

## Clinical trials in cystic fibrosis<sup>☆</sup>

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Received 7 February 2007; accepted 7 February 2007

### Abstract

In patients with cystic fibrosis (CF), clinical trials are of paramount importance. Here, the current status of drug development in CF is discussed and future directions highlighted. Methods for pre-clinical testing of drugs with potential activity in CF patients including relevant animal models are described. Study design options for phase II and phase III studies involving CF patients are provided, including required patient numbers, safety issues and surrogate end point parameters for drugs, tested for different disease manifestations. Finally, regulatory issues for licensing new therapies for CF patients are discussed, including new directives of the European Union and the structure of a European clinical trial network for clinical studies involving CF patients is proposed.

**Keywords:** Drug development; Pre-clinical drug testing; Animal models; Surrogate end points; Safety issues; Drug licensing; European clinical trial network for CF

<sup>☆</sup> This document is the result of an European Consensus Conference which took place in Artimino, Tuscany, Italy, in March, 24–26, 2006, involving 58 experts on antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis patients, organized by the European Cystic Fibrosis Society, and sponsored by Bayer Healthcare, Chiron, Corus Pharma Inc., Forest Laboratories UK Ltd, Chiesi Farmaceutici, Dr Falk Pharma, Boehringer-Ingelheim Pharmaceuticals, Inc., AOP Orphan Pharmaceuticals AG, Transave, Inc., Vivometrics and PTC Pharmaceuticals. The purpose of the conference was to develop a consensus document on Clinical Trials in Cystic Fibrosis based on current evidence.

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<sup>2</sup> Acted as an observer. He contributed on a personal basis and his contribution reflects neither the EMEA position nor the position of its Committees.

## 1. Background

Improvements in clinical care have resulted in a dramatic increase in the life expectancy of people with cystic fibrosis (CF) over the last 40 years [1,2]. In several European countries, the median survival is between 30 and 40 years. The aggressive treatment of lung disease and improvements in nutrition are the major factors in this context. However, 95% of people with CF still die from respiratory failure [1,2]. In addition to optimizing existing therapeutic strategies, effective new agents need to be identified. There are currently a number of antimicrobial and anti-inflammatory drugs in clinical trials and several drugs with potential efficacy in pre-clinical studies which address the abnormal pathophysiology of defective CFTR function. The search for small molecules which correct the mutated CFTR ion channel in the respiratory tract and in other organs needs to be intensified through activities in CF research centres and pharmaceutical companies [3]. It is hoped that the number of promising drugs for CF patients will increase in the next 10 years.

The clinical phase of drug development in people with CF, poses increasing challenges for a number of reasons. Although many European CF care centres have excellent infrastructure for this multi-organ disease and provide optimal facilities for clinical trials, the number of CF patients in these centres may be limited. Many patients are already involved in clinical trials or other observational studies. Phase III studies in CF, powered on FEV<sub>1</sub>, require up to 1000 patients for comparison of active drug to placebo. Such numbers may not be easily available, since approximately only 50,000 CF patients are registered in patient databases in Europe and North America. However, novel methods of statistical analysis, for example those using repeated measures of FEV<sub>1</sub> over a 6 month study period, can reduce the sample size required by over 50% [4]. Age and disease status of the patient, as well as the availability of CF centres with the appropriate infrastructure may further reduce the number of patients for a given clinical trial. It is estimated that currently ~30% of all CF patients are potentially available to participate in clinical trials [5]. In addition clinical trials are regulated by the EU directive on clinical trials [6]. This requires all studies involving an investigational medicinal product (IMP) to be registered and undertaken to the standard set out in this directive. Particular responsibilities of the sponsor, principal investigator and care provider concern research governance and appropriate infrastructure to be in place to ensure high standards in the conduct of clinical trials, in particular to protect patient safety. The increasing bureaucratic demands on researchers have been identified as an important barrier to research.

The European Cystic Fibrosis Society (ECFS) therefore wants to build a European Clinical Trials Network (CTN) to offer a structured cooperation between sponsors and CF care centres and facilitate the conduct of clinical trials in Europe in accordance with the EU directive on clinical trials. This

effort is in line with the aims of regulatory agencies such as European Medicines Agency (EMA) to encourage and support the development of drugs for orphan diseases such as CF.

This document describes the results of discussions on various issues, relevant in the context of clinical trials, during an ECFS consensus conference, held on March 24–26, 2006 at Artimino, in Italy. Section 2 is a state-of-the-art-survey of the different clinical manifestations of CF patients for which drugs have been successfully developed and a critical discussion where clinical studies and drug development is needed. Section 3 describes experimental methods and animal models for pre-clinical testing of drugs for CF; Section 4 covers study design options for phase II and III studies in CF. Surrogate end point parameters, patient numbers, Quality of Life (QoL) questionnaires and safety issues of drug testing and reporting in CF are discussed; Section 5 includes regulatory issues for licensing new therapies for CF, particularly details on orphan drugs designation. Finally, Section 6 includes the mission statement and structure of the proposed CTN, its relations to sponsors, to European Clinical Trials Centres (CTCs) and other bodies. The aim of this consensus document is to provide better and more evidence based care of CF patients in Europe and elsewhere through an increased number of well designed clinical trials.

## 2. Current status of drug development for CF

CF is a multi-organ disease. Drug development has mainly been directed at the treatment of pulmonary disease and pancreatic maldigestion and malabsorption. Drugs have been developed in such different areas as anti-inflammatory drugs, protease inhibitors, antibiotics, quorum sensing inhibitors, vaccines, mucolytic agents, ion channel blockers and enhancers and gene replacement. In the treatment of pancreatic insufficiency, the main focus has been on developing more effective pancreatic enzymes. Here, the current status of drug development in CF is briefly discussed and future directions highlighted.

### 2.1. Treatment and prevention of infection

The currently accepted paradigm in the lung pathophysiology of CF is based on the hypothesis that reduced mucociliary clearance — as a consequence of a defective chloride channel-facilitates bacterial lung infection with opportunistic pathogens [7]. These infections become chronic due to a phenotypic switch from non-mucoid to mucoid variants which are resistant to antibiotics and the innate host response [8–10]. Strategies for antibiotic treatment have evolved significantly over the past 20 years [11,12]. There is now better evidence that commonly used treatments for CF are safe and effective, through large, well designed clinical trials and systematic reviews, such as those undertaken by the Cochrane Collaboration.

The value of intravenous antibiotics for pulmonary exacerbations in CF is well established though significant questions remain as to optimum combinations, duration of treatment and frequency of administration [13,14]. Two different regimens for administering intravenous antibiotics in CF patients were compared in the TOPIC study, a randomised controlled trial of once versus three times daily tobramycin. This study, powered for equivalence, found no difference in efficacy between the two regimens but concluded that once daily treatment might be less nephrotoxic in children [15]. The emergence of multiply resistant clones of *P. aeruginosa*, methicillin resistant *S. aureus* (MRSA) and species such as *Burkholderia cepacia* complex organisms present new challenges for antibiotic treatment [16]. Studies investigating particular approaches to such treatments have not demonstrated any advantages of particular regimes or combinations of antibiotics [17]. A Cochrane systematic review has found that there was insufficient evidence to determine whether regular maintenance antibiotic treatment was more effective than treatment “on demand” in maintaining lung function in CF patients [18]. However a clinical trial of patients randomised to regular versus on demand treatment found that both groups received a similar number of courses of treatment, suggesting that there is a convergence in clinical management [13,14]. The investigation of issues of clinical practice such as this may be best addressed using comparisons of management and care delivery from national and international patient registries.

Chronic nebulised antibiotic therapy improves lung function, suppresses bacterial counts and reduces the frequency of pulmonary exacerbations [12,19]. Early aggressive anti-pseudomonal antibiotic treatment is effective in clearing *P. aeruginosa* infection for a number of years [20–27]. However a recent meta-analysis found only three good quality randomised controlled trials in this context [28]. Eradication treatment is routine in many CF centres and clinical trials comparing alternative eradication regimens may be preferable for pragmatic reasons. Significant questions remain as to the most effective combinations of antibiotics to use for eradication. Further multicentre studies on the treatment of multi-resistant organisms are also urgently needed.

New devices are needed to enhance the speed of drug aerosolization, given the large number of different therapeutic interventions a CF patient has to carry out daily. New inhalation devices including more effective nebulisers using mesh technologies, dry powder inhalers and more effective formulations of antibiotics, including lipid incorporation are currently tested to fulfil this purpose. All these new devices and medications need to be tested in studies of CF patients.

Clinical trials using vaccines to prevent *P. aeruginosa* infections have not yet shown convincing results [19]. Ironically, two large phase III studies using a bivalent *P. aeruginosa* flagella vaccine [29] and a polysaccharide-exotoxin A conjugate vaccine [30] have been initiated at a time when “early eradication therapy” was used by many European CF centres which led to a reduction of the incidence rate of

chronic *P. aeruginosa* lung infection in the centres. Consequently, much longer time frames or larger number of patients have to be envisaged when vaccines are tested in the CF population and end points such as “chronic *P. aeruginosa* lung infection” are chosen. Cross-infection between CF patients has been convincingly shown to be significantly reduced when improved hygienic measures and separation regimes have been implemented in CF centres [31,32].

## 2.2. Anti-inflammatory drugs

People with CF suffer from chronic lung inflammation and several endogenous substances involved in inflammation may harm the airway tissues. This prompted a successful clinical trial with high dose prednisolone more than 20 years ago [33]. Because of severe adverse effects with systemic steroids and a proven effect on airway inflammation in asthma, inhaled corticosteroids have been suggested as a viable alternative [34,35]. A recent study has demonstrated no deterioration in lung function or in time to next pulmonary exacerbation after withdrawal of inhaled steroids [36]. In bronchopulmonary aspergillosis (ABPA), oral corticosteroids in conjunction with itraconazole are the treatment of choice at present [37]. This strategy has in part been extrapolated from the treatment of ABPA in people who do not have CF. Larger multicentre studies of ABPA are needed to know if this treatment is justified or if, for example, inhaled steroids would be as beneficial, inducing less adverse effects. Another strategy to avoid adverse effects of corticosteroids is to use non-steroidal anti-inflammatory drugs. Ibuprofen has been successfully used in CF patients [38,39]. The beneficial clinical effect of ibuprofen was particularly observed in mild patients. However, this has not become a well established treatment partly due to gastrointestinal and other side effects and the need to monitor blood concentrations [40].

Serine protease inhibitors, which neutralise mainly neutrophil elastase, an enzyme which is present in airway specimens of CF patients may be of value in reducing inflammation in CF [19,41–43]. The reported beneficial effect of aerosolized  $\alpha_1$ -protease inhibitor derived from a study in 12 CF patients carried out in the year 1991 [41]. At the time of writing, this result has not been confirmed by other investigators. A recent phase II study of transgenic  $\alpha_1$ -protease inhibitor failed to demonstrate any important anti-inflammatory or clinical benefit [44]. A multicentre trial with EPI-hNE4 has been carried out in CF patients, yet the results have not been published to date.

Azithromycin has been demonstrated to improve FEV<sub>1</sub>, QoL and to reduce exacerbations in randomised controlled trials [45–48]. These results are most likely due to anti-inflammatory effects, although this is not confirmed. Several other anti-inflammatory drugs may have beneficial effects in CF patients [40] and need to be further evaluated. Pre-clinical results demonstrated that a metabolically stable lipoxin analog suppressed neutrophilic inflammation, decreased pulmonary bacterial burden and attenuated disease

severity [49]. The leukotriene receptor antagonist montelukast has been studied in two smaller trials, showing reduction of serum eosinophilic cationic protein, but conflicting results on serum IL-8 and clinical outcomes [50,51]. Although potentially beneficial, anti-inflammatory drugs remain a two edged sword in CF where phagocytic effector cells are needed to control airway infection. A recent trial with the leukotriene B4 antagonist BIIL 284, aimed at reducing inflammation resulted in an increased rate of pulmonary exacerbation in patients on active treatment compared to control patients.

### 2.3. Treatment of pancreatic insufficiency, nutrition and liver disease

Pancreatic insufficiency results in a poor nutritional status which worsens the prognosis of CF patients [52,53]. The development of acid resistant microspheres for delivery of pancreatic enzyme preparations has greatly improved the treatment of fat malabsorption, but in many patients a normal absorption, even with proton pump inhibitors, is not achieved [54]. Better pH-independent pancreatic enzyme preparations need to be developed. Additional interventions to improve the nutritional state in CF are also necessary. Interventions may include oral calorie supplements in CF, enteral feeding by either nasogastric or gastrostomy tubes [55] and microelements [52]. Although a recent study found no significant difference between children randomised to supplements and those given nutritional advice [56], new approaches to energy supplementation are needed. Neonatal screening programs detect CF patients within the first few weeks of life and it is particularly important to optimize nutritional strategies at this early stage.

CF related diabetes (CFRD) is a major complication in older patients with exocrine pancreatic insufficiency. The diagnosis is associated with worse survival and a decline in lung function and body weight. Early diagnosis and intervention may lead to improved prognosis, but further studies are required to answer important questions such as the optimum time to start treatment and whether oral hypoglycaemic agents are beneficial prior to starting insulin [57,58].

A further major complication is osteoporosis [59,60]. The cause of osteoporosis is multifactorial, including the effects of chronic inflammation, cumulative use of steroids, lack of exercise, impaired lung function and nutritional deficiencies. Clinical trials are needed to define the optimal treatment strategy for CF related osteoporosis, including the development of guidelines on follow up therapy [61]. Ursodeoxycholic acid (URSO) has been shown to normalise elevated liver enzyme levels in CF liver disease [62]. However, its long-term effect on the evolution of liver disease remains largely unknown. Since liver disease is a life limiting factor in only a few patients, multi-national studies are needed to determine the efficacy of URSO and other potential drugs for liver disease in CF.

### 2.4. Drugs correcting mucus viscosity

Due to the basic defect, water is extensively re-absorbed from the apical side of the respiratory epithelial cells in CF, leading to a highly viscous mucus layer on the respiratory epithelium. In addition, as a consequence of persisting bacterial pathogens, sputum plugs are created by the high numbers of decaying neutrophils in the airway lumen. Sputum obstructs the airways and reduces lung function. To reduce sputum viscosity, recombinant human DNase has given favourable results in several clinical trials [63–65]. It improved pulmonary function and reduced the number of pulmonary exacerbation in CF patients with moderate and mild lung disease. To re-constitute water to the airway surface liquid, hypertonic saline has been successfully used in CF patients, reducing exacerbations and improving lung function [66,67]. Furthermore, UTP analogues may enhance mucosal hydration and mucociliary clearance. A double-blinded phase II inhalation study of INS37217 in patients with mild CF lung disease revealed promising results [68]. Also Moli1901, an activator of the calcium dependent chloride channel, is thought to improve mucociliary clearance and was shown to be safe in CF patients [69,70].

### 2.5. Pharmacological treatment of CFTR

Intracellular production, trafficking or activation of CFTR are possible targets of therapeutic interventions [7,71,72]. A successful example is the aminoglycoside gentamicin which increased the production of CFTR mRNA in CF patients carrying Class I mutations [73,74]. Certain chemical chaperones, such as phenylbutyrate, CPX and glycerol have been shown to increase F508del CFTR folding *in vitro*, and restore CFTR function including chloride transport at the cell surface [75]. However, efficacy with an acceptable safety profile in patients with CF has not yet been demonstrated. The same is true for the flavonoid genistein which interacts directly with the nucleotide binding domain 2 to stabilise the open channel configuration of CFTR [76], and vitamin C which regulates CFTR-mediated chloride secretion in epithelia [77]. High throughput screening technology is currently being used in the search for new compounds that either rescue the cell surface expression of mutant CFTR (termed CFTR correctors) or enhance the activity of mutant chloride channels present at the cell surface (termed CFTR potentiators) [3,78].

### 2.6. Gene replacement therapy

Several clinical trials have been carried out in CF patients to restore CFTR function by transfecting mutant CFTR expressing cells with wild type CFTR [79]. Successful gene transfer has been demonstrated, though effects on CFTR function were modest and temporary. Improved gene transfer agents and plasmids have been developed and clinical trials are likely in the near future [80].

### 3. Pre-clinical testing of drugs for CF

#### 3.1. Which *in vitro* models are useful for drug testing?

Pre-clinical testing of drugs with potential activity in CF patients is an essential step in drug development. This implicates a thorough knowledge of the methods to test the activity of the drug in question [81]. Experimental protocols for human and murine airway cell cultures are available [82–84]. For testing drugs which improve CFTR function (correctors, potentiators), the following cell lines are useful: Fischer rat thyroid (FRT) epithelial cells, stably transfected with human  $\Delta F508$  CFTR + YFP construct [3] or with  $\Delta F508$  CFTR or G551D CFTR and grown as monolayers on filters [85]; NIH-3T3 mouse fibroblasts or C127 mouse mammary epithelial cells, stably transfected with human  $\Delta F508$ -CFTR [3,85,86]; CFBE41o-cells (CF bronchial, homozygous  $\Delta F508$  CFTR), parental, grown on coated permeable filters or collagen-coated plastic [87], or stably lentiviral-transduced with WT or with  $\Delta F508$ -CFTR [88]; BHK cells, stably transfected with human  $\Delta F508$  CFTR [89] or  $\Delta F508$  CFTR-3HA [90], or stably lentiviral-transduced with WT or with  $\Delta F508$ -CFTR [91]; CFT1-C2 $\Delta F508$ ; CFTE29o-(CF tracheo-bronchial); CFNPE14o-(CF nasal polyps); CF-KM4 (serous airway cells); and CF15 or IB3 cells (CF nasal, co-expressing ENaC channels) [71,91,92]. Additionally, different cell models, expressing N-terminally GFP-tagged human  $\Delta F508$  CFTR, have been described [93].

Furthermore, CHO-mouse and human  $\Delta F508$ -CFTR cells are available, air–liquid interface cultures of human tracheal and bronchial epithelial (HBE) cells ( $\pm$ co-cultures with neutrophils) [85,91], native airway epithelial cells from nasal brushings [71], and *ex vivo* muscle-stripped intestinal mucosa [94]. Finally, CF airway tissue has been used in a xenograft model [95,96], and pig and human airway serous glands are available [97,98].

The detection of CFTR in human and murine tissues [99,100] and in cells obtained by nasal brushings [81] has been published as well as techniques for the study of CFTR protein *in vivo* [101] and *in vitro*. CFTR folding assays have been established [102–104]. Antibodies with specificity for CFTR have been evaluated [99]. Drugs which correct CFTR function can be tested using the electrophysiological patch-clamp technique [105], the Ussing chamber [106], fluorescent dyes and radioisotopes [107,108]. For gene replacement therapy, suitable gene transfer agents and expression plasmids have been described [109]. For testing mucolytics, no standard assay is available. *P. aeruginosa* isolates from CF patients are tested using routine susceptibility testing of antibiotics. To mimic the interaction of antibiotics with bacterial biofilms microtiter assays have been developed [17,110].

#### 3.2. Which animal models are useful for drug testing?

Animals which mimic CF disease are an important cornerstone for drug testing in CF [111]. Transgenic mice

carrying *Cftr* mutations have been generated [112–118]. These include homozygous  $\Delta F508$  CF mice in different backgrounds (FVB, C57/Bl/6, Balb-C) [94], *Cftr*-KO mice [80], human WT-CFTR knockin CFTR $^{-/-}$  mice with the FABP intestinal promoter to prevent DIOS (*Cftr*<sup>tm1Unc-TGN<sup>FABPCFTR</sup></sup> mice [119], hG542X knock-in mice [73], *Cftr*<sup>tm1G551D</sup> mice, *Cftr*<sup>tm2Hgu</sup>, carrying the G480C mutation.

Congenetic CFTR-knockout mice develop a lung disease with signs of fibrosis and recruitment of inflammatory cells. However, lung cultures of these congenic CFTR $^{-/-}$  mice showed no growth of pathogenic organisms [120,121]. Although infectious lung disease can be established in murine CF models, either the pathogen has to be repeatedly administered to the animals [115], an extremely high *P. aeruginosa* cfu has to be used [117] or the inoculum has to be embedded in agar or alginate beads and introduced into the lungs by tracheostomy [122].

Another difference of murine CF models as compared to humans concerns drug clearance [123]. Mice are also available which over-express the sodium channel *EnaC* and show CF like lung disease [124]. The described mouse models have not been used until today for gene therapy or pharmacological interventions. This is most probably due to the lack of potent antibodies, specific for mouse CFTR, and other end points for clinical trials. Consequently, alternative animal models of CF in larger species such as the pig and ferrets are currently developed [125]. Given its gross anatomical, histological and physiological similarities to humans, the pig may be particularly useful in this context [126]. Animals models of chronic *P. aeruginosa* lung infection have been developed [122,127]. Animal models for CF-related diabetes/liver disease are missing.

### 4. Study design options for drug development in CF

An essential requirement for good clinical trials in phase I and II is a valid study design which takes into account the specific disease process in CF patients, sufficient patient numbers, appropriate inclusion/exclusion criteria, meaningful and reliable outcome measures, core laboratories to measure specific variables, and appropriate analysis. Furthermore, the design of clinical trials in CF requires a multidisciplinary approach including clinicians, statisticians, pharmacologists, nurses and patients and their representatives. As pharmacokinetics may be different in CF, phase I studies should be undertaken in people with CF before progressing to phase II. The size of clinical trials conducted in CF patients has often not been large enough to be able to answer important questions [128]. The Clinical Trial Network (CTN) (see Section 6) will insure that clinical trials are conducted in the best possible manner, involving a panel of European expert physicians, biostatisticians and scientists. The following chapter describes outcome measures and study designs for clinical trials in CF patients.

#### 4.1. Which end points are appropriate for clinical trials in CF patients?

Studies in patients with CF are particularly challenging because of the progressive nature of the disease and a wide variation in severity and number of organs involved that influences the outcome of drug testing considerably. Confounding factors for which patients may be stratified include their class of mutation, age, gender, bacterial lung infection, lung function, pancreatic function, hepato-biliary disease and nutritional status. The most relevant and robust end point for interventional studies in CF patients is survival. However, apart from studies using historical controls [129], the use of survival as an end point is no longer appropriate as it would take enormous numbers of patients and many years to see an effect. Thus, surrogate end points and biomarkers are needed. This may pose problems since CF is a complicated disease and drugs generally only ameliorate symptoms rather than lead to a radical change in the disease progression. For example, antibiotic therapy regimes will not lead to eradication of *P. aeruginosa* in the chronic infection state. Thus, it remains difficult to choose a meaningful end point for a clinical trial.

In the treatment of lung disease in CF, forced expiratory volume in 1 second (FEV<sub>1</sub>) and the number of exacerbations have been accepted by the regulatory agencies EMEA and FDA based on published clinical trials [11]. Since lung function is maintained at a higher FEV<sub>1</sub> level in an increasing number of CF patients and on average now decreases by less than 1%/year [130] clinical trials will be increasingly restricted to adults if this end point is chosen. Alternative endpoints, measuring lung function which are more sensitive to disease severity and change following treatment are needed.

A reduction of the number of exacerbations during treatment of lung disease is another accepted end point. The frequency of pulmonary exacerbations is also declining with the use of rhDNase, nebulised antibiotics, macrolides and hypertonic saline and this should be considered in sample size calculations. At present, FEV<sub>1</sub>, time to or frequency of pulmonary exacerbations and QoL assessments are considered to be the three most robust clinical endpoints. A number of other surrogate endpoints such as CT scan changes, exercise tolerance, sputum volume/weight and cough frequency may be useful and may lower the numbers of individuals required for particular studies. Biomarkers of disease such as nasal potential difference and measurements of inflammation are more problematic to use as endpoints. There is usually a high variability in these measures and it is unclear how much change in any of these is required to correlate with an improvement in clinical outcomes. Pulmonary exacerbations have been used as both a primary and secondary end point in CF trials and have been the basis of regulatory approval of both drugs and biologics. Exacerbations can be measured as the number over a fixed period or as time to the first event. A major issue is how to

define “exacerbation”. Historically, it has been defined by the use of antibiotics or hospitalisation. However, since this is at the judgment of a treating physician, differences likely occur between sites. Alternatively, strict definitions have been developed to define an exacerbation on clinical criteria. The problem with this approach is that physicians caring for CF patients have in general become more aggressive using early antibiotic therapies to prevent a potential hospitalization. Thus, many patients may not reach specified criteria. A preferable solution is to require the presence of symptoms that have are highly predictive of an exacerbation which permits early therapy for the patient while achieving uniformity of the end point [131].

QoL or patient reported outcome end points are important for gaining marketing authorization and should be included in all phase III trials, although it remains unclear at present, how they should be formulated. Whereas the FDA requests such end points to be included in pivotal trials, the EMEA remains less restrictive. Where possible, CF specific instruments should be used. In international trials it is advisable to stratify by country to control for any cultural differences in reporting QoL [132] (Table 2).

In phase II trials, several surrogate end points and markers of biological activity and physiologic function are of interest. The selection of certain biomarkers depends on the disease manifestation targeted. For instance, a change in proteolytic activity of sputum with anti-protease therapy may represent the primary end point, whereas the clinical efficacy and further biological activity measures are used as secondary end points. The reason for this is that the measurement of the biological activity directly addresses the change made by the intervention, i.e. reduced proteolytic activity after the application of an anti-protease. No biomarkers have been accepted by the authorities. Thus, after a phase of basic research and proper evaluation in CF studies, demonstrating the link between the biological activity and a clinically meaningful outcome, it is advisable to discuss the use of such markers again with the authorities.

Particularly, when markers are determined more than once, invasiveness of the applied technique is of concern. Non-invasive techniques are highly desirable. Inflammatory markers can be assessed in exhaled air or in collected breath condensate. The markers, measured in exhaled breath condensate so far, are all biomarkers of inflammation, thus again surrogate outcomes. The multiple-breath inert gas washout technique can distinguish between CF children and unaffected controls and between children with CF who have pulmonary infection with *P. aeruginosa* and those who do not [133]. However these methods are currently not sufficiently standardized and validated and thus not accepted as important end points. Furthermore, the requirement for specialist equipment may limit their use in multicentre trials. Table 1 provides a list of possible surrogate markers for CF clinical studies.

A problem in conducting clinical trials in CF is that many CF patients are not antibiotic naïve. Prior use of an

Table 1  
Outcome measures in clinical trials of CF patients

Outcome measure	Treatment target	Reference	Phase I	Phase II	Phase III	Phase IV
Aerosol deposition	ID, GT, P, I, MV, T,	[139]	+++	++		
Airway surface liquid height	GT, P, MV	[10]				
Blood glucose						
Body mass index	NL					
Bronchial PD	GT, P, I, MV, T,	[140]	+++	++		
Bronchial brush, bronchial lavage fluids, cells	ID, GT, P, I, MV, T,	[141,142]	+++	++		
Chest CT, MRI, HRCT	ID, GT, P, I, MV, T,	[143,144]		+++	+++?	
Chest radiograph	ID, GT, P, I, MV, T,	[143,144]	++	+++		
Exercise testing	MV, T,	[145]		++		
Exhaled air and breath condensate analysis		[146–148]		++		
Fat/nitrogen absorption	NL					
Frequency of pulmonary exacerbations	V, ID, GT, P, I, MV, T,	[131]		++	+++	
Illness severity score	ID, GT, P, I, MV, T, NL	[149]		++	+++	
Infant and toddler lung function	ID, GT, P, I, MV, T,	[133,150]		+++		
LCl/gas mixing						
Liver enzymes						
Lung function (Spirometry)	V, ID, GT, P, I, MV, T,	[11]	+++	+++	+++	+++
Mucociliary clearance	P, MV, T,	[67]	+++	++		
Nasal brush, nasal wash fluids, cells	ID, GT,P, T,	[151]	+++	+++		
Nasal/intestinal PD	GT, P, T,	[152–54]	+++	+++		
Quality of life <sup>a</sup>	V, ID, P, I, MV, T, NL	[132,155–163]		++	+++	+++
Serum levels of drug	ID, P, I, T, NL	[11]	+++	+++	++	
Serum antibody titers	V,	[164–166]				
Serum markers of inflammation	ID, P, I, T, NL	[167]	+++	+++		
Sputum cells and inflammation markers	ID, GT, P, I, MV, T,	[168]	+++	++		
Sputum volume	ID, GT, P, I, MV, T,		+++	++		
Sputum microbiology	V, ID, GT, P, I, MV, T,	[169]	+++	+++	+++	+++
Stool Fat						
Symptom score	ID, GT, P, I, MV, T, NL	[170]	+++	+++	+++	+++
Weight, height, skin-fold thickness, Arm muscle circumference	V, ID, P, I, MV, T, NL	[11]		+++	+++	+++

Abbreviations: V: vaccine; ID, anti-inflammatory drugs; GT: Gene therapy; P: pharmacological CFTR treatment; I: infection, MV: mucus viscosity; T: Lung transplantation; NL: pancreatic insufficiency, nutrition and liver disease.

<sup>a</sup> See also Table 2.

antibiotic, however, is likely to attenuate changes in FEV<sub>1</sub> or bacterial cell numbers in patients with moderate to extensive lung disease. This presents some problems in equivalence trials which compare current therapy with new drug of a similar class. A further problem concerns routine medication, which is not likely to be changed in CF patients when a new treatment is tested, particularly, when the new therapy is cyclic as for instance the use of TOBI. Thus, in long-term trials of greater than 28 days that compare monotherapy of inhaled antibiotics, routine medication may confound the analysis of lung function change or bacterial numbers. CF is a condition in which the therapeutic burden is considerable and equivalence studies have an important role in evaluating treatments which may have similar efficacy, but where one treatment is more convenient or less toxic than the other [15].

#### 4.2. Which is the optimal study design?

An “optimally designed” trial will probably never occur in reality, since there is always a disconnect between doing as much as possible in a given trial, using a finite patient population, and getting the trial done in as short a span of

time as possible. However, to optimize a trial, one must keep in mind the following:

- (1) The selection criteria must be broad enough to keep enrolment moving but still be stringent enough to obtain the answer to the clinical questions that are being asked. These criteria should also be customized for early versus late stage disease, mutation or age etc.
- (2) Early studies are usually easier to perform with regard to general primary variables such as PK levels or safety. But as the clinical development moves forward, a measurable, relevant end point(s) needs to be defined. Ideally a scoring system is needed that could correlate clinical efficacy and QoL measures.
- (3) The trial design should not be so complicated that it is acceptable only to a minority of eligible patients. For example, a trial that needs serial PFTs and PK blood draws, repeated often during the course of the trial, is not likely to succeed. This sort of trial is best handled with a small number of patients in a specialized setting.
- (4) Since the CF community has a limited number of patients, this limited resource has to be utilized effectively. The number of patients needed to obtain an answer for a

given variable needs to be carefully considered when designing the study. A crossover design works well in that it uses less patients, each patient can act as his/her own control and the data are generally less variable. However, this design can take longer based on the length and number of treatment and washout periods. There is also the risk of a patient having an exacerbation during one of the arms of the study and discontinuing the participation in the trial.

A parallel group design is preferable to the crossover design in CF. The most important argument against crossover designs are carry over effects, seasonal variation, patient inconvenience of multiple visits and the inherent instability of CF lung disease. Time series designs, in which the participants serve as their own control and a concurrent control group is missing, may have additional disadvantages including learning effects, regression to the mean and secular trends.

Randomization of matched pairs is a strategy for balancing baseline confounding variables that requires selecting pairs of subjects who are matched for important factors like age and sex. Simple randomisation may not always be appropriate in studies CF. Stratification of randomisation allows a balance to be maintained for predetermined important factors which may impact disease progression. Factors such as age, gender, lung function or infecting organisms may be used for stratification. Non-inferiority studies are equivalence studies designed to detect that the efficacy of a drug is not inferior to that of another drug in the same class and are usually hoping to find some advantage in secondary endpoints such as less side effects, better acceptability to patients or less development of resistance in the case of antibiotics. Analysis of non-inferiority studies involve the use of a one-sided equivalence test, rather than two-sided. The key to such trials is determining a pre-specified margin of the end point, which determines non-inferiority, the  $\delta$ . This  $\delta$  is sometimes set in the same range as the improvement which would be seen in a placebo controlled trial.

Non-inferiority studies have the advantage that they allow comparison to conventional treatment but have a number of disadvantages. The number of subjects necessary in such trials is usually larger than the number of subjects required for conventional parallel group trials. There is no comparison to placebo and it is important that the difference from the comparator is carefully defined. Such studies are particularly useful where it is unethical to compare treatment against placebo. However, unless the new treatment, for which equivalence or non-inferiority is being sought, offers an advantage in other directions (safer, more convenient to administer) it is doubtful that such a trial would be a good use of limited resources. Naïve patients may be needed for an equivalence trial, as the comparator may have lost efficacy and the new agent may then be judged

equivalent. However, it too may not be as efficacious as the comparator was when used in naïve patients.

- (5) More Ethics Committees and IRBs are requiring that trials have an active control or, at the very least, have the “usual standard of care” plus the study medication. If a placebo controlled trial is mandated, or a medication has to be taken out of the patient’s normal regimen, enrolment could take longer. Balance is needed between what is needed to have a successful trial and what the enrolment rate might be. It should be noted that comparing a new treatment with placebo, and not best available therapy, is answering at great expense and inconvenience a question that clinicians may not be interested in asking.
- (6) Clinical trials in CF sometimes need to compare changes in formulation or a directly competing treatment, for example, new inhaled or alternative inhaled antibiotics. In these situations equivalence trials may be appropriate. These trials may demonstrate bio-equivalence when the same drug is being compared with two different formulations. In such types of studies a pharmacokinetic (PK) approach is used. Clinical equivalence studies are more complicated. A true equivalence study is usually set up as a parallel group RCT, though it can be a crossover design. Shorter term, placebo-controlled trials, allowing establishment of efficacy, followed by longer open label experience for safety evaluation, may be an acceptable compromise.

#### 4.3. How many patients are needed for phase II and phase III studies according to a given study design?

The number of patients needed for a given clinical study depends on the variation of the primary efficacy variable. It is not possible to determine exact numbers for phase II clinical trials. They are likely to require 50–100 patients but will be very dependent on the primary endpoint for any particular trial. For phase III studies, the numbers required for particular studies, will also depend on the primary endpoint. Primary outcome measures such as FEV<sub>1</sub>, time to next pulmonary exacerbation, or QoL measures usually require between 200 and 600 patients. A significant effect on the number of subjects necessary, i.e. a reduction in patient numbers with a longer duration of a trial, comes into play only when the length of a study extends beyond 2 years. The duration of phase I and phase II clinical trials is usually determined by the expected time to see a change in the primary and secondary outcomes. Phase III trials are often longer in duration in order to allow adequate time to evaluate the safety profile of the particular drug. In order to detect a difference between two groups, it is important to know the standard deviation of the primary endpoint variable. Assuming that the standard deviation of the variable, e.g., FEV<sub>1</sub>, is ~15% of predicted and one wants to detect a difference of 10% between the two groups (unpaired *t* test, significance level ( $\alpha$ )=0.05 (two-tailed)), one needs



~35 subjects in each group to conduct the trial with a power of 80%. If one wants to detect a difference of 6%, one needs ~100 subjects in each group. The smaller the difference to be detected, the larger the sample size or a lower power has to be accepted. These calculations can be easily made with simple programs. For equivalence studies it is important to use the appropriate methods for calculating the statistical power of the study and the sample size [134; <http://home.clara.net/sisa/instr.htm>].

#### 4.4. How is safety of new medicines defined and reported in CF?

In clinical trials involving CF patients, patient's clinical status may vary from almost asymptomatic to severely affected. This may pose a problem regarding the reporting of serious adverse events during studies, since under current definitions, every time a patient is hospitalized, it counts as a serious adverse event. Acute respiratory exacerbations are therefore considered in some interventional trials not as serious adverse event, but regarded as an endpoint. People with CF are taking multiple and diverse medications and, combined with progression of disease, may make them prone to develop serious adverse events. People with CF accept a greater degree of risk over benefits, because of the morbidity and high mortality of this condition. It is important to collect and disseminate nominative data on laboratory parameters and adverse event profiles in the CF population. This data will facilitate development of appropriate eligibility criteria for study participation and provide baseline safety data for comparison during administration of therapeutic agents. A database of current clinical trials in CF would be helpful and may provide a way to monitor adverse events. All clinical trials in CF should be registered on a clinical trials website such as for the European Union: <https://eudract.emea.eu.int/eudract/> or North America: [clinicaltrials.gov](http://clinicaltrials.gov).

## 5. Regulatory issues for licensing new therapies for CF

### 5.1. What are the benefits of orphan drugs status and how it is reached?

All clinical trials in Europe must be in line with ICH-GCP standards, according to the European Union Clinical Trials Directives [6]. There may be important other regulatory issues in each member country in relation to research governance within the research institution and submission of clinical trials to independent ethical review boards. Appropriate licensing with each country's medicines and health-care regulatory authority are also important.

After the US "Orphan Drug Act" in 1983, the Japanese "Orphan Drug Legislation" in 1993, the Australian "Orphan Legislation" in 1998, the European Union has set up similar incentives for development and market authorization of drugs for rare diseases, beginning in December 1999 [135,136]. The Regulation on orphan medicinal products is reserved for

Table 2  
Countries of validation of Quality of Life Questionnaires (QoL) and patients' ages for which QoL is used

Country in which QoL has been validated	Patients' ages for which QoL is used (years)	References
7 European Nations	8–16	[156]
UK	16+	[157]
Germany	16+	[158]
France	8–13; 14+	[159]
Netherlands	14+	[160]
USA	7–13	[161]
USA	14+	[162]
Germany	14+	[163]

medicinal products for human use only, but not for medical devices, food or food supplements, or medicinal products for veterinary use. It has been designed for drugs, aimed to treat, prevent or diagnose life-threatening or very serious diseases affecting less than 5 individuals in 10,000 in the Community. It also concerns drugs for which revenues after marketing would not justify the necessary investment for its development. When authorised methods for treatment, prevention or diagnosis exist for a given disease, the applicants should justify that the product provides a clear benefit to patients.

The current systems have encouraged many smaller and medium sized companies and academic institutions to embark on the development of new medicines for CF. The main EU incentives for sponsors for reaching orphan drug designation include (1) 10 years exclusivity from the date of marketing authorisation, (2) protocol assistance from the EMEA, (3) direct access to the centralised procedure, (4) fee reduction for centralised applications, (5) priority access to EU research programs, and (6) national incentives.

Since November 2005 designated orphan medicinal product can be authorised only through the centralised procedure. Sponsors can apply for orphan designation [135] at any stage of drug development, but always before having applied for marketing authorization. However, orphan drugs designation can also be sought for "old", already authorised, drugs for which a new indication is investigated in rare diseases.

Some pre-clinical and/or clinical data to support the rationale of the application are generally required. Designations have been made based exclusively on in vitro data in cases where the data strongly supported the applicants' intention to treat, diagnose or prevent a rare disease. The application for orphan designation should also include details of any existing diagnosis, prevention or treatment methods, e.g., authorised medicinal products, medical devices and other approaches, such as surgical interventions, radiological techniques, diet, physical means. It is important to include a justification as to why existing methods are not satisfactory or why a significant benefit can be reached with the drug in question.

Orphan drug applications are examined by the Committee for Orphan Medicinal Products (COMP) which consists of a chairperson, one member per EU member state, three representatives from patients groups, nominated by the European Commission, and three members, proposed by the EMEA to

the European Commission. On a case per case basis, the COMP appoints experts that contribute to the opinions on designation. The COMP also advises the European Commission on general EU policies, and international co-operation in the field of rare diseases.

This program has been increasingly used for drug development in the context of rare diseases: From 29 applications received by EMEA since April 2000 for obtaining orphan drug designation for CF, 21 were granted. Six applications had been withdrawn and two are still under assessment. For the designated products 13 procedures for protocol assistance have been initiated for 11 products. Unfortunately, the criteria for orphan designation are not internationally harmonized. For instance, in contrast to FDA, EMEA does not offer grants for development of products to treat CF and sponsors do not receive tax credit for clinical research expenses and it would be of great benefit if a similar system would be adopted in Europe. Thus, an application should be made to the European Commission to link the Orphan Disease Programme to the Framework Granting Programme. There is still a significant bottleneck in development of drug treatments for CF.

### 5.2. *What are the European Union directives on clinical trials in adults and children and how does the European Union support those studies?*

Since the symptoms of CF start very early in childhood, clinical studies must involve children. Most CF children are prescribed drugs which either do not have a marketing authorisation for use in children or are used outside the terms of this authorisation [137]. Drugs may not be available in formulations which are suitable for children. This is major a problem, since the evidence for the use of many drugs in children is inadequate. Considerable work has been undertaken in the last 5 years to remedy this situation. A new programme aims to provide incentives for pharmaceutical companies to undertake appropriate research on the efficacy and safety of new and existing medications in children has been adopted [138], and the proposals should become law by 2007. It will automatically become law in member states.

Importantly, a new Paediatric Committee set up. Article 4 of the Council Common Position concerns the composition of the Paediatric Committee. All members of the EU will be invited to nominate two representatives, one of whom will be chosen. In addition, there will be three places for health professionals and three places for patients' representatives. Members will be chosen by the Commission after a call for expressions of interest, likely to be published in Autumn 2007. This committee will have a large number of very important functions:

1. The scientific assessment and agreement of a detailed paediatric investigation plan (below), and monitoring its implementation;
2. The agreement of waivers and deferrals (below), the monitoring of compliance with the plan, and ensuring that *all* data is in the public domain;
3. To provide free scientific advice to those interested in developing medicines for children. The committee will be concerned to foster good studies, and avoid unnecessary ones. Ethics will be dealt with under existing provisions;
4. They will administer a Paediatric Use Marketing Authorisation (PUMA) a separate category for products exclusively designed for use in children;
5. They will monitor adverse effects — and part of any application will have to be a plan for gathering data on these. In particular high risk situations, specific studies may be required to be put in place;
6. There will be a European wide clinical trials registry;
7. The committee will develop an inventory of paediatric needs, which will set the research agenda in the EU;
8. There will be a network of centres doing clinical studies set up within Europe;
9. There will be an increase in the high quality information, available concerning medicines for use in children, both for professionals and families. Properly tested medications will be marked with a logo (the letter 'P' in blue lettering, surrounded by an outline of a star, also in blue), providing transparency about processes.

The paediatric investigation plan is to become an integral part of the introduction of all medicines in the EU. It should be submitted early. It must include details of which sub-populations of children will be studied. It may be appropriate to delay research in children until there is experience in adults, but early dialogue with the Paediatric Committee will be essential. The plan may be deferred or waived, but only with the agreement of the Committee. The Committee will have the power to revoke the waiver, and will be consulted about compliance with the Investigation prior to the granting of market authorization. The Committee will mark with a symbol medication which has been tested in children. Furthermore, companies will be obliged to place the product on the market within two years of approval, and must notify the Committee if they propose to withdraw them.

Waivers would be on the grounds that the medication is likely to be ineffective, unsafe, or irrelevant (the disease does not occur in children); or offers no new benefit to children. Deferral would usually be because it is appropriate to perform the studies in adults first, or paediatric studies will take so long that there would be an unacceptable delay in marketing for adults. The rewards will include a 6 month extension of patent if companies have submitted a paediatric Plan and adhered to it appropriately, even if it transpires that the medication is not suitable for children and a licence is not authorised. Rewards will be withheld if Investigation Plans are flouted. Furthermore, there will be penalties which are as yet unspecified, but will be 'effective, proportionate, and dissuasive'. Those infringing will be named publicly.

A similar system will exist for off-patent medicines: the Paediatric Use Marketing Authorisation (PUMA). For orphan drugs, the granted 10 years period of marketing

exclusivity will be extended to 12 years, when paediatric data is provided. For medicines where these incentives have not led to appropriate research in children, the EU proposes to fund research under the Medicines Investigation for the Children of Europe (MICE) programme.

In addition to statutory incentives, the paediatric research community needs access to a publicly available database of proposed and ongoing clinical trials. The EudraCT database has been established under the EU clinical trials directive [European Parliament and the Council of the European Union 2001] and provides limited information regarding ongoing trials. More detailed information on paediatric trials is provided by DECnet (<http://www.dec-net.org/>).

## 6. The Clinical Trial Network (CTN)

### 6.1. What is the primary purpose and the structure of the CTN and how does it relate to Clinical Trials Centres (CTC), sponsors and regulators?

There is consensus that a European clinical Trial Network (CTN) should be formed. The intention of ECFS is to create a CTN to optimize the development and evaluation of new and approved treatments for CF in clinical studies in Europe. The CTN will give advice on optimal study design, including recruitment of eligible patient groups, statistical methodology and standardization of outcome measures and promote safety of participants in clinical trials involving CF patients. The CTN will consist of an Coordinating Body (CB) and Clinical Trial Centres (CTC)s. The ECFS board in collaboration with appropriate patient organizations will appoint a working group. This working group will define structure and functions of the CB and criteria for accreditation of the CTCs. The established CTN will be governed by a steering committee (SC) which among others includes representatives from the CTCs. The CTCs will conduct clinical trials within CTN by providing appropriate personnel, facilities equipment, materials and services and access to eligible CF populations. The CTN will collaborate with member state CTNs and internationally with other non-European clinical trial organizations such as the CFF TDN. It is the intention of CTN to include interpretive centres and central laboratories, if necessary. CTN will communicate closely and cooperate with the EMEA (EMA).

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