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Speeding up access to new drugs for CF: Draft manuscripts from the ECFS Strategic Planning Task Force* for input from the CF community (2018)

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Purpose and Scope

The last decade has witnessed developments in the CF drug pipeline which are both exciting and unprecedented, bringing with them previously unconsidered challenges. The Task Force group was brought together to consider these challenges and possible strategies to address them. Over the last 12 months, we have discussed internally and gathered views from a broad range of individuals representing patient organisations, clinical and research teams, the pharmaceutical industry and regulatory agencies. We have identified three main areas of focus: i) optimising trial design and delivery for speed and efficiency; ii) drug development for patients with rare CFTR mutations and iii) inequity of access to approved drugs. Draft manuscripts on the first two of these areas are now available for comment and input; we have possibly raised more questions than provided solutions for some of the challenges. We are disseminating these documents at the draft stage through the ECFS website and the Belgrade conference, June 2018. We hope that you will read them, discuss the ideas with patients, families and colleagues in other disease areas, consider your views, and share your valuable thoughts with us through the e-mail addresses after each section. Many thanks for your time and assistance as we move further towards optimal therapies for *all* people with CF.

DOCUMENT 1:

Considerations for clinical trial design and delivery

Current status of clinical trials pipeline

Optimal, effective care for people with cystic fibrosis has evolved over the past 3 decades with trials leading to the introduction of antibiotics, mucoactive and anti-inflammatory drugs which improve important clinical outcomes such as reducing the frequency of pulmonary exacerbations and increasing related measures such as lung function and quality of life. As these therapies are aimed at the *downstream consequences* of CFTR dysfunction in the lungs (infection, mucous hypersecretion, inflammation), the trials were conducted in people with CF with impaired lung function but were *independent of specific genotype* as the efficacy of antibiotics, anti-inflammatories and mucoactive drugs is generic in CF.

In contrast, the focus of many new drugs in the clinical development pipeline is on the *root cause* of CF, CFTR dysfunction. These agents are thus exemplars of genotype-specific, targeted drugs. The underlying biology of particular mutations can be targeted by specific small molecules with activity directly on CFTR. CFTR potentiators such as ivacaftor *restore protein function*; correctors, amplifiers and stabilisers *increase the amount of mutant* CFTR reaching the cell surface; *wild type* CFTR can be *generated* with gene therapy/ repair, nucleotide therapies and read-through agents. There is also increasing interest in alternative, non-CFTR directed approaches for example inhibition of the epithelial sodium channel (ENaC) and stimulation of calcium-mediated chloride secretion.

In the last decade there has been an unprecedented expansion in a) the number of trials being conducted, many of which compete for the same patient groups and b) the proportion of patients receiving CFTR modulators as evolving standards of care; the latter is very region-specific. This progress is extremely encouraging, but raises major challenges for the field. In this and the accompanying/ future articles, we seek to explore strategies to accelerate access to new treatments in the short to medium term future. We have sought input into these documents from patient organisations, regulatory bodies and pharmaceutical companies developing CF drugs in an attempt to provide multiple views and we actively encourage correspondence from interested parties on these matters. We feel the time is right for a focussed look at current challenges from all angles, clinical, economic, societal, ethical, and regulatory and propose strategies to deal with these in a rare disease such as CF. We hope some of the lesson learned and approaches to this complex field will help inform programmes in other diseases.

Efficient trial delivery

44 clinical trials are either open or have been completed in the past 12 months across the European Clinical Trials Network (CTN; https://www.ecfs.eu/ctn). We are aware that a substantial proportion of these trials are conducted in a relative minority of CF centres, efficiency in recruitment is suboptimal (often very large numbers of sites opened to recruit small numbers of patients each) and significant inequality in patient access to trials exists. Furthermore, concurrent conduct of trials of drugs targeting the basic defect and others

treating down stream consequences may lead to a recruitment skew towards the former due to biases amongst clinicians and/ or patients. Symptomatic therapies will continue to be needed by the majority of the CF population for the foreseeable future: CF modulators will not cure CF in people with already damaged lungs and so far there is no compelling data to suggest they are associated with substantial reductions in infection or inflammation. We consider it important therefore that we maintain some balance in the portfolio of trials.

Increasing participant numbers and geographical reach

Over the previous 12 months at the time of writing, 278 new patient had been enrolled into CTN-sanctioned clinical trials. This equates to only 1.6% of the European CF Society (ECFS) Clinical Trials Network (CTN) population and is likely due to several factors: capacity of trial teams, patient awareness, geography, and specific inclusion/ exclusion criteria. The majority of trials, even those which are not CFTR mutation-specific, have a defined range for lung function based on forced expiratory volume in the 1st second (FEV_1) and exclude patients with certain infecting organisms. There are relatively few trials available for younger children and there may be a reluctance on the part of some investigators to 'increase the burden' for their patients by asking them to participate. There is good evidence that participation in clinical research is beneficial for patients, even those on placebo arms. *So how can we increase the number of patients participating in clinical trials* and *use our networks to their full capacity?*

Competition for patients

Encouraging trial data from sponsors with an established track record of drug development in CF has increased patient awareness and is likely to encourage participation in future trials of the similar or new generation drugs. However, in all fields of clinical medicine, advantages exist with a diverse armamentarium, rather than single drug options and it is encouraging that a number of pharmaceutical companies currently have CFTR modulator programmes (https://www.ecfs.eu/ctn/clinical-trials; https://www.cff.org/Research/Developing-New-Treatments/Clinical-Trials/). Supporting newer sponsors and aiding patients' decisions about participation when several options are available is an important role of our networks and trial teams.

Symptomatic therapies: how their development can continue to be supported

Until full restoration of CFTR function is achieved and even then, possibly only when such drugs are initiated in early childhood prior to the development of complications, people with CF will still have a need for symptomatic therapies: anti-infectives, anti-inflammatories and mucolytics. There may be a perception that trials for such drugs are somehow less 'exciting' or transformative, and thus increased recruitment challenges for these programs. Within both the CTN and TDN, drugs targeting CFTR function will receive higher priority scoring, so how can we ensure that these trials continue to receive support and that patients can access them?

Proposed solutions:

- Clinical trials networks should be expanded, and work more to a hub and spoke model allowing free flow of patients between centres. This will require more communication and perhaps challenge operational systems in some areas, particularly when considering cross-border referrals. Additional manpower costs including those for activities at local referring sites should become an expected component of budgets. This referral system happens frequently within the CFF TDN and in the UK Clinical Trials Accelerator Platform (https://www.cysticfibrosis.org.uk/the-work-we-do/clinical-trials-accelerator-platform) and is reimbursed.
- Consideration should be given to allocation of fewer trials to each site, with higher recruitment goals for each. These could be made up of both CFTR modulator and symptomatic therapies, mirroring the current pipeline. Time and resource will be saved both by sponsor/ CRO in set up and by sites themselves. Such a shift would clearly require significant co-operation between sites/ investigators and will inevitably involve some investigators compromising on their personal aspirations. This will also require engagement with patients and national patient organisation. We recognise that there may be some hesitation from trial sponsors or CRO's over reduced control in site selection, for example running trials in countries/ sites with limited experience, based on concerns related to time delays. These concerns may be reduced by sharing of individual site metrics with prospective sponsors, something which has not been done to date but will be considered by the CTN.
- The CTN and TDN have developed standard operating proceedures which are are now implemented for routine activities such as spirometry and more specialist procedures such as nasal potential difference measurement and lung clearance index; the establishment of central training and analysis hubs where appropriate will impact on logistics and optimise data quality.
- Direct engagement between networks and the CF community needs be increased. The majority of CF patients report not having been approached about clinical trials and unless proactive, they may lack awareness of what is available. Clinical networks and country-specific initiatives such as the Clinical Trials Accelerator Platform led by the UK CF Trust with the searchable 'Trials Tracker' (https://www.cysticfibrosis.org.uk/get-involved/trialstracker) provide useful links, but we consider more could be done for example via blogs or live video streams; with increased awareness may well come improved recruitment
- Geographical reach could be widened by the innovative implementation of protocols incorporating virtual data collection. This will clearly only be suitable in certain instances and may meet with sponsor scepticism initially, but is an approach being used in other patient populations (clinicaltrials.gov NCT02921724) and could conceivably incorporate not only clinical monitoring (eg. uploading of spirometry) but also electronic pill pack monitoring, and blood sampling such as those being developed for remote monitoring of patients with diabetes. Opinions of the regulatory agencies will clearly be of paramount importance in the future direction of travel in this regard so it is encouraging that such novel ideas are being discussed on a number of platforms including through the FDA website (https://www.fda.gov/ScienceResearch/SpecialTopics/RegulatoryScience/ucm535768.htm).

Optimal future trial design

Optimising trial design has become a very high priority to ensure efficient conduct of studies to meet regulatory requirements for safety and efficacy with the minimal numbers of patients and in the shortest time period possible. This will allow more trials to be conducted and translate ultimately into more rapid access to the market for more agents. However, as clinical standards of care evolve, this will require innovative alternatives to current protocols and the consideration of alternative outcome measures. We have identified the following major issues for further consideration:

a) *Testing new CFTR modulators* in subjects already receiving such agents clinically

For most patients with class III mutations, and a growing number with residual function mutations, ivacaftor is available and highly effective. However, lung function is not completely normalised with this drug and exacerbations still occur, albeit at a significantly reduced frequency[1]. It remains a realistic possibility that more effective alternatives will become available, but the ethical and practical implications of testing them pose a challenge. Patients are unlikely to agree to be randomised into a trial including a placebo arm of any substantial duration[2]. A washout period may be acceptable and a short duration may be sufficient to recalibrate sweat chloride, but recalibrating FEV_1 may take longer and carry adverse clinical consequences. Head-to-head comparisons of drugs from different sponsors are unlikely to be feasible, particularly if (as seems likely) the onus falls upon the newer company to purchase and pay for blinding of the prior product. Variable access in different health care regions further complicates any comparisons. Currently, the only globally licensed CFTR modulator for homozygous F508del patients is lumacaftor/ ivacaftor (Orkambi); it is not tolerated by all subjects and the acute efficacy is somewhat modest[3], meaning that patients may be willing to come off drug to trial a potentially 'better' option. However, we are likely to find that this increasingly difficult; during the preparation of this article the field has already evolved with the FDA approved tezacaftor/ ivacaftor (Symdeko). This combination appears to be better tolerated[4] and once established, patient willingness to stop taking the drug may be a limitation in future trials. We urgently need to consider implications of this in designing sufficiently powered non-inferiority or superiority trials and the incorporation of adaptive designs to facillitate the study of new CFTR modulators.

b) *Building on efficacy* of an existing CFTR modulator with 'add-on' molecules

The optimal approach for the majority of CF patients will be a combination of drugs targeting different aspects of CFTR dysfunction in addition to current symptomatic therapies. The results of early phase clinical trials using triple combinations suggest that 3 drugs will be required for optimal benefit for homozygote and heterozyote Phe508del patients[5]. Some companies are developing molecules which may most useful be applied as 'add-on' therapies to an existing modulator, for example CFTR amplifiers, stabilisers or ENaC inhibitors. Similarly, the potential of genetic-based therapies could be maximised by concomitant use of CFTR modulators. Translating these theoretical benefits into reality will, however, be a hugely complex undertaking. Competing drug

companies are unlikely to work together. In the above scenario, the sponsor of the drug B trial may be unwilling or unable to fund drug A; this will almost certainly be the case if drug A is not one of their own as costs/ access may be absolutely prohibitive. Secondly, assessing safety of a new agent which is being built upon a foundation of another drug with only limited patient-years of use raises issues which have not concerned us in the past. There will be complex issues of dose and timing of multiple drugs, assessment and optimisation of PK/ PD and the potential for drug /drug interactions both within multiple-component drugs and with other concomitantly administered therapies. We are struggling to provide solutions to this internally and propose calling upon the experience and skills of those working in other disease areas, such as cancer, where this approach is more established and from whom we may best learn.

c) Alternative outcome measures: why should we consider them?

In clinical trials, 'direct' endpoints are clinically meaningful measures of how a patient feels, functions or survives. They characterize the clinical outcome of interest: survival, disease exacerbation, symptom score, 'health related quality of life'. A surrogate endpoint such as FEV_1 is a measure of effect of a treatment that may correlate with a direct clinical endpoint and are used when the primary endpoint is undesired (eg. death), or when the number of events is very small, thus making it impractical to conduct a clinical trial to gather a statistically significant number of endpoints. The commonest of these employed in CF is the pulmonary function measure, FEV_1 which, measured by spirometry, has been acceptable as an end point in support of registration of chronic CF respiratory therapies to regulatory agencies. Over the last two of decades, almost all pivotal CF studies have been required to use FEV_1 by regulatory agencies for approval of therapies for routine clinical care. Widespread implementation of these therapies has resulted in significantly improved outcomes for patients with CF with reduction in FEV_1 decline and better survival. As a result, more patients maintain FEV_1 in the normal range, which has an impact on the size of an eligible population, most commonly between 40-90% of predicted values.

In most of the clinical trials of CFTR modulators, the increase in FEV_1 is observed in the first weeks of the study. Consequently, the proposed duration of a trial will not influence sample size calculations for studies employing FEV_1 difference end points. Of increasing interest are pulmonary exacerbations (PEx) which are associated with lung function decline and contribute to significant morbidity and mortality. Differences in relative risk of exacerbation or median time to next exacerbation have been employed as key secondary clinical end points for clinical trials of a variety of chronic CF therapies. Studies employing PEx end points need to be longer and require more subjects than sustained FEV_1 difference end points, but proactive enrichment for subjects with exacerbation risk factors may reduce sample size requirements. The FDA has recently agreed to PEx rather than FEV_1 as the primary endpoint in a phase II program for an anti-inflammatory agent (clinicaltrials.gov NCT03451045). PEx is also being used increasingly as an outcome measure for interventional trials in CF lung disease in relatively healthy patients and infants and young children who have normal or minimally reduced pulmonary function. However, the relative infrequency of exacerbation in very young children with CF precludes its use as an end point in infant studies due to sample size requirements. Furthermore, although there is a consensus that PEx represents an important outcome in CF clinical trials, the definition used in most

studies has been a broad one of new or increased pulmonary and systemic symptoms, reduced FEV_1 and commencement of new antibiotic treatment. Large multicenter clinical trials that have been conducted over the past years have used some variations of physician-derived definitions. Due to the crucial importance of PEx, it is very important to have standardized, validated criteria for diagnosis and treatment for both clinical practice and in clinical trials. In longer term studies, many of which are post-marketing, phase 4, rate of lung function decline is a focus. This is highly relevant, as it relates to survival, but many such studies have employed suboptimal designs, for example using an observational or registry group as controls, which have led to some concern over interpretation.

Other outcomes include patient-reported measures of quality of life such as the CFQ-R and for systemic treatments, weight gain and change in BMI. These will not be covered in further detail here, where we have focussed on the adoption of newer end-points with a view either to a) improving efficiency of trial delivery or b) allowing assessment of efficacy in a group for whom FEV_1 is not useful.

Elevated chloride levels in sweat are characteristic of CF. In populations, correlations between disease severity, degree of CFTR dysfunction and level of sweat chloride has been shown. It is therefore expected that treatment with CFTR modulators which improve CFTR function will decrease sweat chloride levels. In the ivacaftor phase 3 trials[1, 6, 7] a marked and sustained decrease in sweat chloride by approximately 50% was demonstrated indicating improved CFTR function, which, in turn, *should* result in improved pulmonary function. However, on an individual patient basis, there was little to no correlation between the decrease in sweat chloride levels and FEV_1 improvements with ivacaftor, nor did there appear to be a threshold level for change in sweat chloride above which an improvement in FEV_1 could be observed. Therefore, for the ivacaftor program, change in sweat chloride, while of interest, was not predictive of an improvement in lung function in individual patients with CF, as determined by FEV_1. We consider that more evaluation of the reasons for this apparent discordance in some individuals is required. This may be partly related to the day to day variation in both measures within individual subjects[8]; individuals experiencing respiratory exacerbations on the day of study visits; and possibly under-reported deficient concordance on the day of the sweat tests. This may also relate to organ-specific differences in responsiveness to a drug or to the contribution to the measurable abnormality made by CFTR dysfunction. Whereas in the sweat gland, raised sweat Cl^- is completely attributable to lack of CFTR, in the lung, decreased lung function is substantially contributed to by downstream consequences of this ion channel abnormality, infection and inflammation.

The nasal potential difference (NPD) test measures the transepithelial potential difference, another marker of CFTR function. It has been used as a proof of concept in phase II clinical studies of CFTR modulators to demonstrate CFTR activation. Again, in the ivacaftor phase 3 trials there was little to no correlation between improved NPD levels and decrease in chloride levels in sweat tests or improvement in FEV_1 in patients treated with ivacaftor. In the VX-809 clinical trial, there was a smaller, dose-dependent decrease in sweat chloride with no change in NPD[9]. So far, a close relationship between NPD and pulmonary function has not been demonstrated. NPD measurement is prone to variability, is time-consuming and in our opinion is only suitable as a marker of CFTR function in proof of concept phase I or II studies; it cannot be a surrogate marker of

severity of lung disease or survival of patients with CF. Likewise intestinal current measurement (ICM) is a surrogate electrophysiological measurement of CFTR function in the intestinal cells. No correlation was shown with sweat chloride levels or pulmonary function or other clinical parameters of severity in CF and therefore like NPD, it can mainly be used in phase-I or -II clinical trials when proof-of-concept for a specific compound is explored.

Lung clearance index (LCI), a sensitive measure of gas mixing inhomogeneity, is abnormal early in the life of a CF patient before FEV_1 falls; measured by multibreath washout (MBW) and based on tidal breathing, it can also be performed by very young children who are unable to perform forced expiratory maneuvers. As a clinical trial outcome, LCI has been particularly useful in adolescents and adults with well-preserved FEV_1 ; in a population with FEV_1 >90% predicted, ivacaftor led to a substantial improvement in LCI[10]. Although changes could also be measured in FEV1, post-hoc analysis demonstrated that ~1/4 the number of patients would be needed to power a study with LCI as a primary outcome, than with FEV_1. Thus, LCI could be considered in groups with well-preserved lung function as a means to improve efficiency of trial delivery. LCI also demonstrated improvements in a trial of Orkambi in 6-11 year olds[12] and is in use in even younger children (clinicaltrials.gov NCT02725567). Standardization, training and certification processes have taken place globally, enabling this procedure to be utilized in a uniform manner in multi-centre trials, although further data, including correlations with long term outcomes are likely to be required before the technique is an accepted primary outcome. Similar standardization efforts are being made globally in lung imaging techniques, in particular CT scans, which may also be most useful in early disease.

Determining 'sufficient improvement' for adoption of a new drug into SoC

The 10% absolute improvement in FEV_1 in class III mutation groups receiving ivacaftor[1, 6, 7] was regarded by most as highly clinically relevant. In contrast, opinion is less consistent on the clinical importance of ~3% improvement with the dual combinations of lumacaftor/ivacaftor[3] and tezacaftor/ivacaftor[4]. Despite being licensed in Europe, the former is not reimbursed in many areas. For example in the in UK, reimbursement was declined specifically on the basis of inadequate 'cost-effectiveness'. While there are well developed methods to determine cost effectiveness, these are blunt tools and not fit for purpose in rare disease. For example, a drug which did not improve FEV_1 acutely, but reduced its rate of decline and pulmonary exacerbations, could be of great value if introduced at an early stage in the disease and perhaps more effective than a drug leading to large, acute FEV_1 improvement but lacking a disease modifying effect. These are deliberately binary examples for the purposes of illustration. Restoration of CFTR function by ivacaftor in appropriate mutations leads both to acute improvements in lung function and longer terms benefits including fewer exacerbations and a slowing in the slope of decline of FEV_1[13]. However, improvement in FEV_1 is not predictive of a reduction in pulmonary exacerbations and it is intriguing that the latter and longer term benefits of Orkambi[14] are similar to those of ivacaftor despite the more impressive acute benefits of the latter. In addition, substantially more data (subjects and duration) are needed to demonstrate the benefits on slope of decline, making powering of such studies difficult. In patients with early stage disease, FEV_1 may be well-preserved and any improvement

difficult to show. As discussed above, more sensitive measures of lung function for example using MBW are a potential solution to outcomes measures in this group of patients; the minimal clinically important difference (MCID) is yet to be determined, and may be disease-stage specific. The benefits on nutritional and metabolic measures and patient reported outcomes are also considered important by regulatory agencies. Formal approval of alternative outcome measures may be possible in the future, but in our opinion, the onus is on us as clinical researchers to create opportunities to generate compile high quality datasets with which to convince the regulatory agencies of their utility. Even when/ if we do, we will need to communicate well with payers and clinical prescribers to convey the clinical implications of any change.

Testing CFTR modulators in *extremely rare mutations*

The use of *ex-vivo* predictive models for extremely rare mutations is the focus of the subsequent paper in this series. The recent FDA approval of ivacaftor for an expanded list of residual function mutations based purely on non-clinical data (https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm559212.htm) came as a surprise to many, but is one pragmatic solution to this issue. So-called n of 1 trials[15], where a patient undergoes a strict protocol of on-off drug cycles with sensitive outcomes, is also feasible, but clearly more cumbersome and expensive. Short trials, building on safety and efficacy in larger populations with other mutations, could be conducted with the use of a CFTR biomarker, most likely sweat chloride, although the lack of correlation with clinically-meaningful outcomes in trials must be borne in mind.

We have highlighted major issues in the current clinical trial arena alongside several proposals to challenge the status quo that we consider will accelerate the development of, and access to, new drugs for CF. Our intention herewith is to stimulate multidisciplinary discussion and generate ideas to be incorporated in a formal proposal document; we seek to provide not only an illustration of our challenges but a also clear path forward into the medium term future. Ultimately, we seek to maximise benefit to as many patients and in the shortest time frame possible.

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DOCUMENT 2

Ex-vivo prediction and testing of CFTR modulators: the current status complemented by a strategic plan

Introduction

Today's great challenge is monitoring and treating patients more precisely and effectively in ways that better meet their individual needs, i.e. introducing 'personalized' or 'precision' medicine into clinical practice [1,2].

As a paradigmatic rare disease, it is not surprising that CF leads the way in this field. More than 2,000 different *CFTR* variants have been reported so far [3] and of these only 374 variants have at present established CF disease liability [4]. The *CFTR* mutation F508del is by far the most frequent, with 85% of patients worldwide carrying F508del on at least one of their 2 *CFTR* alleles. By comparison, all other *CFTR* variants or mutations are rare to extremely rare. The European Cystic Fibrosis Society Patient Registry (ECFSPR), reports on 35,500 subjects with CF. In this data more than 1,300 different *CFTR* variants are represented, but only 5 have a prevalence above 1% of *CFTR* alleles [5]. About 1000 *CFTR* variants occur in <5 people in the whole world. It is thus a huge undertaking to find adequate mutation-specific therapies for all patients with CF. The vast mutation diversity indeed comes with the near impossibility of establishing classical drug development programs for very low patient numbers and even unique patients.

The alternative lies in *ex vivo* prediction of response to CFTR modulator drugs. With the pipeline of CFTR modulators continuously expanding [6], *ex-vivo* prediction as a starting point is a more feasible approach than testing every CFTR modulator drug for clinical benefit in an N-of-1 trial [7]. The prerequisite is of course the availability of a robust and standardized pre-clinical test that reliably predicts the clinical response to different drugs [8]. Importantly, and once we have a toolkit of drugs targeting the same CFTR defect (e.g. F508del), such an *ex vivo* predictive test can also be used to select the best drug for each individual patient, in a personalized therapy approach. Indeed, the marked variability in clinical benefit in phase 3 trials with CFTR modulators in patients with at least one G551D mutation [9] or in patients homozygous for F508del [10] indicates that drug responsiveness depends not only on the *CFTR* genotype but also on the genetic background of the individual and likely as well on multiple environmental factors. Large inter-patient differences have been documented for improvements in both surrogate outcomes like FEV_1 and also in CFTR biomarkers like the sweat chloride concentration [10]. Just as *CFTR* mutations alone cannot fully predict the clinical phenotype and disease prognosis [11], it is not surprising that an individual's response to CFTR-modulators does not solely depend on his/her *CFTR* genotype but also on a large number of other modifier genes and biological pathways [12]. Once we accept that every person with CF is unique, we need to move to the personalized assessment of the most adequate therapy for every individual. This requires using new tools that pre-assess the effectiveness of drugs *ex vivo* so as to predict the best (combination of) medicines *in vivo*. Classifying mutations and patients

according to 'theratypes' thus introduces '*theranostics*'[1] (ie., employing diagnostic tests to select targeted therapy) into the CF field [13, 14].

In this paper, we discuss the current work on *ex vivo* predictors of response to CFTR modulators, including the available clinical evidence from *ex vivo* and *in vivo* correlations. Furthermore, we define a strategy with which we can speed up the use of these new techniques so as to bring treatments to patients with rare *CFTR* mutations, as well as- in the future- bring the best possible treatment combination to individuals with more common *CFTR* mutations. By preselecting the most suitable candidate drugs, the field of theranostics can lead to higher efficiency via targeted clinical trials. Especially in patients with rare mutations, theranostics will hopefully be used as evidence for early access to treatment via 'Medicines Adaptive Pathways for Patients (MAPPs)' the initiative piloted by the European Medicines Agency (EMA) [15].

The recent FDA approval for label extension of Kalydeco to patients with 23 different residual function mutations based primarily on laboratory evidence of efficacy, in a non-human cell line (Fisher Rat Thyroid, or FRT cells) has been a game changer and demonstrates the potential and importance of *ex vivo* efficacy assessment.

Models to measure efficacy of CFTR modulators reliably *ex vivo* in a personalized way

Bronchial cells

Well-differentiated primary human bronchial epithelial (HBE) cell cultures are considered the gold standard to validate pathophysiology pathways in CF research and have proven very successful for drug development as a preclinical validation of CFTR modulator efficacy [16, 17]. However, although HBE cells can be accessed from explanted lungs in large amounts, obtaining them through bronchoscopy is invasive and the number of cells obtained for culture is limited.

Nasal cells

Human nasal epithelial (HNE) cells, freshly obtained via nasal brushings, have recently become an alternative on which to perform measurements of ion transport [18]. A major technical breakthrough that boosted the use of HNE cells, consisted in their expansion as conditionally reprogrammed cultures (CRC) in a "stem cell-like" state in which they can be expanded *in vitro*, and then be differentiated into primary (polarized) epithelial cultures. [19, 20] The application of HNE-CRCs to pre-assess the effect of CFTR modulator drugs *ex vivo* in a given individual, comprises bioelectric approaches, e.g. in Ussing chamber [21]. HNEs can also be cultured into nasospheroids for use in multiple assays, including electrolyte and fluid transport, ciliary motility, epithelial polarity, cellular metabolism, and drug cytotoxicity [22].

In a random choice of patients with residual CFTR function, the benefit from ivacaftor was explored in N-of-1 trials. In a subset of 7 subjects, the effect of ivacaftor was tested both *in vivo* and also *ex vivo* on HNE's: an

[1] Theranostics is the molecular/functional diagnostic and therapeutic test with the goal of individualizing treatment by targeting a patient's specific disease subtype/ genetic/functional profile.

increase in ion flux after application of ivacaftor on HNE's was only seen in the 3 patients who had responded *in vivo* with a drop in sweat chloride in the clinical trial [23]. At present, additional data are accumulating in labs across the world further exploring *ex vivo* responses on HNE's. These findings indicate that CFTR-mediated chloride secretion can indeed be measured in monolayers of HNE cells, similarly to studies in HBEs.

The challenges of working with HNEs are however significant, including: the need for significant expertise and time to realize successful HNE expansion and the subsequent air-liquid interface (ALI) differentiation phases. Moreover, the existing multiple culture conditions, lead to heterogeneous data accumulation making it hard to determine the best consensus protocol.

Intestinal organoids

In contrast to nasal cells, intestinal organoids [24, 25] have already a good track record of demonstrating their robustness to predict drug efficacy in individuals with CF [26], reviewed in [27].

A major advantage is the relative ease and robustness of culturing intestinal organoids compared to primary HNE cell cultures associated with the much higher expression levels of CFTR. Another significant advantage of intestinal organoids is that they can be biobanked as living cellular systems with indefinite capacity and high reproducibility [28-30]. They can later be thawed and expanded again for testing of responsiveness to new CFTR modulators in the pipeline. This avoids the need for repetitive biopsies thus avoiding patient further discomfort.

With intestinal organoids, 2-3 weeks after culture and expansion, a significant amount of data can be generated to accurately determine the best CFTR modulator response in individual patient samples [31]. The main readout to quantify the CFTR function and how it can be rescued by CFTR modulators is the forskolin induced swelling (FIS) assay. Some authors report even shorter culture time before analysis, at least for enterospheres derived from mice [32].

Validation of CFTR function measurements in organoids is indeed already well advanced. Results in intestinal organoids have been correlated with Intestinal Current Measurements (ICM) and sweat chloride concentration in the same patient [25, 26]. Mean lung function improvements observed in the clinical trials with CFTR modulators in patients with different genotypes, also correlated well with mean FIS response to CFTR modulators in organoids from subjects with these same genotypes [26, 33]. Even more so, in 2 subjects with the rare G1149R mutation a large response to ivacaftor in their organoids led to an N-of-1 clinical trial in these same subjects. The subsequent positive clinical benefit formed the basis of drug reimbursement for these subjects [26]. Conversely, there was no FIS response to ivacaftor in organoids from subjects with the G970R mutation [34], and concordantly no decrease in sweat chloride during ivacaftor treatment was seen in subjects with this mutation. These patients had been included in a clinical trial that grouped patients with rare mutations [35] presumed to belong to mutation class III based on observations in CFTR-cDNA transfected FRT cells [36]. However, the defect caused by the G970R mutation is not an amino acid change but rather alternative splicing, which was not recapitulated in the cDNA/FRT system (without introns), in contrast to the patient-derived organoids.

Additional trials are in progress, namely involving the A455E [26] and 3948+10 kb mutations [37]. By testing efficacy in the patient's own tissue, organoid testing not only integrates the effects on the two *CFTR* mutations present, but also recapitulates any effects that modifier genes may have on enhancing/ precluding the *in vivo* benefit.

So far, it has been proven that for common mutations, the magnitude of the mean response in organoids corresponds to the magnitude of the mean clinical benefit. In organoids from subjects with rare *CFTR* mutations the response or absence of response to CFTR modulators predicts the presence (or absence) of clinical benefit. In patients with common mutations like F508del there is a marked inter-patient variability in FIS response to CFTR modulators which is a consistent and thus patient-specific response [38]. To what degree these variations among individuals with the same genotype also correspond to the *in vivo* differences in lung function or sweat chloride, still needs to be further established.

Next steps to optimize use of *ex vivo* biomarkers to predict responsiveness to CFTR modulators

To advance in the widespread use of these novel biomarkers, strict standard operating procedures (SOPs) are needed: from how to obtain patient samples all the way through to determining sample quality, growth conditions, test procedures and data analysis. Using these SOP's, proof of test repeatability (multiple samples from the same patient as well as repeated measurements on banked samples) and reproducibility between labs must be given. In the context of personalized medicine, intra-patient repeatability is more important than differences in inter-patient measurements.

Ideally, convergent validity and discriminate validity must be assessed: convergent validity by proving a correlation between the read-out at baseline and sweat chloride concentration, nasal potential difference (NPD) read-out, ICM read-out; discriminate validity by comparing the baseline values among patients with known differences in baseline CFTR function (e.g. pancreatic sufficient - PS - versus insufficient - PI - patients; patients with and without residual CFTR function). However, when biomarkers are used in the context of personalized medicine, their predictive validity is the most important aspect. For organoids, the database of concordance between *ex vivo* drug response and clinical benefit from treatment in the same patient must be further expanded; for HNE's such data need to be collected. The magnitude of change that constitutes a significant *ex vivo* drug response (i.e, threshold benefit) must also be established. With more CFTR modulators entering the clinic, the time is optimal to prove this relationship. These data must be compiled using both standardized *ex vivo* biomarker assessment and standardized *in vivo* drug evaluation.

Another key aspect to be discussed is: how often must a close correlation between an *ex vivo* test and the *in vivo* drug response be shown before the test can be seen as a 'stand-alone' valid predictor of treatment benefit? This discussion must of course involve the regulatory authorities. To advance this plan, a strong alliance will be needed among cell biologists, clinician researchers, patients and patient organizations, regulators and also pharmaceutical industry.

What type of clinical trial is appropriate to further validate *ex vivo* biomarkers in patients with rare and common *CFTR* mutations?

Some may argue that drug responses observed *ex vivo* can never substitute evidence obtained from *in vivo* studies, i.e. 'real life' benefit. However, previous classical clinical trials with CFTR modulators in patients with more common mutations had as starting point evidence from effects of drugs in HBE's [11, 39] or, even in non-human cell lines ectopically expressing *CFTR* mutation [36]. Drug pre-assessment in individual patient's own tissues - which have the superior quality of integrating the complete patient-specific genetic background (i.e., modifier genes)- are thus expected to be better predictors than those previously accepted cellular systems. Another argument for rejecting *ex vivo* prediction in intestinal organoids may be that the response to CFTR modulators in the intestinal tissue may not correlate with that in the respiratory epithelium. Although good strong correlations have been demonstrated between the two tissues in the past, additional confirmatory testing can be made by comparing results in intestinal organoids and nasal cells [40, 41]. But lower CFTR expression levels, significantly higher damage of the CF nasal tissue and much higher culture variablity may argue for the use of intestinal tissue vs HNE's.

Whatever argument exists against *ex vivo* prediction in patients with rare mutations, additional proof of efficacy can be gained in N-of-1 trials. In these trials a sequence of interventions is randomly assigned to one patient, e.g. alternate time periods of standard treatment with placebo or with study drug, to allow selection of the best intervention. In contrast to classical clinical trials, the participant is thus not only a trial participant but also the ultimate beneficiary of the clinical trial. It is part of a personalized or precision medicine strategy. Even more so than in classical trial design, for optimal N-of-1 trial design the onset of action of the study drug and the duration of washout which is needed to get back to baseline must be known [7]. When the results from serial related N-of-1 trials are aggregated, wider inferences can be drawn. N-of-1 trials are considered most useful for rare diseases with a chronic and indolent course; hence, quite suitable for patients with rare *CFTR* mutations. In Europe a 'provisional drug approval' process is in place. This is an opportunity for patients with a serious condition: they can benefit from new therapies as early as it seems reasonable and possible, whilst long-term efficacy and safety data are established during further drug exposure. Thus *ex vivo* prediction of drug benefit could be integrated into a MAPP [15].

"Basket" trials have mainly been used in oncology. They use the hypothesis that the presence of a molecular biomarker will predict the response to a targeted therapy [42]. Basket trials thus also make sense in CF when the treatment effect is expected to be large based on 'biological' selection of patients with a specific theratype. Basket trials are thus of interest to group patients with different rare *CFTR* mutations but with one similar biomarker characteristic into one clinical trial: e.g. patients are recruited into a particular trial on the basis of the magnitude of their *ex vivo* response to a specific (combination of) CFTR modulator(s). In such a non-random patient group, the new treatment is then compared to the standard of care.

In "umbrella" trials, a larger population is stratified into 'biomarker-defined' subgroups. Again, in the context of CF, the best *ex vivo* response to a reference CFTR modulator in individuals are homozygous for F508del could be used as the stratifying biomarker. Different new treatments (according to best response *ex vivo*) would be tested in each subgroup. Using this design several parallel and independent phase II trials could be conducted simultaneously [43]. Whereas working with a common molecular platform seems within reach in the field of CF, working across pharmaceutical companies to supply the needed range of targeted therapies may be the big challenge. But as stated above, the rapidly developing field of novel biomarker tests for molecularly targeted therapies also asks for standardization, quality control, accreditation, reimbursement models and regulation [8].

What outcome measures could be used as proof of significant benefit during a clinical trial in patients with rare or common *CFTR* mutations?

The appropriate set of outcome measures to quantify the clinical benefit from CFTR modulators should be agreed upon. It should take into account parameters such as: age, disease state and drug delivery mode (inhaled or systemic) of the compound. This list should include biomarkers of CFTR function (sweat chloride concentration, NPD and/or ICM), assessment of lung function (FEV_1, and more sensitive parameters like lung clearance index), nutritional status and 'quality of life' measures. Just as for *ex vivo* biomarkers, strict definitions and agreed upon SOPs for these outcomes are needed.

Some suggest the development of a 'model phase 3 trial template' to evaluate the clinical benefit of a CFTR modulator or modulator combination. We argue this is unrealistic given the differences in mode of action, pharmacokinetics, safety profile etc. among different compounds. We have learnt that changes in sweat chloride values correlate to changes in FEV_1 during treatment with CFTR potentiator ivacaftor [J Cyst Fibros. 2017 Jan;16(1):41-44. doi: 10.1016/j.jcf.2016.10.002. Epub 2016 Oct 20. Correlation of sweat chloride and percent predicted FEV1 in cystic fibrosis patients treated with ivacaftor. Fidler MC, Beusmans J, Panorchan P, Van Goor F]. By analogy, validation of *ex vivo* biomarkers could be advanced more rapidly by correlating the individual *ex vivo* drug response to changes in FEV_1 as surrogate outcome in clinical trials. This would provide a major benefit for subjects with rare *CFTR* mutations. However pharmaceutical companies might feel less at ease with this approach, for fear that it could have as 'side effect' that the *ex vivo* biomarker is requested as companion diagnostic also in subjects with more common mutations, for which 'conventional' trials can still be applied. Efforts towards validation of *ex vivo* biomarkers against surrogate outcome measures bring specific challenges: how to translate *ex vivo* drug concentrations to *in vivo* drug distribution or drug concentration at target site? How to monitor real-life adherence to drug treatment?…

Towards a pan-European and global strategy for *ex vivo* testing of response to CFTR modulators in patients with CF and rare or ultra-rare *CFTR* mutations

Currently there is no CFTR modulator treatment for patients who do not have an F508del, a known class III, residual function or an R117H mutation on one of their CFTR alleles. More worrisome even, subjects with a rare *CFTR* mutation who are not compound heterozygous for F508del are at present not included in any drug development program. Neither is this expected to occur in the short-term due to the low numbers of patients who carry such "orphan" mutations. Nevertheless, also these patients should have the prospect of a treatment that targets their basic CF defect.

In the present days, irrespective of the country where patients with CF live, all should have both *CFTR* mutations identified. Using an ongoing ECFS grant and respecting patient privacy and information, we can search for mutations in patients with an elevated sweat chloride but without 2 *CFTR* mutations identified. The proportion of subjects without F508del is higher in Southern European countries hence a higher prevalence of rare mutations is also expected there.

The next step will be designating sites within each country, which are proficient in obtaining patient samples for *ex vivo* drug efficacy testing and conform ethical review board approval. By a joint effort of CF Europe and ECFS, we can promote access to this service for patients with rare ("orphan") *CFTR* mutations: patients can be informed via the lay persons' community , namely patients' associations, patients can be reached via the CF care centres and/or via consultation of the ECFSPR database. Response to current CFTR modulator drugs is most likely in subjects with missense mutations and most promising in those with residual CFTR function (usually PS). Hence, priority could be given to test *ex vivo* efficacy to CFTR modulators in tissue from these patients. Linking CF basic science labs to ECFS-clinical trial network centres will be very useful to facilitate CFTR drug development and label expansion.

The most feasible personalized biomarker platforms for testing of CFTR function and CFTR modulator responsiveness will likely be organoids and/or nasal cells (see above). At a first stage, for the validation of SOP's a small number of labs will be involved. Once robust SOP's are established, these will be rolled out over an increasing number of sites to cover each country/region. Quality control, standardization and compliance with technical standards possibly via certification will be extremely important throughout. In a next phase, qualified advice from EMA and FDA should be asked for and provided for these *ex vivo* biomarkers.

Patients with rare *CFTR* mutations reaching a predefined *ex vivo* positive response to CFTR modulators could then progress to an N-of-1 trial, a basket trial or possibly just get early access to CFTR modulator treatment, pending 'real-life' proof of benefit during treatment, which would necessarily involve a close follow-up. Rigorous adherence to standardized data collection via disease specific clinical trial network sites (like ECFS-CTN), will be key to success. Of course, this latter phase should be in close consultation with and supervised by the national/local regulators and health authorities, so that the data obtained will result in drug approval for the individual. Upon proof of benefit in patients with specific rare mutations, patient registries should be re-consulted to find other patients who also carry them and who will most likely benefit from treatment.

Concluding Remarks

Biomarkers to determine *ex vivo* response to CFTR modulators in organoids and/or HNE's open new avenues towards personalized medicine in subjects with rare *CFTR* mutation. They will become equally useful in subjects with more common *CFTR* mutations once multiple treatments correcting the same defect become available.

Remaining challenges with organoids as a validated biomarker are: the difficulty to relate the FIS read out to %WT function, insufficient information about the linearity and the best dynamic range for the assay.

Remaining challenges associated with nasal cells validation are: harmonization of the many techniques and culture conditions in use; more difficult storage and re-expansion of cells; very limited validation database.

Other remaining open questions are: patient acceptance of nasal brushings versus rectal biopsy; degree of concordance between nasal cell responses and intestinal organoid responses; dynamic range for HNEs vs organoids. To address them further work is required.

And the ultimate challenge will be to efficiently and constructively align drug companies, patients, researchers and regulators, so that *ex vivo* biomarkers can be used to bring existing and new CFTR modulators faster to patients with rare mutations and in the long run also to bring the most efficacious drug to individual patients who carry more common mutations.

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