseline v 12 mths
Variable	N	Mean	Std Error	N	Mean	Std Error	p-value
% Bronchiectasis	55	3.27	0.449	41	2.49	0.365	0.1769
% Disease	55	5.64	0.738	41	3.09	0.396	0.0030
% Trapped Air	55	13.90	1.790	41	9.01	1.666	0.0485
% Mucous plugging	55	1.35	0.335	41	0.07	0.033	0.0003
% Bronchial Wall Thickening	55	0.98	0.110	41	0.52	0.080	0.0010
F508del/Minimum function							
	Baseline (N=30)			12 months (N=23)			Baseline v 12 mths
Variable	N	Mean	Std Error	N	Mean	Std Error	p-value
% Bronchiectasis	30	4.44	0.819	23	4.09	0.809	0.7605
% Disease	30	9.03	1.317	23	4.86	0.825	0.0098
% Trapped Air	30	27.33	2.856	22	13.96	3.644	0.0057
% Mucous plugging	30	3.08	0.727	23	0.16	0.098	0.0002
% Bronchial Wall Thickening	30	1.43	0.235	23	0.58	0.121	0.0022

Table 4. PRAGMA CF scores from the RECOVER study demonstrating no change to bronchiectasis scores but substantial improvements in overall disease, trapped air, mucus plugging and bronchial wall thickening with ETI.

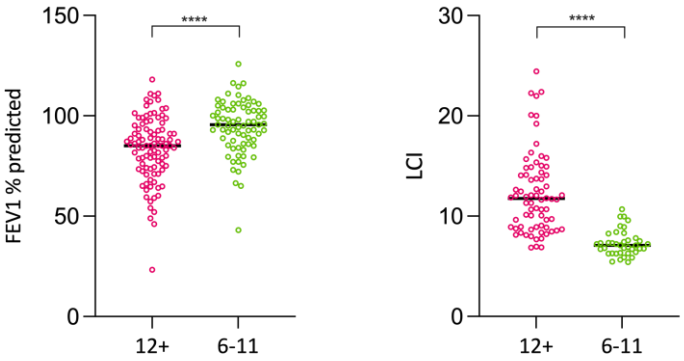


Figure 4. Baseline (pre-treatment) FEV1 and LCI in 12+ and 6-11 cohorts in the RECOVER study.

Study Team and Collaborators Strengths
RECOVERwill utilize our existing collaborative clinical research experience, and develop collaborations with leading international experts to ensure we can deliver on the great potential that exists. We have worked collaboratively with the Guy's and St Thomas' NHS Foundation Trust on a previous multi-centre investigator led study(124) in addition to CFORMS. The adult CF centres at St Vincent's University Hospital and Belfast City Hospital have considerable experience in CF clinical trials and investigator led studies and are both part of the ECFS clinical trial network. **Our collective experience with clinical trials and collaborative investigator led studies puts us in a strong position** to deliver on this multi-centre real-world project.

We have brought together a team of collaborators who are leading experts in their individual areas and will bring experience along with their expertise in terms of helping us to ensure the study achieves its aims.

Optimising the Research Potential from RECOVER
The design of RECOVER is specifically aimed at maximizing the research benefit (and ultimately the benefit to people with CF) from this cohort of children, adolescents and adults with CF. Our aim with this study is collect as comprehensive as possible an array of biological samples and clinical data from this cohort, and to follow them longitudinally. After the end of RECOVER, we envisage an ongoing surveillance study involving this group, in collaboration with our registry partners so that we can continue to learn about the longer-term impacts of these treatments. In addition, our sample storage plan associated with RECOVER will allow us to work with collaborators and collect, aliquot and store biological samples that will magnify the benefit of the study in light of the fact that these samples will be linked to a well phenotyped cohort with a wide array of useful clinical outcome measures.

EXPERIMENTAL DESIGN AND METHODS

Study Design
RECOVER is a multi-centre, cohort study which takes part in seven paediatric and adult sites across Ireland and the UK over a two-year period (parent study) and a further five years follow up (extension study) in conjunction with the CF Registry of Ireland (CFRI) and the UK CF registry. On the basis that drug will become available first for people aged 12 and over, the first phase, RECOVER12+ will recruit children 12 years and older and adults. This will be followed by the second phase, RECOVER6, recruiting children aged 6-11 years who will likely be starting on drug approximately a year after the older cohort. Data collection for each of these phases will occur over a two year period (Parent Study), and will overlap by 1-2 years. Subsequent to the two-year period, participants will enter an extension study for a period of five years, with a scaled back programme of assessments occurring annually or biennially. For subjects in both these cohorts, the decision to implement Kaftrio treatment is made completely independently of the decision to enter the study. The investigators will

follow the requirements of the local approved Kaftrio SmPC for the patient management as detailed in the 'special warnings and precautions for use' (e.g. management of hepatic impairment, rash, ophthalmological monitoring, between others), and for the management of interaction with other medicinal products and other forms of interactions.
The 6-11 year cohort will only be enrolled when the license is extended to this age group, and treatment with Kaftrio will only occur in the context of prescription by a physician in compliance with marketing authorisation and the SPC.

The study will have its base in RCSI and Children's Health Ireland (Crumlin) in Dublin, where the PI and project manager will be based and will co-ordinate all aspects of study conduct, training and management. The co-Lead PI, Prof Jane Davies is based at Imperial College and Guy's and St Thomas' NHS Foundation Trust. The statistical, data management and bioinformatics aspects of the study will be coordinated through CFRI for the parent study and CHI for the extension study. Guy's and St Thomas' NHS Foundation Trustwill be the central LCI training and over-reading site. All sites will have their CT scanning protocols for the study optimized and standardized through the Lung Analysis core at the Erasmus Medical Centre in Rotterdam.

List of Parties

Name	Role	Primary Affiliation	Secondary Affiliation
Core Study Team for Parent Study			
Paul McNally, MD	Lead PI	RCSI, Dublin	Children's Health Ireland, Dublin
Jane Davies, MD	Co-PI	Imperial College, London	Guy's and St Thomas' NHS Foundation Trust London
Karen Lester	Project Manager	RCSI, Dublin	Children's Health Ireland, Dublin
Paola Della Porta	Sponsor Contact	RCSI, Dublin	
TBD	RECOVER Monitor	RSCI, CHI at Crumlin, Dublin	Children's Health Ireland, Dublin
Sharon Sutton	Adherence Sub-Study lead, PhD Student	RCSI, Dublin	Children's Health Ireland, Dublin
Laura Kirwan	CFRI Representative	CF Registry of Ireland, Dublin	University College Dublin
Kenny Lynch	Data Manager	Children's Health Ireland	
Aidan Beegan	Statistician	Children's Health Ireland	RCSI, Dublin
Rachel Cregan	Project manager	RCSI, Dublin	Children's Health Ireland, Dublin
PPI Representatives			
Caroline Heffernan	CF Ireland Rep	Cystic Fibrosis Ireland	
Benat Broderick	Patient Representative		
Carolyn Thornton	Parent Representative		
Clinical site Investigators			
Des Cox, MD	Site PI	Children's Health Ireland at Crumlin	University College Dublin
Basil Elnazir, MD	Site PI	Children's Health Ireland at Tallaght	Trinity College Dublin
Mike Williamson, MD	Site PI	Children's Health Ireland at Temple Street	RCSI, Dublin
Barry Linnane, MD	Site PI	University Hospital Limerick	University of Limerick
Ed McKone, MD	Site PI	St Vincent's University Hospital, Dublin	University College Dublin
Jane Davies, MD	Site PI	Imperial College London, London	Guy's and St. Thomas' NHS Foundation Trust, London
Damian Downey, MD	Site PI	Belfast City Hospital	Queens University Belfast
Alastair Reid, MD	Site PI	Royal Belfast Hospital for Children	Queens University Belfast
Collaborators			

Felix Ratjen, MD	Collaborator	The Hospital for Sick Children (SickKids), Toronto	University of Toronto
Harm Tiddens, MD	Collaborator	Eramsus MC, University Medical Centre Rotterdam	
Jochen Mainz, MD	Collaborator	Klinikum Westbrandenburg	Brandenburg Medical School
Marion Rowland, MD	Collaborator	University College Dublin, Dublin	
Emer Fitzpatrick	Collaborator	University College Dublin, Dublin	
Hartmut Grasemann., MD	Collaborator	The Hospital for Sick Children (SickKids), Toronto	University of Toronto
Alexandra Quittner, PhD	Collaborator	Nicklaus Children's Hospital, Florida	University of Miami
Cliff Taggart, PhD	Collaborator	Queen's University Belfast	
Paul Cotter, MD	Collaborator	Teagasc Food Research Centre, Ireland	APC Microbiome Institute, Cork Institute of Technology
Billy Bourke, MD	Collaborator	Children's Health Ireland at Crumlin	University College Dublin
Aidan McCormick, MD	Collaborator	St Vincent's University Hospital, Dublin	University College Dublin
Sinead Weldon, PhD	Collaborator	Queen's University Belfast	
A.H. Maitland - van der Zee, MD	Collaborator	Amsterdam University Medical Centre	
Anna Georgiopulous	Collaborator	Massachusets General Hospital	Harvard Medical School
Sarah Carroll, PhD	Collaborator	Children's Health Ireland, Dublin	University College Dublin

Table 5: Parties Involved in the RECOVER Study

Patient Population
Recruitment to the Parent Study is dependent on clinical prescription of ETI to individuals with approved genotypes at the participating centres and their willingness to consent to the study.

Inclusion and Exclusion Criteria for Parent Study

Inclusion criteria
Participants may only be selected for inclusion in RECOVER if they have been independently determined by their treating physician to be suitable for treatment with ETI in compliance with the official marketing authorization and summary of product characteristics (SPC). The decision to include participants in the study is independent of decision to prescribe ETI. Participants will receive treatment only through prescription by their physician through usual clinical treatment pathways.

Subjects on ETI
In exceptional circumstances where baseline clinical data has been collected prior to the start of treatment either through clinical care or ethically approved research projects (including a cohort of subjects initially recruited to this study on the understanding that it was a non-regulated observational study) subjects already receiving ETI may be recruited to this study and undergo on-treatment visits. Any additional patient data can only be added with written informed consent from the patients/parents concerned.

All subjects must have a signed informed consent form and/or signed assent form when appropriate, as determined by the subjects age and individual site and country standards.

Male and female participants of childbearing potential must agree to adhere to contraception requirements as detailed in the local ETI SmPC and in line with the standard of care.

Exclusion criteria
Patients not willing to comply with study procedures or assessments.

Individuals on clinical trials of investigational CFTR modulators.

Clinical instability at baseline assessments. Subjects undergoing an active exacerbation and at the beginning of their treatment should be excluded from the study as this is likely to skew the data.

Any contraindication to ETI treatment as per the local approved SmPC.

Severe hepatic impairment.

Pregnant and breastfeeding women.

Inclusion and Exclusion Criteria for Extension Study

Inclusion criteria
Children and adults with CF who have completed two years participation on the parent study, and are willing to provide informed consent for continued data and bio-sample collection for a period of five years.

Exclusion criteria
Participants not willing to comply with study procedures or assessments.

Individuals on clinical trials of investigational CFTR modulators.

Drug Interruption or Discontinuation of ETI

If a patient experiences a drug interruption, discontinues ETI and/or is prescribed TEZ/IVA, LUM/IVA or IVA, they can continue trial participation.

Advanced Testing v Standard Testing for the Parent Study
As outlined in Table 5, we will have a standard and advanced testing group for the parent study. In addition to all elements of the standard testing group, the advanced testing group will undergo:

- Ultra-low dose spirometry-controlled CT scanning
- Sputum collection
- Nasal lavage collection

Table 6 outlines projected recruitment targets for both groups. Advanced testing will be offered to all participants to the cap in table 6, on the basis of fully informed consent. Progress in recruitment to both groups will be monitored by the project manager and monitor.

	F/F		F/M		F/G		TOTALS		
Ages	6-11	12+	6-11	12+	6-11	12+	6-11	12+	All
ADVANCED	47	60	17	26	17	0	64	86	**150**
STANDARD	27	36	9	15	0	0	36	51	**87**

Total	**74**	**96**	**26**	**41**	**17**	**0**	**117**	**137**	**254**

Table 6. Projected recruitment to standard and advanced testing arms for parent study.

Primary and Secondary Outcomes
The primary outcome measure for this study is the absolute change from base line values and CT scores.

The secondary outcome measures are as follows;

- height/weight/BMI
- FEV1
- Airway microbial culture
- Airway microbiome determination
- Nasal Lavage inflammatory markers
- Nasal microbiome determination
- Exhaled Nitric Oxide
- Liver function testing
- Liver ultrasound Liver
- Examination score
- Sputum inflammatory markers
- Sputum microbiome determination
- Stool inflammatory markers
- Stool microbiome determination
- Sweat Chloride
- Genetic modifiers of CF phenotype
- Abdominal symptom score
- CFQ-R
- Treatment Adherence
- Medication Pick up rates

Core Data to be Collected
Eligibility assessment
Prior to study enrolment, the investigator will assess subject eligibility. This will include reviewing the subject's current medication and medical history in detail to ensure no exclusion criteria are met and all inclusion criteria are met. As part of the eligibility assessment, a urine pregnancy test (CE marked) must be conducted in female participants considered of childbearing potential (WOCBP). WOCBP are defined as females that are fertile, following menarche, until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A negative test must be produced prior to enrolment and documented within the subject's medical records.

Demographic data
Core demographic data will be collected on all individuals including genotype, sex, date of birth, CF centre and medications.

Sweat Chloride
Sweat tests will be carried out on all individuals prior to initiation of treatment and then at 6 months after treatment, at one year and at two years for the parent study, and annually in the extension study. Sweat will be collected using the Macroduct sweat collection system as per manufacturer's instructions using pilocarpine iontophoresis to induce sweating. Sweat tests will be performed on both arms. Sweat will be stored in 0.2ml

PCR tubes and immediately transferred to the laboratory and frozen at -80°C. Sweat chloride will be analyzed in all stored sweat samples in batches by the Chloro-check Chloridometer (EliTech Group Inc.) at all sites.

Nutritional Indices
Height and weight will be measured at three monthly intervals throughout the duration of the parent study at each out-patient visit, and annually during the extension study. Height, weight and BMI z-scores will be calculated from these measurements.

Patient Reported Outcomes – Respiratory Symptoms
CFQ-R (including the CFQ-R treatment burden subscale) will be administered to study participants every six months during the parent study and annually during the extension study. Scores in all domains of CFQR will be compared between baseline and follow up visits.

Investigational Medicinal Product (IMP)

The IMP for this trial is ETI (EU/1/20/1468/001). This IMP is licenced for use in the United Kingdom and will be administered to study subjects in line with the prescription and in line with the SmPC. To be enrolled in the Parent Study, all patients were prescribed ETI. The decision to implement treatment with IMP is made completely independently of the decision to enter subjects into the study. If a patient experiences a drug interruption, discontinues ETI and/or is prescribed TEZ/IVA (EMEA/H/C/004682), LUM/IVA (EMEA/H/C/003954) or IVA (EMEA/H/C/002494) after enrolment, they can continue trial participation. IMP will be dispensed in accordance with usual clinical practice (i.e. by hospital pharmacy/community pharmacy) and therefore no additional labelling, QP batch release or drug accountability is required.

Recording and reporting of adverse events and reactions

Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
Adverse Reaction (AR)	Any untoward and unintended response in a subject to an investigational medicinal product which **is related** to any dose administered to that subject. *This includes medication errors, uses outside of protocol (including misuse and abuse of product)*
Important Medical Event	These events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events should also be considered 'serious'.
SUSAR	Suspected Unexpected Serious Adverse Reaction A serious adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out: (a) in the case of a product with a marketing authorization, in the summary of product characteristics for that product, (b) in the case of any other investigational medicinal product, in the investigator's brochure relating to the trial in question.

Recording adverse events

All AEs occurring during the study observed by the investigator or reported by the subject, whether or not attributed to the study medication, will be recorded on the Case Report Form and in the medical notes unless they are stated as non-reportable events, in which case they will not be recorded. AEs will be collected from the time of consent until the final study visit

Non-reportable adverse events
Certain clinical and laboratory events in cystic fibrosis have a high background incidence or are considered to be typical clinical features of cystic fibrosis and would not need to be reported. These include the following;

- Pulmonary exacerbations and hospitalizations due to pulmonary exacerbations.
- Clinical signs and symptoms commonly associated with pulmonary exacerbations (nasal congestion, nasal discharge, cough, sputum production, drop in lung function, wheeze, crackles, positive bacterial/fungal cultures)
- Clinical signs and symptoms commonly experienced by patients with CF in their normal daily routine (abdominal discomfort/distension, headaches, fatigue, malaise, clubbing, nasal polyps)
- Symptoms of known CF complications such as liver disease (rise in liver function tests), diabetes, sinusitis, constipation and distal intestinal obstruction syndrome (DIOS)

NOTE: Any of the above events deemed by the clinicians/ study investigators to be possibly related to the study drug or occurring to a disproportionate degree should be reported as an adverse event/serious adverse event.

Adverse Events of Special Interest

-Influenza is an adverse event of special interest, consistent with the risk management plan for Kaftrio. Occurrence of Influenza will be recorded as an adverse event or serious adverse event in every subject.

Assessments of Adverse Events

Each adverse event will be assessed for the following criteria:

Severity

Category	Definition
Mild	The adverse event does not interfere with the subject's daily routine, and does not require intervention; it causes slight discomfort
Moderate	The adverse event interferes with some aspects of the subject's routine, or requires intervention, but is not damaging to health; it causes moderate discomfort
Severe	The adverse event results in alteration, discomfort or disability which is clearly damaging to health

Causality

The assessment of relationship of adverse events to the administration of IMP is a clinical decision based on all available information at the time of the completion of the case report form. The following categories will be used to define the causality of the adverse event:

Category	Definition
Definitely:	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Probably:	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely
Possibly	There is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant events).
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
Not related	There is no evidence of any causal relationship.
Not Assessable	Unable to assess on information available.

Expectedness

Category	Definition
Expected	An adverse event that is classed in nature as serious and which is consistent with the information about the IMP listed in the SmPC
Unexpected	An adverse event that is classed in nature as serious and which is not consistent with the information about the IMP listed in the Investigator Brochure (or SmPC if Licensed IMP)

The reference document to be used by the Sponsor (RCSI) to assess expectedness of the event against the IMP is the SmPC for:
ETI (EU marketing authorisation number EU/1/20/1468/001)

Seriousness

The Principal Investigator (PI) should make an assessment of seriousness as defined below:

A Serious Adverse Event (SAE) is any adverse event that:
- results in death,
- is life-threatening,
- requires hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity, or
- consists of a congenital anomaly or birth defect
- is considered to be an important medical event (in the opinion of the investigator)*

** These events may jeopardise the subject or may require an intervention to prevent one of the above characteristics/consequences. Such events should also be considered 'serious'.*

Procedures for recording and reporting Serious Adverse Events

All serious adverse events will be recorded by the site staff in the hospital notes, on the CRF, and the sponsor's SAE form.
All serious adverse events will need to be reported to the sponsor on a SAE form unless they fall under the category of non-reportable adverse events.

The Principal Investigator (PI) or appropriate designee is responsible for capturing SAEs on the appropriate trial specific SAE forms and reporting all SAEs to RCSI Pharmacovigilance (Pharmacovigilance@rcsi.ie) within 24 hours of first becoming aware of the event (as per the RCSI Sponsor SOP on Expedited Safety Reporting). It is important to note that in the event that an SAE was not previously documented as an AE, the PI or designee must also fill out an AE form in junction with an SAE form.

SAEs will be collected from consent until the final study visit. SAEs that are related to the investigational drug and continue beyond the normal collection period (i.e., are ongoing at the time a subject exits the study) will be followed until resolution or until stabilized with sequelae. SAEs that begin after the subject's participation in the study is complete, but that the PI considers to be related to study drug, may be reported at any time. Reporting to the sponsor will be completed as per the Sponsor's Expedited Safety Reporting SOP.

Reporting SUSARs
The sponsor will notify the main REC, competent authority and marketing authorisation holder of all SUSARs. SUSARs that are fatal or life-threatening must be notified to the CA and REC within 7 calendar days after the sponsor has learned of them. Other SUSARs must be reported to the REC and CA within 15 calendar days after the sponsor has learned of them.

Development Safety Update Reports
The sponsor will provide the main REC and the competent authority with Development Safety Update Reports (DSUR) which will be written in conjunction with the trial team and the Sponsorship office. The report will be submitted within 60 days of the Developmental International Birth Date (DIBD) of the trial each year until the trial is declared ended. Any safety data from this study will be reviewed and assessed by the investigator and by reference to safety data already contained in the approved SmPC.

Annual progress reports
An annual progress report (APR) will be submitted by the Sponsor to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. The sponsor will prepare the APR.

Pregnancy

Any pregnancy occurring during the clinical study and the outcome of the pregnancy should be recorded and followed-up for congenital abnormality or birth defect. The Principal Investigator (PI) or appropriate designee is responsible for capturing all reported pregnancies in trial participants (or partners of trial participants) on the appropriate trial specific pregnancy form and reporting to RCSI Pharmacovigilance (Pharmacovigilance@rcsi.ie) as per the RCSI Sponsor SOP on Expedited Safety Reporting.

Specific Aim 1 for Parent and Extension Study – Pulmonary Function

Forced Expiratory Volume in 1 second (FEV1)
FEV1 measured by spirometry is the most widely used evaluation of lung function in CF. As FEV1 has been used as the primary endpoint in numerous clinical trials in CF to date, it is important that the rate of FEV1 change is recorded and analyzed as a secondary endpoint in this study. Spirometry will be carried out on each subject at three monthly intervals throughout the duration of the parent study, and annually during the extension study as part of routine clinical care. Spirometry will be performed according to the American Thoracic Society (ATS) guidelines(125). Spirometry values collected as part of routine clinical care will be used for this study, rather than repeating this testing specifically for the purposes of the study. Other spirometric measures such as FVC, FEV1/FVC and FEF25-75 will be collected for inclusion in later analyses. The absolute and relative changes and rate of change in % predicted FEV1 (GLI – Global Lung Initiative) will be assessed from baseline to annual subsequent follow up periods.

Lung Clearance Index
LCI will be performed with the Exhalyzer D multiple breath washout device (Eco Medics AG, Duernten, Switzerland). The test itself is usually completed within twenty to thirty minutes (the test takes longer the more airway disease is present) and will be performed according to the European Respiratory Society / American Thoracic Society consensus statement 2013 on the performance of multiple breath inert gas washout(126).. MBW operators will be trained and certified by the team at Guy's and St Thomas' NHS Foundation Trust) under the supervision of Prof Jane Davies. Centralized over-reading will be completed at the RBH centre. All tests will be performed in replicate, and average values calculated for acceptable trials. LCI will be performed pre-bronchodilator in all subjects. LCI will be performed three months prior to the baseline visit and then at baseline and at 6 monthly intervals subsequently for the parent study, and annually during the extension study..

Analysis plan
The primary endpoint for LCI for the parent study will be the absolute difference in LCI in children on ETI compared the pre-treatment baseline. An interim analysis will be completed after one year of treatment, and a final parent study analysis at the end of two years. Subsequent annual and multi-annual change in LCI will be assessed during the extension study.

Risks with FEV1 and LCI Data Collection
The main foreseeable risk factor here is protocol deviation in terms of collection and timeliness of the data. FEV1 is an easy to perform test and is part and parcel of most clinical interactions with patients. Some individuals, particularly at younger ages may not be able to produce acceptable or reproducible data despite the work of trained physiologists. This we cannot control but hope to have LCI data on all these subjects, an easier to perform and more sensitive test of lung function in this setting.. For RECOVER we will seek to minimize missed data by having an LCI testing frequency of 6 monthly with a one-month window each side during the parent study, and annually thereafter. Having a site monitoring plan and continuously vetting data as it is uploaded in real time to the study database will ensure that opportunities for assessment windows are not missed. All LCI operators will be fully trained under the supervision of the GST site and all studies will be over-read by the GST team, minimizing any prospect of poor-quality data.

Specific Aim 2 for Parent Study – Lung Imaging

CT Scanning
CT scanning protocols will be standardized at all centres by the Erasmus LungAnalysis group at Rotterdam (127). It is vitally important when using CT chest as an outcome measure in clinical trials that the protocol for imaging acquisition is standardized(127). Differing imaging quality can reduce the sensitivity for monitoring CF lung disease. As part of this study LA will ensure scanner outputs from all centers are standardized under the supervision of the group lead by Prof. Harm Tiddens, a collaborator on this study. During the parent study, spirometry-controlled CT scans will be performed at baseline, year one and year two. During the extension study, spirometry-controlled CT scans will be performed along two different potential pathways in those who have had CT scans as part of the parent study: In some centers, where biennial low dose surveillance CT scanning occurs as part of routine practice, these scans will be spirometry-controlled and included in the study database. In centers where routine surveillance does not occur as part of clinical care, spirometry-controlled CT scans will be performed four years after the final RECOVER Parent Study CT to correspond with the second of the routine surveillance CTs in centres performing this biennially.

Spirometry-Controlled CT Scanning
In order to obtain an optimal chest CT scan at each time-point, we will obtain a motion free scan at total lung capacity (TLC) and at residual volume (RV). In order to achieve reproducible and standardized chest CTs, all the scans will be spirometry-controlled by a trained research coordinator. Each centre will undergo a certification process that will include e-training modules through the LA-website. Staff involved in the study will be trained in the importance of correct execution of the spirometry-controlled CT scanning procedure. The e-training includes a background information of the study, background information of the spirometry-controlled CT scanning procedure, staff training on how to instruct patients for breath hold procedures, background information to the radiology department on study specific CT scanning protocols. When all e-training is successfully completed and the site has successfully proven to be able to execute a scan using the site and scanner specific CT scanning protocol by scanning a water phantom, the site receives a study certificate and is ready to scan patients for the study. All patient scans will be monitored for correct use of the CT scanning protocol by LA. The site can find the monitoring information of the patient CT scans on the LA website. Supplementary hands on central training for spirometry controlled CT technique will be carried out in CHI Crumlin under the supervision of two trained respiratory physiologists.

The Erasmus Medical Center LungAnalysis (LA) group lead by Prof. Tiddens have been conducting research in imaging and image analysis for over 20 years. LA has participated in over 40 international studies. LA takes care of standardization of chest CT protocols across centres and across different CT vendors. In addition, LA takes care of safe transport of images through secured internet connections. Finally, LA executes image analysis using image analysis strategies and software developed by the LA group. Since 2019 LA is making use of its study website developed to handle studies that include images as outcome measures. For each study protected dedicated webpages are constructed that can be accessed through a secure log in procedure that contains all imaging related issues in relation to the specific study. In addition, training and certification of site personnel is taken care of through the website.

CT Scoring

Image analysis of the patient CT scans determining the presence, extent and severity of CF lung disease on a CT chest will be executed by LA(128). Scoring systems combine the scores for individual features on CT such as bronchiectasis, bronchial wall thickening, air-trapping, consolidation and mucus plugging. The best validated manual CF CT scoring system is the CF-CT score (upgraded version of the Brody score)(128, 129). Manual scoring systems are prone to subjectivity and can lack sensitivity when it comes to early CF lung disease. A recently developed partially automated method of CT scoring, the Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) system(130) has been shown to be more sensitive in terms of quantification of airways disease than the CF-CT score(131). Bronchial Artery Ratio Score (BA-ratio) will also be performed on the scans. The BA-ratio is a more objective method to diagnose bronchial wall thickening and bronchial widening. BA-ratio analysis uses a three-dimensional image of the lung reconstructed from a chest CT. An automatic artificial intelligence (AI) BA-ratio analysis algorithm was developed and validated(132–135). This automatic BA-ratio analysis can detect a large number of BA-pairs in children older than 6 years(133, 134).

For RECOVER, all CT scans will be locally anonymized, coded and uploaded via an online portal for PRAGMA and BA-ratio scoring at the LA core laboratory at the Erasmus Medical Centre in Rotterdam. The scans in this study will also be utilized in the validation of an automated scoring system currently in development at the LA core under the direction of Prof. Harm Tiddens. Anonymized CT images will be archived to allow for any current or future scoring systems to be applied to this dataset.

Analysis Plan

PRAGMA and BA-ratio scores and sub-scores of CT scans at baseline (prior to treatment), year one and year two of the Parent Study will be compared. Scores will also be compared with scores from historic controls (F508delall). PRAGMA and BA-ratio scores will be compared with other key RECOVER outcome measures including sweat chloride, pulmonary function, LCI, lung infection, airway inflammation and medication adherence. Several subjects at CHI will have been enrolled in CFORMS, a cohort study examining PRAGMA scores and LCI outcomes in children on Orkambi, a predecessor of ETI. Where data is available from subjects moving from CFORMS to RECOVER, PRAGMA scores will be compared to CFORMS scores (pre and post treatment). CT scores in the extension study will be incorporated into a mixed-effects model seeking to understand longitudinal disease progression on CFTR modulators.

Risks with CT Data Collection

The main foreseeable risk factor here is missing data. As scans will be performed in clinical radiology departments and many factors out of our control may impact on timing and scheduling, missing scans or scans performed outside of timing windows may occur. In order to mitigate this we will ensure that there is clear communication between the local research coordinators/nurses and the clinical team about timing and windows, and in parallel we will ensure that the lead PI will communicate regularly with site PIs to maintain engagement and encouragement with regard to adherence to study protocols and timing. There is a risk that scan quality may not be up to the required standard in certain cases given that the performance of scans (apart from the spirometry control) is outside of the remit of the study team. We will mitigate this by ensuring clear lines of communication between local PIs and their radiology colleagues, and clear written communication from the RECOVER team to radiology departments in relation to the vital role they play in the study, and the

necessity for production of reproducible data. We will regularly feedback to the local teams and radiology departments in relation to study progress in an effort to maintain engagement and active participation.

A concern with the use of ionizing radiation is cumulative cancer risk. RECOVER Parent Study involves three ultra-low dose CT scans performed over a two-year period. Many centres in the study perform ultra-low dose CT surveillance every two years, in which case only one extra scan will be required for the purposes of the study. At sites performing routine biennial CT, the extension study does not involve any additional scans over and above what would be performed as part of standard clinical care. At sites not performing biennial surveillance, one additional CT within a five-year period will be required. The technology involved in dose optimization with modern scanners and software, particularly for lung imaging, has led to dramatic reductions in the dose necessary to achieve satisfactory images. The involvement of the Erasmus standardization of scanner output in this study will ensure that all centres are delivering the lowest dose necessary. Accurate measurement of the delivered dose of radiation to an individual, and the calculation of the risk involved from that radiation to the individual is complex and challenging. The lifetime risk of fatal cancer in the general population is thought to be approximately 23.3%. The excess risk of fatal cancer from a single inspiratory and expiratory CT scan for children with CF was estimated by Kuo et al to be 0.023%(135). Rosenow et al have calculated that for a clinical trial with two ultra-low dose CT scans, the extra radiation dose is equivalent to the increase in background radiation exposure from moving from Nevada to Colorado for a period of 9 months(136, 137). Background radiation in Ireland is estimated to be approximately 4 milisieverts per individual from all sources (with an average of 0.55 milisieverts coming from medical imaging). Ultra-low dose chest CT scans are estimated to deliver 1.4 milisieverts per scan in adult patients.

Our site monitoring plan and the continuous vetting of data as it is uploaded in real time to the study database will minimize missing or delayed data, and ensure all sites are keeping up to date with study timelines. All Spirometry operators will be fully trained under the supervision of the Erasmus and the project management team in Dublin, minimizing any prospect of poor-quality data.

Specific Aim 3 for Parent and Extension Study – Airway Infection and Inflammation

Sputum samples will be collected and stored on all participants annually, and these samples will be used for collaborative projects, however detailed analysis of these specimens will not be part of the extension study.

Sputum processing
As part of standard clinical care, sputum or oropharyngeal specimens (OPS) will be collected and processed in clinical laboratories. This data will be stored on local hospital systems and uploaded in the usual way from each center to the relevant national registry, our collaborators in this study. Separate to this, sputum samples for research purposes will be collected at study visits and immediately processed in the laboratory. Measurement of markers of inflammation in sputum is greatly facilitated by having a detailed and reproducible protocol for processing of fresh sputum specimens after collection. We will use such a system to ensure that our samples are collected, handled and processed to the highest level of quality during the study (138). Briefly, according to the TETRIS protocol, sputum will be collected from subjects, either spontaneously or after hypertonic saline induction, and immediately placed on ice for transport to the laboratory. The two-step temperature controlled process will be followed according to the protocol, however, once sputum plugs have been homogenized with cold phosphate buffered saline (PBS), and prior to centrifugation, an aliquot of this homogenized sputum will be mixed with an RNA preservative (RNAlater) and frozen at -80°C for later 16s shotgun sequencing. Sputum supernatant, once processed by TETRIS, will be aliquoted and stored at -80°C.

If a subject is not in a position to produce sputum, the research nurse/coordinator will arrange for sputum induction to be carried out. Sputum induction will be performed by using 7% hypertonic sodium chloride solution will be nebulized for 15 minutes with the subject encouraged to carry out airway clearance maneuvers during and after nebulization. All sputum collected over a 30-minute period will be utilized.

Nasal Lavage

Nasal lavage will be collected as previously described(21) at baseline, 6 months and one year. Briefly, 10ml of sterile isotonic saline will be slowly injected into each nostril sequentially using a 10ml syringe while the subject occludes their soft palate and reclines their head. The saline is allowed to remain in the nasal airways for 10 seconds before being drained into a sterile collection container by the subject leaning forward. If a clinical specimen is required, 5ml of fluid will be sent separately to the local microbiology lab for routine processing. Lavage fluid will be put on ice and transferred to the laboratory for aliquoting and immediate freezing at -80°C. An aliquot of the nasal lavage fluid will be mixed with an RNA preservative (RNAlater) and frozen at -80°C for later 16s shotgun sequencing. Nasal lavage will be performed in individuals 12 years and older.

Airway Infection

Microbiological processing of samples will be completed as per usual clinical practice at clinical microbiology labs at each clinical site. As part of the study, consent will be sought from parents to access historic and current clinical microbiology sample results uploaded to CFRI and the UK CF Registry. Using a similar approach to national registries we will track the percentage of individuals culturing positive for particular organisms prior to and over the course of the study period. In addition, we will analyse trends at an individual level prior to and during treatment with ETI. We will be able to compare pulmonary function and imaging findings collected via RECOVER to clinically collected airway infection data.

Microbiome Analysis

DNA extraction and processing will be performed by our collaborators at the APC Microbiome Ireland in Cork, Ireland who have extensive experience with gut and lung microbiome projects in CF(139-141). Samples will undergo deep shotgun sequencing using the Illumina NextSeq platform. Detailed descriptions of microbial community composition and metabolic profile will be obtained.

Breath Sample Collection

A breath sample will be collected from a cohort of 60 subjects in the 6-11 cohort at CHI at Crumlin, CHI at Temple Street, CHI at Tallaght and Guy's and St. Thomas' NHS Foundation Trust and sent to collaborators in Amsterdam University Medical Centre (AUMC) to evaluate the changes in the composition and the function of the respiratory microbiome after the initiation of Kaftrio. AUMC have developed a novel method to standardize the procedure of trapping volatile organic compounds (VOCs) in exhaled breath onto sorbent tubes. Kaftrio is shown to have a major effect on pulmonary function, mucociliary clearance and reduction in sweat chloride test. VOCs can give insight into both the metabolic pathways and inflammation caused by microorganisms in the lungs that are commonly associated with CF.

Airway Inflammation

Aliquots of sputum supernatant ± protease inhibitors from each individual and time point will be sent to the laboratory of Dr Weldon and Prof Taggart, at Queens University Belfast for analysis of inflammatory markers. Although inflammation is a major feature of CF there is no universally accepted way to measure it in subjects. On the basis of our experience and data to date, we have compiled a compendium of airway inflammation biomarkers of particular relevance to the CF lung (Table 7). The sample collection and storage plan for RECOVER will allow further expansion of this panel based on initial findings or future work.

Host defence family	**Members**	**Method of analysis**
Proteases and neutrophil products	Serine (neutrophil elastase, proteinase 3), cysteine (cathepsin S, B, L), matrix metalloproteases (MMP-8, -9, -12), myeloperoxidase (MPO)	Fluorogenic activity assays, ELISA
Antiprotease	SLPI, cleaved-SLPI, tissue inhibitor of metalloproteases (TIMP-1, -2), cystatin SN and C	ELISA, Western blotting

Cytokines/chemokines	Interleukins -1β, -6, -8	Luminex MILLIPLEX multianalyte panel assays

Table 7. Host factors to be analyzed in CF sputum supernatant and nasal lavage

All data will be compiled on an on-going basis over the course of the task and inputted into the study database as samples are analyzed. We would hope, that through this comprehensive approach, and analysis of the data comparing individuals before and after treatment that we will gain important insights that could be of benefit in terms of monitoring of disease activity and burden in individuals with CF.

Fraction of Exhaled Nitric Oxide (FeNO)
FeNO will be measured with the Niox VERO™ device via a mouthpiece in spontaneously breathing children in all centres by a trained research coordinator according to manufacturer's instructions. FeNO will be assessed at minus three months, baseline, 6 months and one year after commencement of Kaftrio. FeNO levels will be compared to markers of lung function, particularly LCI, markers of lung inflammation and total and specific (particularly air trapping) CT scores.

Airway Arginine/NO Metabolism
Sputum samples will be collected and processed as per TETRIS protocol and then shipped for central storage at NCRC. An aliquot of sputum supernatant from each individual and time point will be sent to the laboratory of Drs Grasemann and Ratjen in Toronto, Canada, for centralized testing of indices of L-arginine and NO metabolism.

Sputum concentrations of NO metabolites nitrate and nitrite will be measured by Griess reagent as previously reported(142). Concentrations of arginase 1 will be determined by use of a commercial ELISA kit (human ARG-1 ELISA KIT, cat.# ELH-ARG1-1, RayBiotech).
Liquid chromatography-mass spectrometry (LC-MS/MS) will be used to quantify L-arginine metabolites as previously reported(75). L-arginine, L-ornithine, L-citrulline and asymmetric dimethylarginine (ADMA) will be measured, as well as the L-ornithine downstream products proline and the polyamines putrescine, spermine and spermidine(143).

Changes in laboratory outcomes (NO metabolites, arginase and L-arginine metabolites) between visits will be assessed using paired t-tests and Wilcoxon sign-rank tests for skewed distributions, or ANOVA. Correlations between measure laboratory outcomes and clinical outcomes (FeNO and pulmonary function) will be assessed with the Pearson's correlation coefficient.

The ability of NOS to produce NO depends on the availability of substrate L-arginine as well as the competitive inhibitor ADMA. Therefore, the ratio of L-arginine over ADMA (L-arginine/ADMA) is used as a marker of NOS impairment(83, 144). The L-ornithine derived polyamines are important in tissue repair and can act as inhibitors of NOS and contribute to airways obstruction(143), while proline contributes to collagen deposition and airway remodeling. Therefore, these ratios will also be used for the analyses.

Risks in Airway Inflammation data collection
The main foreseeable risk factor in this part of the study is the inability to collect a sputum specimen. Many patients, especially younger or healthier ones may not routinely produce sputum on a regular basis. We further suspect that, in light of the significant improvements in clinical outcomes demonstrated already for Kaftrio, that many individuals will go from being sputum producers to non-producers on treatment. We will work closely with the clinical teams to ensure that sputum specimens are optimized in terms of the split between laboratory and research processing. Each clinic has their own mechanism for trying to facilitate sputum production either at clinics or annual assessment – we will work with teams to ensure that saline induction, where used, can be facilitated by the research team to try to ensure a suitable sample sputum volume can be collected for all uses. We are aiming to collect 4 sputum samples over 2 years for RECOVER, with the baseline and year one samples prioritized. Where possible the research team will work with the subjects and clinical teams to ensure that all efforts are made to obtain a sufficient sample volume at these timepoints in particular, including sputum induction. The use of the TETRIS sputum processing protocol will mean that it is acceptable to use

spontaneously produced and induced sputum interchangeably when obtaining samples. This will greatly facilitate optimal sampling.

We expect that an incomplete set of samples will be collected and have factored this into our study calculations in terms of numbers and budget. With a recruitment of 150 individuals to the advanced group we would ideally expect 600 sputum samples and 414 nasal lavage samples to process (1014 total). In reality we estimate that it is likely that we will have 90% of individuals produce one sputum sample, 80% to produce 2 samples, and 70% to produce 3 samples and 60% to produce 4 samples. We estimate that 80% of eligible individuals will produce one nasal lavage sample, 70% two samples and 60% three samples. The main foreseeable risk factor with nasal lavage is aspiration of isotonic saline. In practice this is a very similar procedure to sinus rinse which is commonly performed by many people with CF on a daily basis where we see almost no adverse effects in those who learn how to do the procedure. It is likely that some people may find this an uncomfortable or awkward procedure and elect not to do it.

One of the reasons we selected FeNO as an outcome measure in this study was that we felt we might have individuals struggle to produce sputum samples. We will likely have less in the way of available sputum samples to use for testing NO metabolites than expected, however we do not foresee any particular issues with FeNO measurement as the technique and equipment is straightforward and the test is quick to perform.

Specific Aim 4 for Parent Study and Extension Study – Gastrointestinal Manifestations

The aim of the GI arm of this study is to gain a greater understanding of the changes in abdominal manifestations of CF on modulator therapy and the relationships between these changes and other important treatment outcomes such as sweat chloride response, nutritional outcomes and pulmonary outcomes.

Abdominal Symptom Scores
Our collaborator Jochen Mainz and his team at Medizinische Hochschule Brandenburg (MHB, formerly based in Jena, Germany) have developed a specific abdominal symptom questionnaire for individuals with CF. The CFAbd score is validated in children and adults with CF and scores correlate well with ultrasound findings (28-30) and gut inflammation (unpublished). The CFAbd score has been used in investigator led studies in leading international centres and translated into 9 languages. The score can be delivered by a trained researcher for the first time in 5-8 minutes, and on subsequent occasions in 3-5 minutes. Questionnaires will be delivered at centres by the local research coordinator. Questionnaire results will be collated and centrally analyzed by post-doctoral researchers at Dr Mainz's Centre. Abdominal symptom scores will be collected in tandem with stool samples as below in table 8 for the parent and extension study. Score results will be compared between baseline and follow up visits and compared to markers of gastrointestinal inflammation, pancreatic sufficiency and microbiome community structure.

	Parent Study							**Extension Study**				
	-3/12	0	1/12	2/12	6/12	1yr	2yr	3yr	4yr	5yr	6yr	7yr
Survey	X	X	X	X	X	X	X	X	X	X	X	X
Stool sample		X	X		X		X	X	X	X	X	X

Table 8. Sampling schedule for CFAbd scores and stool samples.

Stool Sample Collection
Stool samples will be collected as per the sample collection plan in Table 8. A 5-10g stool sample will be collected from study participants at routine study visits. Sample collection equipment will be provided in advance to subjects and parents so that samples can be collected and refrigerated (if necessary) on the morning of a study visit. Stool samples will be manually divided at the study site. During the parent study one aliquot of stool will be sent to our collaborators in Brandenburg, Germany for analysis of inflammatory mediators and elastase. A further aliquot will be frozen for analysis of microbiota. The balance will be frozen for

storage and later analysis. As part of the extension study analysis of inflammatory mediators and elastase will be performed for all sites at Children's Health Ireland.

Markers of Gut Inflammation
Stool samples will be processed using prepared commercial assay tubes as per manufacturer's instructions for calprotectin and M2-PK. Calprotectin will be measured from frozen stool samples using the PhiCal kit (Calpro, San Diego, CA, US) and M2-PK will be measured from stool samples using the ScheBo M2-PK kit (ScheBo Biotech, Giessen, Germany).

Markers of Pancreatic Exocrine Status
Fecal elastase-1, a validated marker of exocrine pancreatic insufficiency will be measured on stool samples as per manufacturer's instructions with specific kits (ScheBo® Biotech AG, Giessen, Germany) at Brandenburg, Germany, after initial separation at local centres.

Gut Microbiome
After separation, stool samples will be immediately frozen at the local sites and subsequently shipped for central storage at Dublin. DNA extraction and processing will be performed by our collaborators at the APC Microbiome Ireland in Cork, Ireland who have extensive experience with gut and lung microbiome projects in CF(114-116). Samples will undergo deep shotgun sequencing using the Illumina NextSeq platform. Subsequent bioinformatic analysis will reveal the taxonomic profile and functional potential of the microbiomes within samples.

Risks with GI Data Collection
The main foreseeable risk factor here is missing data. As abdominal USS scans will be performed in clinical radiology departments and many factors out of our control may impact on timing and scheduling, missing scans or scans performed outside of timing windows may occur. In order to mitigate this we will ensure that there is clear communication between the local research coordinators/nurses and the clinical team about timing and windows, and in parallel we will ensure that the lead PI will communicate regularly with site PIs to maintain engagement and encouragement with regard to adherence to study protocols and timing. There is a risk that scans may not capture all requisite elements we are planning to collect data on. We will mitigate this by ensuring clear lines of communication between local PIs and their radiology colleagues, and clear written communication from the RECOVER team to radiology departments in relation to the vital role they play in the study, and the importance of collecting data as outlined on the study sheets. We will regularly feedback to the local teams and radiology departments in relation to study progress in an effort to maintain engagement and active participation.

Some individuals may be reluctant or unable to give stool specimens at the nominated times. Study nurses/coordinators will work closely with subjects to encourage/remind them in relation to timelines and facilitate collection of specimens as much as possible. We expect that an incomplete set of stool samples will be collected and have factored this into our study calculations in terms of numbers and budget. With a recruitment of 201 individuals we would ideally expect 772 stool samples. In reality we estimate that it is likely that we will have 85% of individuals produce one sample, 80% to produce 2 samples and 70% to produce 3 samples and 60% to produce 4. This is outlined in table 9.

Visit	**0**	**1**	**6**	**24**	**Total**
Numbers	156	117	131	113	517

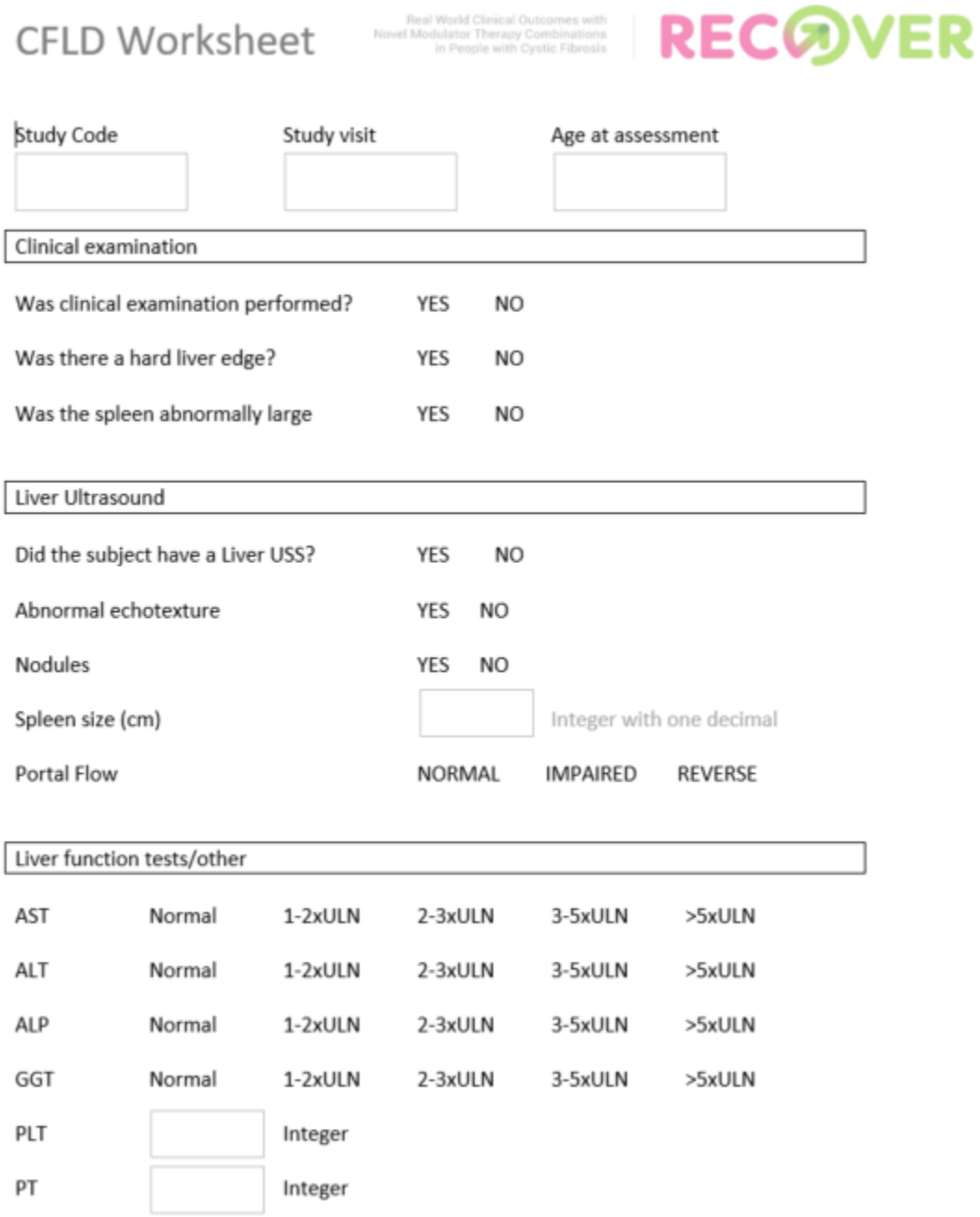
Table 9. Estimated total number of stool samples

Specific Aim 5 for Parent Study and Extension Study – Liver Manifestations

The aim of the CF liver disease arm of RECOVER is to gain a greater understanding of the changes in CF liver manifestations in people on CFTR modulator therapy. This is done by analyzing data from liver ultrasounds, liver function tests and other blood tests. For the parent study, LFTs are monitored every 3 months for the first year and at year two. Liver exams and ultrasounds will be carried out at baseline, year one and year two. For the extension study, LFTs are monitored annually as part of normal clinical care. Ultrasounds will be performed where indicated as part of normal clinical care at the participating center (most RECOVER sites perform annual ultrasound screening).

Liver Examination for the Parent Study

As part of the formal assessment of classification of CFLD (CFLD, non-specific [NSCFLD] and No LD), site investigators at all sites will be asked to clarify specifically on physical examination whether there is a hard liver and splenic enlargement at study baseline and at year one and two. Specific guidance will be provided around clinical examination via a training video by the lead hepatologist (BB) on the study. The baseline liver disease status of participants in the Republic of Ireland will be updated/confirmed using data from the Irish longitudinal study on cystic fibrosis liver disease study (ILSCFLD) based on previously collected data. The data from examination, ultrasound and blood tests will be collated on the CFLD worksheet (figure 7). A single paediatric (BB) and a single adult (AM) hepatologist will review the worksheets together in batches after each assessment window and determine which group subjects should be allocated to. If there is a discrepancy between ILSCFLD allocation and RECOVER baseline allocation these will be reviewed as a group by the study hepatologists and a confirmed allocation agreed to enable longitudinal assessment using ILSCFLD data.



CFLD Worksheet

Real World Clinical Outcomes with Novel Modulator Therapy Combinations in People with Cystic Fibrosis

RECOVER

Study Code | Study visit | Age at assessment

Clinical examination

Was clinical examination performed?	YES	NO
Was there a hard liver edge?	YES	NO
Was the spleen abnormally large	YES	NO

Liver Ultrasound

Did the subject have a Liver USS?	YES	NO
Abnormal echotexture	YES	NO
Nodules	YES	NO
Spleen size (cm)		Integer with one decimal
Portal Flow	NORMAL	IMPAIRED REVERSE

Liver function tests/other

AST	Normal	1-2xULN	2-3xULN	3-5xULN	>5xULN
ALT	Normal	1-2xULN	2-3xULN	3-5xULN	>5xULN
ALP	Normal	1-2xULN	2-3xULN	3-5xULN	>5xULN
GGT	Normal	1-2xULN	2-3xULN	3-5xULN	>5xULN
PLT		Integer			
PT		Integer			

Figure 7. RECOVER CFLD worksheet for the Parent Study.

Liver Function Test

Liver function test results (AST, ALT, GGT, ALP, Bilirubin, albumin) will be collected from medical notes prior to drug initiation, 3 monthly for the first year for the parent study and annually thereafter for the remainder of the parent study and the extension study (this is a mirror of the requirements put in place by the health service for sites in ROI when all previous CFTR modulator therapies were introduced). For UK sites LFTs results will be collected from medical notes in accordance to required schedule of collection based on drug approval and funding. The following data from annual labs will be entered into the eCRF: Hemoglobin, hematocrit, platelets, white cell count, prothrombin time. The mean levels, and incidence of elevations of AST or ALT to greater than specified multiple (1-2xULN, 2-3xULN, 3-5xULN and >5xULN) times the upper limit of normal will be compared between those on ETI and (1) their liver function tests in the 2 years prior to starting ETI and (2) age matched historic (modulator naïve) controls from within the last 5 years.

Ultrasound

As per routine clinical practice in most centers, people with CF from 6 years of age onwards will have annual liver ultrasound scans as part of annual assessment. In centers where this is not the case, ultrasound scans will be performed for the purposes of the study. Ultrasound scans will be performed using a proforma schedule for reporting the findings including liver texture, the presence of nodules and liver edge irregularity and spleen size with reported measurement of AP length (figure 5). The presence of abnormal liver echotexture, evidence of retrograde portal venous flow and spleen size will be compared between those on ETI and (1) their liver ultrasounds, where available, in the 2 years prior to starting ETI and (2) age matched historic CF (modulator naïve) controls from within the last 5 years (Irish sites).

End of parent study assessment

At the end of the parent study, the liver disease status of participants (CFLD, non-specific [NSCFLD] and No LD) will be updated based on study assessments and review of study worksheets. Proportions of subjects in each group will be compared before and after modulator therapy in addition to determining how many individuals from each given group move to another group on ETI. This will be compared to historic natural history data from the ILSCFLD study for ROI participants.

Classification as part of extension study

Classification will be assigned to each participant as having CFLD, NSCFLD or NoLD annually during the extension study as above.

Specific Aim 6 for Parent Study and Extension Study – Pulmonary Exacerbations and Antibiotic Use

IV Antibiotic Therapy

The number of courses of IV antibiotics (hospitalizations and home IVs) and their indication (elective or unplanned) will be recorded over the duration of the study. This data will be collected in conjunction with clinical staff at local sites. The number (and number of days) of IV antibiotic courses over the length of the study timeframe will be compared with the number of IV antibiotic courses (and days) in the five years prior to commencement of ETI. Some individuals will have started on previous generations of CFTR modulators in the two to three years prior to the initiation of ETI, in which case data will be available for IV antibiotic courses before and on previous CFTR modulator therapy as well as on ETI.

Oral Antibiotic Use

The number of courses, total number of days and type of oral antibiotics will be collected from pharmacy records submitted with parent/guardian consent (and ethical approval) at the end of the study period as part of the adherence arm of RECOVER. The number of courses and number of days of oral antibiotic use will be documented in the eCRF. Given the complexity of the system at UK sites for collection of this information, data on oral antibiotic use from community pharmacies will be collected only from sites in Ireland.

Analysis of Data

Given the depth of information collected through RECOVER we will be in a position to compare antibiotic use to pulmonary outcome measures such as lung function, structure and inflammation, and non-pulmonary indices such as sweat chloride and medication adherence.

Definition of Exacerbation

We are specifically avoiding defining exacerbations in this study. While a definition of an exacerbation might assist in terms of standardization of between site comparisons, it will introduce some confusion with so many sites involved, is prone to some level of subjectivity and may influence decision making around treatment. In essence comparisons between groups in terms of antibiotic use will be based on internal comparisons between an individual's treatment by the same medical team using similar criteria before and after the introduction of Kaftrio, so will still be valid without specifying criteria. In this case we will be assessing, in a real-world setting, differences in use of antibiotics as opposed to differences in exacerbation frequency. This is

ultimately a more meaningful outcome measure in our view as it is what is of importance to the individual in terms of their treatment.

Risks with Exacerbation Data Collection
We do not foresee any risks in relation to collection of data for IV antibiotic use as this will be readily available and accessible at all sites. Oral antibiotic usage data will be collected through pharmacy records. In previous studies we have used parent report of oral antibiotic courses but found this to be unreliable and subject to considerable bias. The extrapolation of courses of antibiotics from the pharmacy records is likely to overestimate antibiotic use as some courses or parts of courses may be collected but not be taken. This is likely to some degree to even out over time as we are measuring courses per year. We do not foresee difficulties in obtaining pharmacy records. We have used this approach in previous studies and found the uptake to be good. Our study team setup and monitoring plan is likely to ensure that data is collected in a timely and complete way.

Specific Aim 7 for Parent Study – Treatment Adherence

This specific aim will form part of the RECOVER parent study but not the extension study.

We will undertake a detailed assessment of adherence as part of RECOVER so that we can link our findings in relation to treatment adherence to important disease outcomes. The treatment adherence arm of RECOVER will be led by Sharon Sutton, a clinical pharmacist at CHI Crumlin, who will undertake a five-year, part time PhD with RECOVER as the central theme. Prescribed medications at an individual level will be tracked throughout the study to ensure we have a reliable denominator on which to base adherence measures. A number of methods will be used in RECOVER to monitor and understand treatment adherence in the real-world setting:

- Electronic medication event tracking systems e.g. (MEMS®)
- Self-reported adherence
- Medication possession ratio (MPR) based on pharmacy pick up rates
- Qualitative assessment of beliefs and attitudes around adherence to ETI and other treatments

Medication Event Monitoring System (MEMS®)
This is the gold standard for electronic monitoring but can only measure monotherapy. It mimics a traditional pill bottle with the enhanced ability to track the date and time of each bottle opening.(92) Non adherence to MEMS® is less likely as the adherence pattern is recorded by the opening of the container for every dose. Therefore, it is used as a reference standard for validating other reference tools.(92) This data can be used to calculate the medication possession ratio per patient. Due to the high cost and additional support required this method of measuring adherence is under-utilized. We will measure adherence to ETI with MEMS® in a subset of RECOVER subjects in the Dublin Centres (approximately 100 individuals). MEMS® will not be used for adherence measurements with treatments other than ETI.

Parent/Subject Self-reporting of Adherence
This will be carried out using the up to date version of a specifically designed adherence scale for children and adults with CF(145) with the permission of the author Dr Alexandra Quittner (a RECOVER collaborator). The treatment adherence questionnaire, barriers to adherence questionnaire and prescribed treatment plan will be used to monitor self-reported adherence.

Treatment Burden
The CFQ-R treatment burden subscale, part of the CFQ-R questionnaire, has been used to assess treatment burden in people with CF(113). The treatment burden subscale, which comprises 3 questions, is scored on a standardized 0- to 100-point scale with lower scores representing a higher treatment burden. The questions are as follows: to what extent do your treatments make your daily life more difficult? How much time do you currently spend each day on your treatments? How difficult is it for you to do your treatments each day? CFQ-

R, including the treatment burden subscale, will be delivered on a scheduled basis to participants as per study protocol in table 14.

Medication Possession Ratios
MPR will be calculated using pharmacy records. MPR is calculated on the basis of the supply of medications for a given period divided by the time in that period. For example, if three scripts for 3 months of a treatment are given over a timeframe of one calendar year, the MPR would 0.75. MPR will be calculated for each individual medication for each study participant. Study consent will be sought for dispensed reports from hospital pharmacy, community pharmacy and homecare providers and GP medical records to be submitted to the subject's center at the end of the study period. Records will be analyzed locally and data on antibiotic use, enzyme use and MPRs per 6-month period calculated by the local study team before being uploaded in an anonymized fashion to the study database.

Qualitative Assessment
Using a combination of previously published data(146), adherence theory (e.g. WHO five dimensions of adherence) as well as the findings of the observational research, a semi-structured interview guide will be designed. Using the same cohort invited to participate in the MEMS component of the study, 20-40 participants will be interviewed with interviews halted when data saturation is achieved. Only subjects from the Dublin centers will be invited to participate for ease of access (the adherence lead for RECOVER is based in Dublin). These data will be analyzed using a qualitative analysis plan involving phenomenography/thematic analysis with constant comparative analysis to the WHO adherence framework. Through this process we hope to be able to explore the experiences of patients/parents on commencement of CFTR modulator and adherence to CF regimens. We hope to be able to establish individual factors that influence adherence in this 'new world' for this large cohort of people with CF.

Data Analysis
Analysis of data will be conducted as follows based on our key research questions:

- What level of adherence with ETI is seen in the real world?

Measures of weekly adherence (using the three measures independently) will be reported and compared to previously published data on adherence rates with Ivacaftor(115, 147). Adherence over time, using all three methods, will be reported in a weekly fashion as treatment progresses. Changes in adherence over time (weekly, quarterly) will be assessed per individual and cross sectionally. We will explore the relationship between weight gain and subsequent adherence to ETI (because of concerns on behalf of some PWCF about excessive weight gain on modulators).

- Does adherence with other CF therapies decrease after ETI?

Adherence rates, using MPR and self-reported adherence, to medications individually and in groups (vitamins, mucolytics, nebulized antibiotics etc.) will be collected from 3 months prior to the introduction of ETI. The absolute change in adherence for 4 quarters (one before and three after) will be compared using chi squared and ANOVA. The rate of change of weekly adherence will be compared prior to and in the 9 months after (in 3 quarters) initiation of ETI using ANCOVA.

- Does poor adherence adversely affect outcomes?

This question will be addressed from the point of view of firstly, adherence with ETI, and secondly, adherence with other groups of medications. Medium term effects will be determined by comparing overall 12 and 24-month MPRs for given medications and groups of medications and specific relevant outcome measures such as CT scores, LCI, FEV1, nutritional status, antibiotic use and exacerbations at the end of 12 and 24 months respectively.

- Is adherence related to treatment burden?

Results from the various adherence measures will be compared, within individuals, to the CFQ-R treatment burden subscale results at each timepoint.

Risks with Adherence Data Collection
Measurement of adherence is inherently challenging. Other than direct measures such as MEMS®, other measures of adherence such as MPR and self-reported adherence carry some degree of inherent inaccuracy. Notwithstanding this, the measures we use here are widely used in the adherence literature. The organization of the study team and the presence of a monitoring plan and a centralized eCRF is likely to ensure that data is collected in a timely and complete way. Collection of pharmacy data introduces the risk of inaccurate or incomplete datasets. People with CF in the study centers almost exclusively use a single community pharmacy for their medication needs. Study consent will include permission to access community pharmacy records allowing us to retrieve data from source and reducing the potential for inaccuracies.

Specific Aim 7 for Extension Study – Mental Health

Psychosocial functioning will be measured using the Pediatric Symptom Checklist (PSC), a brief, well validated and widely used parent-report questionnaire(148). The PSC measures cognitive, emotional and behavioral difficulties in children aged 5-17 years(149, 150). Cut-off scores for clinical levels of dysfunction have been derived for Internal (Anxiety/Depression), External (Conduct) and Attention subscales, providing a comprehensive measure of developmental-behavioral difficulties.

Young people's subjective report of their mood will be measured using the Patient Health Questionnaire - 9 (PHQ-9) and the Generalized Anxiety Disorder -7 (GAD-7) which are well validated self-report measures of depression and anxiety respectively (151, 152). These brief questionnaires are frequently used in both research and clinical practice and are recommended in international guidelines as measures of depression and anxiety symptoms in adolescents and adults with CF (118). These measures will be completed by children aged 12 years and over.

The PROMIS Pediatric Anxiety Scale (Short Form – 8a) and Depressed Mood Scale (Short Form – 8a) are brief self-report measures that can be administered to children from the age of 8 years, providing discreet measures of depressed mood and anxiety for younger children (153). This will represent the first self-report of younger children's emotional experience of CF in a large-scale study (118, 153). In addition to the parent-report PSC Attention subscale, neurocognitive functioning will be measured by the PROMIS Pediatric Cognitive Function Scale (Short Form 7a). This is a brief self-report measure of children's experiences of attention, memory, and comprehension, which is validated for use from the age of 8 years. There is also an adult version (Short Form 8a) which will be used for patients over the age of 18 (153). Body image will be assessed using a three-item questionnaire designed for this study, completed by children from the age of 8 years.

A linear mixed model for each of the mental health outcomes will be used to investigate impacts of patient characteristics over time. Fixed effects in the model will be: age at ETI initiation, genotype, sweat chloride level, nutritional status, caregiver burden scores, number of treatments, year. The model will use random intercept for patient.
The RECOVER distress protocol as shown in Table 10 below will be utilised as needed for participants completing questionnaires.

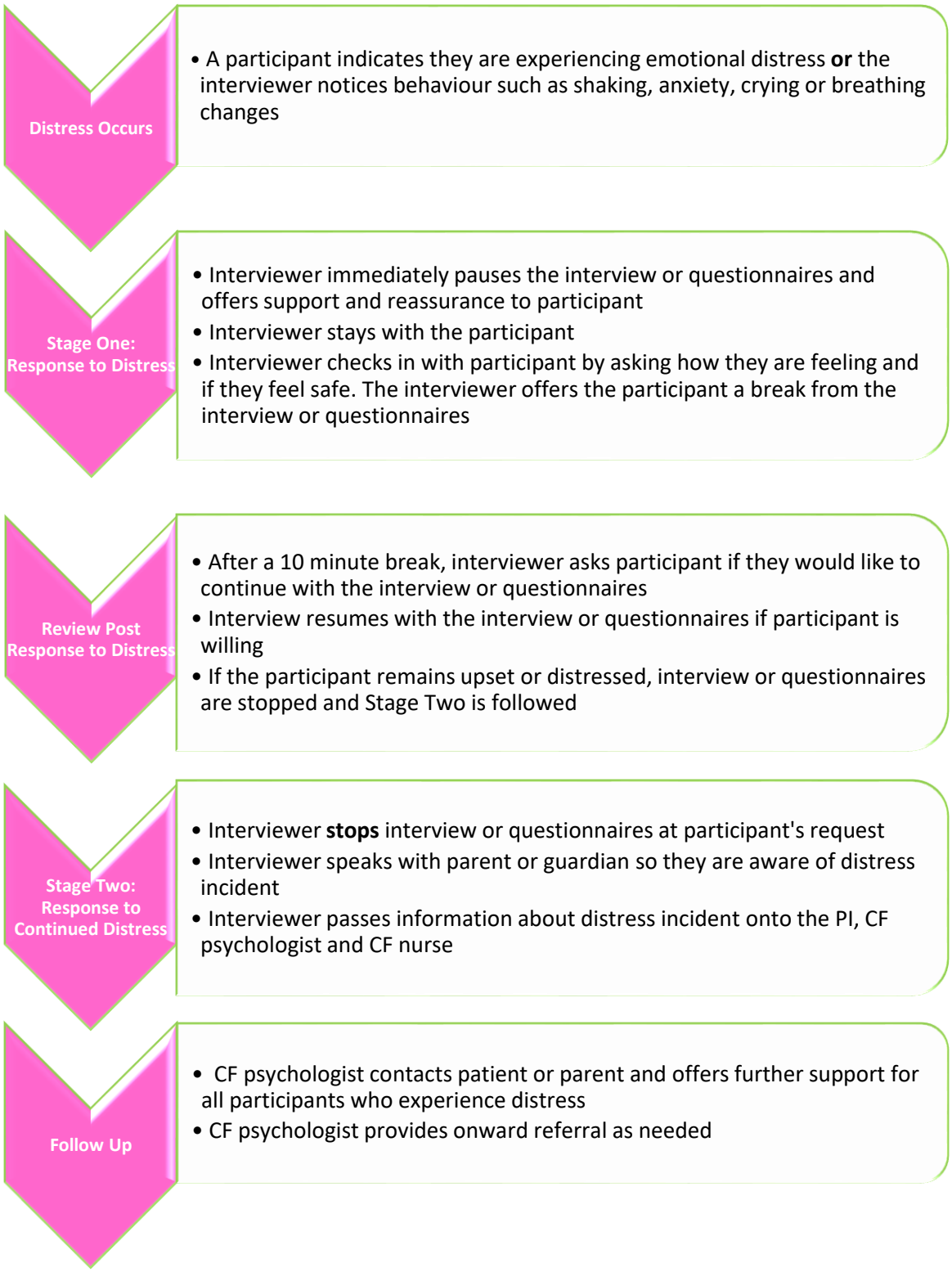


Table 10: Distress protocol for participant completing interview or questionnaires. (154, 155)

Statistical Analysis

Statistical support and analysis will be provided by CFRI for the parent study and by the Research Data Science team at CHI in conjunction with the Data Science Centre in RCSI for the extension study. Data management and analysis will be carried out using SAS version 9.3 for the parent study and STATA V17.0 for the extension study.

Sample Size and Power for the Parent Study
In this multi-centre longitudinal study of subjects starting on ETI we will be examining many different outcomes, and so sample size requirements are different for different aspects. Patients will be recruited to the study up to a cap of 254 participants. On the basis of the phase two trials of ETI(57), we are expecting a significant treatment effect in line with what was previously seen with Ivacaftor treatment in individuals with a gating mutation, and in excess of what was seen with Lumacaftor/Ivacaftor in individuals homozygous for F508del. Our sample size is higher than we expect to be required for most outcomes where data exists (see tables 11, 12, 13 below). We have aimed high deliberately for a number of reasons. Firstly, clinical research naturally carries risks in terms of adequacy of data collection. Secondly, we are keen to ensure that outcome measures can be cross referenced between each other and with previously collected, yet to be collected and data and samples. This will require excess power. With missing or incomplete samples across different outcome measures, complete datasets on a core number of individuals will necessitate collection in a larger cohort. Thirdly, this study represents a unique opportunity, which may not be replicated, to collect this data, and promises very significant insights into CF disease pathogenesis and response to therapies. In light of this, a large sample size with adequate sample collection we feel is important. Where data is available, we have performed sample size calculations (table 10). Where data is not available, we present a summary of key studies and evidence in the area.

Power and sample size calculations were produced for LCI as a main primary endpoint, using the GLMPOWER procedure in SAS 9.3 (Table11, Figure 5). A range of scenarios were considered for standard deviation from 2 (expected) to 3 (conservative) and power was computed for F/F participants only and for F/F and F/M participants combined. We estimated a conservative treatment effect at 1 unit of improvement in LCI (as seen with lumacaftor/ivacaftor), and an expected treatment effect at 2 unit of improvement in LCI (as seen with Ivacaftor in gating mutations).

Group	Sample Size	Std Dev	Power (1 unit)	Power (2 unit)
F/F only	166	2	91.9%	99.4%
F/F & F/M	201	2	96.4%	99.9%
F/F only	166	2.5	76.6%	94.8%
F/F & F/M	201	2.5	85.3%	98.0%
F/F only	166	3	61.0%	84.7%
F/F & F/M	201	3	70.8%	91.7%

Table 11. Computed power for 1 LCI unit difference between baseline and 1 year

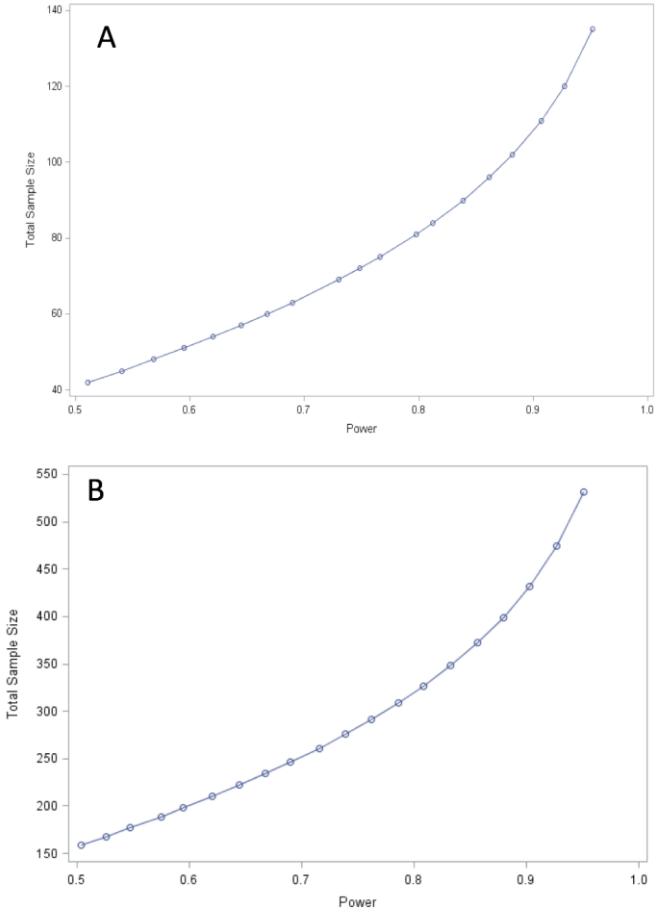


Figure 5 Sample size versus power for LCI. Power calculations are for an absolute difference of A) 2 LCI unit and B) 1 LCI unit between baseline and 1 year, assuming a conservative estimate for standard deviation of 3.

Endpoint	Reference Study	Comments
LCI	Ratjen et al(76), Ratjen et al(56)	Significant improvement in LCI was seen with Ivacaftor with 20 subjects and Orkambi with 206 participants
PRAGMA CT score	Rosenow et al(130)	Sample size estimates in this paper suggested 100 children required to see a 50% reduction in %disease over 3 years and 76 for an approximate halving of bronchiectasis progression over 3 years. This is in very young children with mild disease.
FeNO	Grasemann et al(89)	A significant increase in FeNO was seen with 15 subjects on Ivacaftor
Liver disease parameters	Rowland et al.(40)	Limited relevant data available. 10 year follow up in 84 individuals revealed significant differences in mortality. Unpublished data on 522 individuals, some of whom will be part of RECOVER has again shown significant changes in status over time and mortality.
GI inflammation	Ooi et al (32)	Significant reductions in calprotectin and M2PK seen in 16 individuals on Ivacaftor
Abdominal symptoms	Tabori et al (29)	114 subjects were studied. Significant differences in abdominal symptom scores were seen between individuals with PS and class 4 or 5 CFTR mutations and those with class 2 and PI suggesting that reported symptoms may be relatable to CFTR function in a cohort of this size

CFQ-R	Keating et al (59)	Total recruitment was 123 participants (F508del homozygotes and heterozygotes with differing dose levels. Between group comparisons using 7-21 subjects per group showed significant improvements in CFQ-R compared to controls
Airway Inflammation	Hisert et al (12)	Significant and sustained reductions in sputum inflammatory markers were seen in 12 subjects after the initiation of Ivacaftor
Nasal inflammation	Muller et al(23), McCormick et al(24)	Nasal inflammation in 19 individuals after IV antibiotic therapy showed a trend to improvement. Ivacaftor treatment in 153 individuals was associated with significant improvements in nasal symptom score. Data for nasal inflammation and CFTR modulator use is not available.
Sweat Chloride	Keating et al (59)	Total recruitment was 123 participants (F508del homozygotes and heterozygotes with differing dose levels. Between group comparisons using 7-21 subjects per group showed significant improvements in sweat chloride compared to controls

Table 12 Sample size examples in CF literature pertaining to RECOVER endpoints

Variable	**Expected effect size**	**Mean**	**SD**	**Power (n=166)**	**Power (n=201)**	**Sample size for 90% power**
CT scores	50% relative reduction in %disease at 2 years	2.62	2.59	62.0%	71.8%	325*
FeNO	100% increase in FeNO	8.5	5	>99%	>99%	39
GI inflammation	Reduction in stool Calprotectin by 50% (median [IQR]: 154.4 [102.1–284.2])	NA	NA	>99%	>99%	39
Abdominal scores	Reduction in CF Abd score by 5 points	19.9	5.6	89.2%	94.7%	169
CT scores	reduction by 20 points	71.2	17.3	>99%	>99%	65
Sweat chloride	reduction by 40 mmol/L	99.5	9	>99%	>99%	13
Nasal inflammation	Reduction of IL-8 by one third	1145	656	85%	92%	195
*See detailed discussion of this in Rosenow et al(105) who suggest a sample size requirement of 100 individuals to detect a 50% relative reduction in %disease at 2 years.						

Table 13 Sample size calculations for RECOVER parent study where data is available

Treatment of Missing Data

To prevent loss of information and introducing potential selection biases, missing values may be imputed. Where patterns of missingness are not correlated with outcome variables, multiple imputation will be used under the missing at random assumption. Multiple imputation accounts for uncertainty in the standard errors, confidence intervals and p-values. Complete-case analyses will also be performed, and the results compared to the imputation results as a sensitivity analysis.

Descriptive Statistics

Descriptive statistics will be performed on all measured outcome variables and derived variables. A baseline table will present the demographic and clinical characteristics by treatment group and overall. Continuous variables will be summarized using means and standard deviations. Medians with interquartile ranges will be presented where appropriate. The distributions of continuous variables will be assessed to determine whether variable transformation may be required. Categorical variables will be summarised using counts and percentages.

Data Analysis

Statistical analysis of longitudinal data requires methods that can properly account for the intra-subject correlation of response measurement. Preliminary analysis of treatment group means over the time periods will inform more complex analyses of derived variables such as slopes, pre/post analysis and response trajectory over time. Linear mixed models will include demographic and co-morbidity covariates to adjust for confounders. Random effects will account for correlation among repeated measurements. Data transformation and distributional assumptions will depend on the types of data. A Normal distribution will be assumed for continuous variables. A Poisson distribution and log transformation will be assumed for count data. Categorical data will be analyzed using logistic or multinomial regression where appropriate. Model diagnostics will be applied to assess model fit, distributional assumptions and potential outliers. Where distributional assumptions are not met, non-parametric alternatives will be employed. Where there are multiple outcome measures relating to a specific aim, multiple comparison adjustments may be necessary. False discovery rate (FDR) adjustments will be applied where appropriate.

Biosample Collection, Processing and Storage Plan For Parent Study

Blood

Blood (15ml) will be collected, where possible, during clinically indicated venepuncture. And aliquoted as follows:

- 5ml in EDTA tube for DNA extraction. Sample transferred to the lab and frozen at -80°C for later transfer to Central labs and thereafter to our collaborators in Toronto (FR). This sample will be used for the CF Canada funded genetic modifier study.
- 10ml (or the remainder) will be transferred to a serum tube and transferred to the laboratory for processing. Samples will be centrifuged at 3000rpm and the supernatant (serum) aliquoted to 5 separate 1.5ml Eppendorf tubes. Aliquots will be labelled and frozen at -80°C. These aliquots to be sent to central labs and stored for future use.
- For the year 1 blood collection, 10ml blood will be collected and processed as above, frozen at -80°C, and sent to central labs.

Nasal Lavage

Nasal lavage will be collected as per the SOP described by Dr Jochen Mainz (appendix) with some modifications to reduce background noise in microbiome analyses. Briefly sterile room temperature normal saline will be aspirated with a sterile 10ml syringe and needle. After lavage, nasal lavage samples will be drained into sterile sample collection containers. Once per year per centre, a control sample will be generated whereby saline will be aspirated the same way and collected in a sterile container. Samples will be brought on ice to the laboratory for processing. Where appropriate, at a centre level, 5ml of nasal lavage will be sent to the laboratory for culture. The remainder will be processed as follows:

- 2ml of lavage will be mixed with 2x volumes of RNAlater and aliquoted into 3x2ml RNAse free tubes, labelled and frozen at -80°C. These will be sent to central labs in Dublin.
- 1.5ml samples x 6 will be stored in 1.5ml Eppendorf tubes, labelled and frozen at -80°C. 3x1.5ml samples will be sent to the Belfast Centre for inflammatory mediator measurements. The remainder will be sent to central labs in Dublin and stored for future analysis. An aliquot will be sent to Teagasc in Cork for microbiome analysis.

Sputum

Sputum samples for research purposes will be collected at study visits and immediately processed as per the TETRIS protocol(113). Briefly, sputum will be collected from subjects, either spontaneously or after hypertonic saline induction, and immediately placed on ice for transport to the local processing facility. The two-step temperature-controlled process will be followed according to the protocol, however, once sputum plugs have been homogenized with cold phosphate buffered saline (PBS), and prior to centrifugation, 2ml of this homogenized sputum will be mixed with RNAlater. Processing will occur as follows:

- 2ml of homogenized sputum will be mixed with 2x volumes of RNAlater and aliquoted into 3x2ml RNAse free tubes, labelled and frozen at -80°C

- The sample will be centrifuged as per the TETRIS protocol and 1.5ml supernatant samples x 6 will be stored in 1.5ml Eppendorf tubes, labelled and frozen at -80°C. If there is further supernatant remaining this will be stored in 1.5ml Eppendorf tubes. 3x1.5ml samples will be sent to the Belfast Centre for inflammatory mediator measurements. The remainder will be sent to central labs in Dublin and stored for future analysis. An aliquot will be sent to Teagasc in Cork for microbiome analysis.

Breath Sample

A 1 litre breath sample will be collected on a cohort of 60 patients aged 6-11 at CHI at Crumlin, Temple Street, Tallaght and Guy's and St. Thomas' NHS Foundation Trust. Sample collection equipment such as the intersurgical mask, the carbon filter, the GASTEC air pump and adapter, the Tenax® GR Sorbent tube, plastic bag, T-piecce and Otoscoop tip will be provided by collaborators at Amsterdam University Medical Centre. Briefly, the T-piece will be attached to the anti-viral/bacterial filter. This will be attached to the carbon filter. The correct size of face mask for the patient will be attached to the anti-viral/bacterial filter. The setup is pictured below in image 1.

®GR Sorbent tube is then attached to the sample pump on the grooved side. The remaining nut from the Tenax® tube is removed and attached to the otoscoop tip. The cap of the adaptor is opened and the otoscoop tip is inserted. The GASTEC pump will then be started by pushing the START/STOP button. The pump will start running for about 2 minutes. At the time 500 ml has been sampled, the pump will stop automatically. The Tenax® will be removed from the sampling bag and the adaptor closed. The sorbent tube will be sealed with the nut. The end of the Tenax® tube in the GASTEC pump will then be removed and sealed with the other nut. Tube number and sample date will be noted on a sample log. The capped Tenax® tube will be wrapped in aluminium foil and stored in the refrigerator (around 7 degrees) prior to shipment. They will be transported in a closed plastic bag to AUMC for breath analysis. Breath samples

Stool

A 10-20g stool sample will be collected from study participants at routine study visits. Sample collection equipment will be provided in advance to subjects and parents so that samples can be collected and

refrigerated (if necessary) on the morning of a study visit. Stool samples will be manually divided at the study site. One 5-10g aliquot of stool will be frozen at -80C and sent to our collaborators in Brandenburg in Germany for analysis of inflammatory mediators and elastase. A further aliquot (5g) will be mixed with 2xvolumes of RNAlater and frozen for subsequent analysis of microbiota. The balance will be aliquoted to 1.5ml Eppendorf tubes and frozen for storage and later analysis. All samples other than for inflammatory mediator analysis will be sent to central labs. An aliquot will be sent to Teagasc in Cork for microbiome analysis.

Biosample Collection, Processing and Storage Plan for Extension Study

Blood
Blood (15ml) will be collected during clinically indicated venepuncture and aliquoted as follows:

- 5ml in EDTA tube for DNA extraction. Sample transferred to the lab and frozen at -80°C for later transfer to Central labs for biobanking.
- 10ml (or the remainder) will be transferred to a serum tube and transferred to the laboratory for processing. Samples will be centrifuged at 3000rpm and the supernatant (serum) aliquoted to 5 separate 1.5ml Eppendorf tubes. Aliquots will be labelled and frozen at -80°C. These aliquots to be sent to central labs and biobanked for future use.

Stool
A 10-20g stool sample will be collected from study participants at annual study visits. Sample collection equipment will be provided in advance to subjects and parents so that samples can be collected and refrigerated (if necessary) on the morning of a study visit. Stool samples will be transferred to the lab and frozen at -80°C for later transfer to Central labs for inflammatory mediator and elastase analysis. Remaining sample will be bio-banked for future use.

Sputum
Sputum samples for research purposes will be collected at study visits and immediately processed as per the TETRIS protocol(115). Briefly, sputum will be collected from subjects, either spontaneously or after hypertonic saline induction, and immediately placed on ice for transport to the local processing facility. The two-step temperature-controlled process will be followed according to the protocol, however, once sputum plugs have been homogenized with cold phosphate buffered saline (PBS), and prior to centrifugation, 2ml of this homogenized sputum will be mixed with RNAlater. Processing will occur as follows:

- 2ml of homogenized sputum will be mixed with 2x volumes of RNAlater and aliquoted into 3x2ml RNAse free tubes, labelled and frozen at -80°C
- The sample will be centrifuged as per the TETRIS protocol and 1.5ml supernatant samples x 6 will be stored in 1.5ml Eppendorf tubes, labelled and frozen at -80°C. If there is further supernatant remaining this will be stored in 1.5ml Eppendorf tubes. Samples will be sent to central labs in Dublin and stored for future analysis.

Sample Storage and Transportation
The central storage point for study samples will be at the existing NCRC labs. Sites will send locally processed sputum and nasal samples to Belfast for inflammatory markers and stool to Brandenburg in Germany for inflammatory mediator/elastase analysis for the Parent Study. Balances of samples for storage, as well as all blood samples will be sent to NCRC labs at CHI for the parent study. Blood, sputum and stool samples collected as part of the extension study will be send to the NCRC labs at CHI for storage and analyses.. Samples will be stored locally at -80C and shipped on a schedule to testing and coordinating sites.

International Transport of Human Biological Samples
National legislation on all proposed transfers will be strictly enforced. Partners will only be in a position to receive samples or material further to the signature of Material Transfer Agreement (MTA) and in accordance with EU Directive 2004/23 of the European Parliament and of the Council and The General Data Protection

Regulation (Regulation (EU) 2016/679) (GDPR). The exact usage of the samples will be described in these MTAs and will be reflective of the RECOVER work programme. An inventory of samples will be maintained. The destruction of samples will be documented. The traceability of the samples will be guaranteed by the material transfer agreements (MTA) that will be signed by relevant Partners. In this agreement, the Partner receiving samples will commit to terms and conditions set forth by the sending Partner in order to ensure that the sending Partner remains in compliance with the requirements of their regulatory approval in accordance with EU Directive 2004/23.

Study Visit Timetable

RECOVER	Parent Study												Extension Study				
	Lead In		Year One						Year Two				Year Three	Year Four	Year Five	Year Six	Year Seven
Month/Frequency	-3	0	1	2	3	6	9	12	15	18	21	24	Annually	Annually	Annually	Annually	Annually
Pregnancy Test	X	X															
Eligibility Assessment	X	X															
Sweat Chloride		X				X		X				X	X	X	X	X	X
LCI	X	X				X		X		X		X	X	X	X	X	X
Spirometry-controlled CT (A*)		X						X				X		X[1]		X[1]	
Height/Weight/BMI*	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
FEV1*	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
Airway Sampling (Micro)*	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X
Nasal Lavage (A*)		X				X		X				X	X	X	X	X	X
FeNO	X	X				X		X									
Liver Function Testing*		X			X	X	X	X				X	X	X	X	X	X
Liver Ultrasound		X						X				X	X	X	X	X	X
Sputum Collection (A*)		X				X		X		X		X	X	X	X	X	X
Liver Examination		X						X				X					
Stool Collection		X	X			X						X	X	X	X	X	X
Blood Collection		X						X				X	X	X	X	X	X
Breath Sample Collection		X			X	X	X	X									
Abdominal Symptom Score	X	X	X	X		X		X				X	X	X	X	X	X
CFQ-R		X				X		X				X	X	X	X	X	X
Pharmacy Records MPR								X				X					
Adherence Questionnaire	X	X			X	X	X	X	X	X	X	X					
MEMs Caps Reading (M)								X				X					
Antibiotic Use		X						X				X					
Adverse Event Check			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Mental Health Questionnaire													X	X	X	X	X

* Collected as normal clinical care,

A - Advanced arm testing in RECOVER parent study

[1] Only for patients who performed spirometry-controlled CT in parent study. Scan at years four and six at sites doing routine clinical CT. Scan at year six for sites not performing biennial CT

M - MEMs study only

Table 14. Schedule of assessments. Each visit has a study window. For the RECOVER parent study the baseline visit (0 month visit) will have an 8 week pre commencement on ETI start window. The 1, 2 and 3 month visits have +/- 2 week window. The 6, 9, 12, 15, 18, 21 and 24 month visits have +/- 1 month window. For the RECOVER extension study each annual visit will have a window of +/- 4 month.

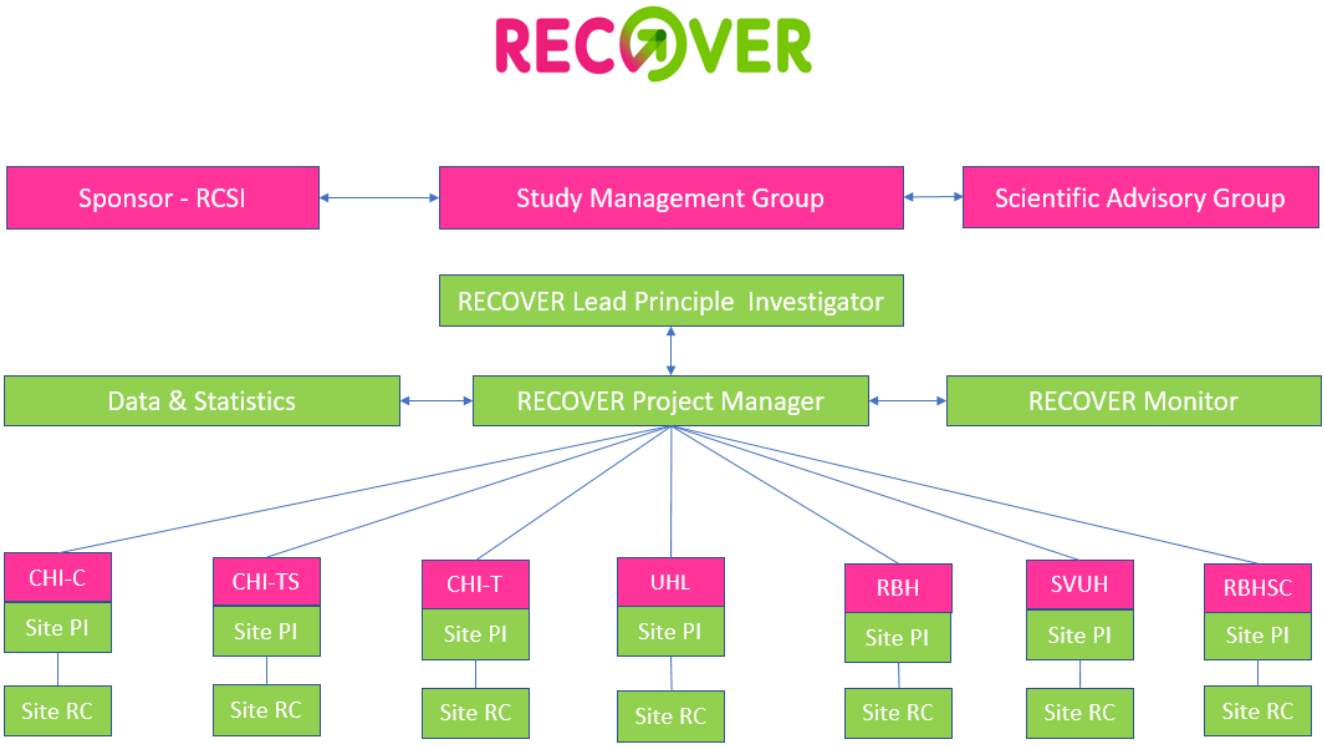


Figure 6 RECOVER study management structure.

RECOVER will be Sponsored by RCSI and centrally operated and coordinated through RCSI/Children's Health Ireland in collaboration with Prof Davies at Imperial College. The Study Management group (SMG) will oversee the progress of the study and ensure that it is operating in such a way as to achieve its aims. The SMG will meet via videoconference on a regular basis prior to and during the study. The SMG will meet 3-monthly until the start of the study and 3-6 monthly as required during the study. Regular information will be disseminated to all members of the SMG during the study by email and via the RECOVER website (for information and training purposes).

The role of the Study Operations Group (SOG) (Table 12) will be to ensure a smooth execution of study operations and provide a platform for feedback and information dissemination between sites, study teams and collaborators. The SOG will meet monthly, or more frequently if required. Face to face meetings and training sessions will be used, particularly at the start of the study, and videoconferencing will be utilized throughout. Site visits by the project manager and monitor will occur throughout the study as required.

The lead PI, in conjunction with the co-PI, will coordinate and manage all aspects of the research program supported by the Study Management Group and the Study Operation Group. The project manager will be responsible for communication within and between the sites, organizing meetings, reporting on technical and non-technical aspects of the project and the coordination of legal, contractual and ethical aspects of the project, as well as overseeing the data management, risk management, and the timely delivery of project and financial reports and obligations. A secure, password protected, Content Management System (CMS) will be used to facilitate the communication and exchange of relevant documents of RECOVER among project partners only. The management activities, based upon the principles of the ISO 10006 European project management standard, will be split into two levels of management activities: 1) operational and 2) scientific management.

Overall Scientific Project Management will be the responsibility of RCSI (Scientific Coordinator), which has substantial experience in leading large scale national and International projects.

RECOVER STUDY MANAGEMENT GROUP for Parent Study		
CHAIR	**COLLABORATORS**	**RECOVER MONITOR**
Paul McNally (PI)	Felix Ratjen, Toronto	TBD
Co-PI	Harm Tiddens, Rotterdam	**CFRI REPRESENTATIVE**
Jane Davies	J Mainz, Brandenburg	Laura Kirwan
PROJECT MANAGER	Marion Rowland, Dublin	**CF IRELAND REPRESENTATIVE**
Karen Lester	H Grasemann, Toronto	Caroline Heffernan
SITE PIs	Alexandra Quittner, Florida	**PARENT REPRESENTATIVE**
Crumlin – Des Cox	Paul Cotter, Cork	Carolyn Thornton
Tallaght – Basil Elnazir	Cliff Taggart, Belfast	**PATIENT REPRESENTATIVE**
Temple St – Mike Williamson	A.H. Maitland - van der Zee	Benat Broderick
Limerick – Barry Linnane		**STUDY SPONSOR**
SVUH Dublin – Ed McKone		RCSI representative
Belfast **– Damien Downey**		
Guy's and St Thomas' – Jane Davies		

Table 15 RECOVER Study Management group for the parent study

RECOVER STUDY OPERATIONS GROUP	
CHAIR	**SITE**
Paul McNally (PI)	Crumlin
Co-PI	Tallaght
Jane Davies	Temple St
PROJECT MANAGER	Limerick
Karen Lester	Belfast
RECOVER MONITOR	Guy's and St Thomas'
TBD	SVUH Dublin
CFRI REPRESENTATIVE	
Laura Kirwan	

Table 16 RECOVER Study Operation group for the parent study

Extension Study Management Group		
CHAIR	**COLLABORATORS**	**STUDY MONITOR**
Paul McNally (PI)	Harm Tiddens, Rotterdam	TBD
Co-PI	J Mainz, Brandenburg	**Data Management**
Jane Davies	Jane Davies, London	Kenny Lynch

PROJECT MANAGER	Paul Cotter, Cork	**Biostatistics**
Rachel Cregan	Katheleen Bennett, Dublin	Aidan Beegan
SITE PIs	Emer Fitzpatrick, Dublin	**CFRI Representative**
CHI Crumlin – Des Cox	Felix Ratjen, Toronto	TBD
CHI TS – Michael Williamson	Anna Georgiopolous, Boston	**CF Ireland Representative**
CHI Tallaght – Basil Elnazir	Sarah Carroll, Dublin	TBD
Limerick – Barry Linnane		**UK CF Trust Representative**
Belfast – Damien Downey		TBD
Guy's and St Thomas' – Jane Davies		**CF community Reps**
		TBD

Table 17. RECOVER Study Management group for the extension study

Study Operations Group	
CHAIR	**SITE RC/RNs**
Paul McNally (Lead PI)	CHI-Crumlin
PROJECT MANAGER	CHI-Tallaght
Rachel Cregan	CHI-Temple Street
STUDY MONITOR	Limerick
TBD	Belfast
Data Representative	Guy's and St Thomas'
Kenny Lynch	SVUH

Table 18. RECOVER Study Operation group for the extension study

The PIs, Project manager and all members of the RCSI research office have experience in the ethics of project management and project management on international projects and will act in such a manner as to ensure the responsible conduct of research and to uphold the highest standards of scientific integrity and ethical conduct during the implementation of the grant agreement. Those involved with all aspects of program management will act in compliance with the RCSI Statement on Research Integrity and the RCSI Policy on Investigation into Allegations of Research Misconduct. This policy adopts the definition of research misconduct produced by the Office of Research Integrity, US Department of Health and Human Services namely "the fabrication, falsification, or plagiarism in proposing, performing or reviewing research or in reporting research results."

Regulatory approval and Research Ethics

Regulatory & REC Approval

The sponsor will ensure that the trial protocol, patient information leaflet, informed consent form and submitted supporting documents have been approved by the appropriate competent authority and a research ethics committee, prior to any patient recruitment. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethical and regulatory approval prior to implementation.

Before the site can enrol patients into the trial, the Principal Investigator or designee must apply to the hospital for permission to conduct the study (if required). It is the responsibility of the Principal Investigator at each site to ensure that all subsequent amendments gain the necessary approval locally. This does not affect the individual clinician's responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

Within 90 days after the end of the trial, the Sponsor will ensure that the main REC and the competent authority is notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.
The CI /sponsor will prepare a clinical trial report, which will then be submitted to the competent authority and main REC within 1 year after the end of the trial.

Compliance with Legislation For Biological Samples
Biological samples will include blood, sputum, stool, nasal lavage samples, and DNA samples (SickKids Toronto). Permissions for the use of samples specific to RECOVER are planned for and will be in place before the start of the research. All necessary measures for the ethical aspects relating to the collection of the samples including informed consent, and in the case of minors from the participant's legally authorised representative, harvesting, collection, storage and ensuring confidentiality of patients will be settled before processing data. The research will comply with international/EU conventions and declarations, including the Declaration of Helsinki (Oct 2003 and in its latest versions), the Council of Europe Convention of Protection of Human Rights and Biomedicine, Clinical Trials Directive 2001/20/EC and Directive 2001/83/EC, and Good Clinical Practice Directive (2005/28/EC).

Collaborators

This study involves multiple collaborations, the details of which are outlined below. Letters of support are included from each collaborator. Detailed collaboration agreements will be developed once funding is in place, and prior to the study start date.

Laura Kirwan (CFRI)

The CFRI's contribution to the RECOVER study will include data collection, data management program support and advanced statistical analysis (subject to budget and contract). CFRI has vast experience of data collection in an environment where a limited amount of data is electronically recorded. Certain data variables for the RECOVER study will be sourced from the CFRI in order to enhance data collection efficiency and quality for RECOVER.

Jane Davies (LCI)

The site at Guy's and St Thomas' NHS Foundation Trust, in addition to being a clinical study site, will perform all training and over-reading for LCI testing throughout the study. This will involve training research coordinators/nurses in performance of LCI, certifying training, and reviewing an initial number of traces to ensure adequate quality prior to certification. During the study, all LCI tests performed at the various sites will be centrally over-read at the RBH site to ensure consistent quality.

Felix Ratjen (Genetic modifiers)

The group at SickKids Toronto has a specific interest in genes that modify the CF phenotype. Prof. Ratjen's team have recently shown that certain modifier genes also affect the response to CFTR directed therapies such as Ivacaftor and lumacaftor. Samples of blood (EDTA) will be collected at each participating site as outlined above and sent directly to the laboratory at SickKids for genotyping. Funding is in place for this as part of a Genome Canada funded project at any time when DNA samples will become available. As a collaborator on this project Prof Ratjen will have access to coded RECOVER outcome data that can be matched to genotype data.

Jochen Mainz (GI symptoms and inflammation, nasal inflammation)

Prof. Dr. Mainz's research group has a specific interest in non-respiratory manifestations of CF, in particular gastrointestinal and nasal complications. They have developed a specific abdominal scoring system for CF called the CFAbd score and demonstrated that this associates well with gut inflammation and ultrasound imaging findings. Furthermore, they have developed a simple procedure for sampling nasal fluid, and have published on its utility in the measurement of infection and inflammation in people with CF. Both of these tools will be utilized, with permission, as part of RECOVER. Prof. Dr. Mainz's research group will carry out testing of inflammatory mediators and elastase on stool samples sent from each participating site.

Harm Tiddens (Imaging)

Prof. Tiddens' group have specific expertise in lung imaging in CF. The LungAnalysis core at Erasmus will support all sites in the RECOVER study in relation to standardization of CT protocol optimisation and image output. The LungAnalysis core will organize for image upload from all sites, collate the transferred images, process them, and carry out PRAGMA and BA-ratio scoring. LungAnalysis will provide central training for the spirometry control, and provide backup during the study for the sponsor, central co-ordinating and local sites to help to maintain a high level of training and quality in terms of CT data collection.

Paul Cotter (Gut and lung microbiome)

Dr Cotter's lab at Teagasc has a large high throughput DNA sequencing platform and an associated bioinformatics platform, which facilitates the in-depth investigation of microbial communities. Dr Cotter's team will carry out DNA extraction, shotgun sequencing and bioinformatic analysis for all stool, sputum and nasal lavage samples in the study.

Hartmut Grasemann (NO metabolism)
Measurement of exhaled nitric oxide will be carried out at a site level by research coordinators. Sputum and nasal lavage samples will be collected locally, and after local processing, an aliquot sent to Prof. Grasemann in Toronto where the team will carry out analysis of Nitric oxide metabolites in sputum and nasal lavage.

Marion Rowland, Billy Bourke, Emer Fitzpatrick (Liver)
Our liver collaborators will assist in identifying and matching participants in their longitudinal study of CFLD in Ireland to the RECOVER participants. They will assist in assigning liver disease status to all RECOVER participants at all-time points. They will assist in data interpretation of liver disease data from this study.

Sinead Weldon/Cliff Taggart (Inflammation)
Dr Weldon and Prof. Taggart will accept nasal and sputum samples on a scheduled basis from central labs in Dublin and carry out inflammatory and innate defense measurements as listed in the study protocol. They will enter the results from these experiments onto the eCRF.

Alexandra Quittner (Quality of life and adherence)
Dr Quittner has extensive expertise in assessment of quality of life and treatment adherence in people with CF. She will share CFQR resources with the study team and allow us to utilize adherence measures designed by her team in RECOVER. She will provide advice and guidance in relation to adherence and quality of life aspects of RECOVER and assist in data interpretation.

A.H. Maitland - van der Zee (Breath sample analysis)
Prof. Maitland van der Zee will assist in the analysis of the breath samples collected in cohort of 6-11 year olds at four of our clinical sites to evaluate the changes in the composition and the function of the respiratory microbiome after the initiation of ETI.

Kathleen Bennett (Biostats)
Prof. Bennett is head of the RCSI data science center and will lead the biostats and database aspects of the RECOVER extension study.

Anna Georgiopulous and Sarah Carroll (Mental Health)
Dr Georgiopolous is a child and adolescent psychiatrist at Massachusets General Hospital and an assistant professor at Harvard medical school. Dr Carroll is a Senior Clinical Psychologist supporting children and families with CF at CHI. They have a particular interest in understanding the psychological needs of PwCF and will lead on the mental health outcomes study of the RECOVER extension.

Data Management

Electronic Case Report Form (eCRF) and Database
A unique Electronic Case Report Form (eCRF) and database has been generated for RECOVER. The software design will allow for electronic collection of study data from multiple study sites in Ireland and the UK, as well as streamlined amalgamation of data from the RECOVER eCRF and CF Registry of Ireland, subject to participant consent. This eCRF has been designed on the same platform as the Irish and European CF registry and a previous widely deployed industry sponsored study (VOICE). This will allow for streamlined amalgamation of data between the registry and the RECOVER eCRF subject to consent. Furthermore, this platform has been demonstrated to be a safe, user friendly, robust and effective tool for CF clinical data collection. Each data field is specifically designed for the piece of data being collected, and in mind of what type of analysis will be carried out on that data.

The most up-to-date encryption software will be employed to protect the study database. Data will be processed in a manner that complies with the General Data Protection Regulation (GDPR) (2018) and the Data Protection Act 2018 in Ireland, to ensure study participant's right to data privacy is safeguarded. CFRI will

provide support in the development of the software, and also in day-to-day data management, where required. The study variable list will be approved by Research Ethics Committees at each study site. Study team members will receive training in best data collection practices and data protection. A data quality assurance plan will be implemented. Patient registry protocols and practices will be adapted for use in this study, given their experience of processing large, longitudinal datasets of CF patient data. Univariate and multivariate data screening methods will be employed to identify data errors and outliers and evaluate the amount and distribution of missing values. We will not automatically discard values identified as possible outliers so as to not bias the outcomes of analysis. If there are significant outliers, we will perform a sensitivity analysis to evaluate the effect of excluding them.

Confidentiality and Data Coding
Participant data will be coded and only indirectly identifiable. Confidentiality of the participants is ensured by using a specific code that does not carry any personal identifier. The link between the codes that could identify the donor, enabling correlation with the data is only accessible to authorised personnel at the collection sites but not to the scientists involved in the RECOVER project or anyone in a receiving hospital, or institution. Data analysis will be performed in a blinded manner. Results of a study may be communicated at scientific meetings and will contribute to the scientific literature. At no time will this be done in such a way that an individual participant may be identified.

Internal Data Sharing:
For network organization, coordination of materials and documentation exchanges and data that is to be shared amongst the consortium (e.g. project results, deliverables, documents, datasets, reports, SOP's, presentations, publications, MTA's, contracts, communicating agendas, minutes of meetings, up-to-date information on the project etc), a secure GDPR and HIPPA compliant cloud platform with end to end encryption will be used (Tresorit) for efficient transfer between partners. Partners will be advised that documents containing data to be shared are saved in an appropriate format, encrypted, uploaded to Tresorit with appropriate nomenclature. This will provide for a data standardization concept to facilitate data queries, transfer and joint analyses. This data sharing platform is secured using restricted login details accessible only to consortium partners, controlled by the RECOVER Management Group. However, this platform will not be used for data storage long-term. Each individual partner is responsible for their own (meta)data collection, storage and security.

Sensitive Personal Genetic data
Participants will be asked to explicitly consent to the collection of their genetic material or data for the purposes of the study. They will be assured that their material/data will be pseudonymised to protect their privacy, and securely stored, with access tightly controlled, to safeguard the confidentiality of their genetic material and data with regard to both family members and others. The link between the codes that could identify the donor, enabling correlation with the data is only accessible to authorised personnel at the collection sites.

However, potential participants will also be advised that by its nature, genetic material is in principle identifiable, even if personal identifiers are not collected or are removed. Layering consent will be used so that individuals can select from a graduated set of consent options concerning the storage and future use of their material or data and be clear on their right to withdraw.

Regulation and Compliance with National, EU and Federal Legislation
RECOVER research will take place in Ireland, Germany, Netherlands, UK and Canada. When the research begins, the GDPR will cover all consortium partner countries except the UK and Canada (see below). Within Europe, the use and transfer of personal or patient data are covered by the EU GDPR, which ensures the protection of individuals with regard to the processing of personal data, including sensitive data, and the exchange of such data. The three countries (Ireland, Germany, Netherlands) have legislated domestic data protection laws to implement the GDPR as below:

Ireland
· The GDPR

· Data Protection Bill 2018 (Data Protection Act) (repealing Data Protection Acts 1988 and 2003)

Germany

· The GDPR
· German Federal Data Protection Act (Bundesdatenschutzgesetz – 'BDSG')

Netherlands

· The GDPR
· The Dutch GDPR Implementation Act (Uitvoeringswet AVG, the Implementation Act)
· Dutch Data Protection Act (Wet bescherming persoonsgegevens)

UK

· When the UK leaves the EU on 31 October 2019, the Data Protection Act 2018 ("DPA"), currently allows for the continued application of the GDPR in UK national law.

Canada

· Privacy Act, Sections 7-8 (1983)
· Personal Information Protection and Electronic Documents Act ('PIPEDA') (2001)
· Personal Information Protection Act ('PIPA BC')
· Personal Information Protection and Identity Theft Prevention Act ('PIPITPA') (not yet in force)
· An Act Respecting the Protection of Personal Information in the Private Sector ('Quebec Privacy Act'), (collectively, 'Canadian Privacy Statutes')

National, European and federal standards and guidelines for ethics and data protection will be rigorously applied. The GDPR covers all collaborator partner countries except the UK (after Brexit) and Canada. Where data is needed to be transferred to a non-EU country, in the normal course of events this should only occur if such a country also has satisfactory legislation *or practices* in place that will ensure data protection adequacy status. The GDPR contains various mechanisms where data can be transferred to third countries subject to contracts/agreements between the transferring and receiving entities involved, provided suitable safeguards are put in place.

The legal basis for processing pseudonymised personal data is that the data subjects will give explicit consent for the specific purposes of RECOVER (GDPR, Art. 9). We will ensure the protection of individuals with regard to their right to privacy and processing of personal data, including sensitive data, and the exchange of such data in compliance with the GDPR. Specifically, the intent with which any data is accessed and used will be lawful, fair and transparent, and for specified explicit and legitimate purposes. Data collection will be limited to what is adequate and relevant to the purpose of the research project (in accordance with the GDPR 'data minimisation' principle). We confirm that appropriate technical and organisational measures are in place to protect against unlawful or unauthorised processing, as well as accidental loss or destruction. To ensure that personal data is protected and that the GDPR is complied with, data controller and data processors responsibility and liability for further processing will be clearly defined, and data security and transparency will be paramount considerations. Data handling standards, procedures, and best practices will be adhered to.

Data Storage Post RECOVER

At the end of the project, data will be stored at the respective collection sites and corresponding institutions. Data will be made available to all partners that contributed to the data generation and data analysis after completion of the program for an indefinite time

Only designated members of the study management group will have user and administrative privileges to view the data. Data will be securely stored for a designated length of time in line with statutory and legal obligations imposed on the data controllers before being deleted or disposed of securely in line with Data Protection guidelines. The RECOVER ethics application will seek permission to retain data for a period of up to 20 years to complete all analysis, publish results, and to seek funding for further follow up.

Monitoring requirement for the trial

The sponsor will assign an independent monitor who will visit the investigator site(s) intermittently to validate compliance of the protocol to the GCP, the maintenance of the study related records, and the extensiveness

and accuracy of a proportion of CRF entries compared to source data. The investigator will co-operate with the monitor to ensure that any potential discrepancies are resolved.

Monitoring procedures include a site initiation visit designed to clarify all prerequisites before the trial commences at the site, interim site monitoring visits and study close-out visits. The study will be monitored by regular scheduled visits to site and on-going communication via telephone and e-mail.

At a minimum, source documentation will be available to substantiate subject identification, eligibility, and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures, adequate reporting and follow-up of AEs, dates of subject completion, discontinuation from treatment, or withdrawal from the study, including the reason if appropriate.

CRF entries will be verified with the source documentation, if applicable (in some cases there are no source pages, therefore verification is not necessary). If any data, signatures, or forms are missing or incorrect, the Investigator or designee will be informed and corrections will be made. Direct access to all source documents must be guaranteed by the PI, who must provide support at all times for these activities.

Insurance

RCSI holds insurance against claims from participants for injury caused by their participation in the clinical trial. Hospitals selected to participate in this clinical trial shall provide malpractice cover for harm caused by negligence on the part of a hospital employee.

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