Primary nasal epithelial cells from patients with cystic fibrosis hold promise for guiding precision medicine and expanding treatment

Stephen R. Reeves[1,2]

[1]Dept of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA. [2]Center for Immunity and Immunotherapies, Seattle Children's Research Institute, Seattle, WA, USA.

Corresponding author: Stephen R. Reeves (Stephen.Reeves@seattlechildrens.org)

Shareable abstract (@ERSpublications)
Sette *et al.* should be commended for their excellent work appearing in the current issue of the *ERJ*. These studies provide a roadmap for expanding treatments and delivering personalised medicine for people with cystic fibrosis. https://bit.ly/31qe9zZ

Cite this article as: Reeves SR. Primary nasal epithelial cells from patients with cystic fibrosis hold promise for guiding precision medicine and expanding treatment. *Eur Respir J* 2021; 58: 2102735 [DOI: 10.1183/13993003.02735-2021].

Received: 15 Oct 2021
Accepted: 25 Oct 2021

In recent years, improving treatments for cystic fibrosis (CF) have dramatically enhanced the longevity and quality of life for people with CF. The pathogenesis of CF has been well characterised and has been directly linked to a dysfunctional chloride channel termed the cystic fibrosis transmembrane conductance regulator (CFTR), which is predominately found in epithelial tissue layers [1]. Until recently, most treatments for CF have focused on supportive care including enhanced airway clearance therapies, improved nutritional support, management of CF sequelae such as CF-related diabetes and liver disease, and/or treatment of pulmonary exacerbations with antibiotics [2]. These interventions have significantly improved life expectancy for people with CF, yet they do not address the underlying cause of the disease [3]. Since the identification of the CFTR gene, >2000 mutations have been identified, with at least 300 variants now known to be disease causing [4]. The most common mutation is the F508del variant, which in the homozygous situation leads to classical multi-systemic manifestations of CF. Importantly, additional disease-causing variants, either in combination with F508del or other significant disease-causing variants, contribute to the diversity of disease presentation, severity and outcomes. Significant progress in characterising the molecular consequences of CTFR mutations in recent years has led to the development of a classification system that correlates CFTR mutations to specific defects in CFTR production, trafficking and function [5].

Over the past decade, a greater understanding of the functional consequences of pathological CFTR mutations has led to the development of several new drugs, termed CFTR modulators, which target CFTR defects and restore its function [6]. While these drugs do not repair the underlying disease-causing mutations, they do dramatically restore CFTR function for certain types of mutations, resulting in substantial clinical improvement for people with CF who are eligible to receive these treatments. A significant limitation of currently available CFTR modulators is that these compounds target and correct specific defects in CFTR expression. For example, the mechanism of action of ivacaftor, the first available CFTR modulator, consists of maintaining the CFTR in the open position and thus potentiates CFTR function [7]. As a result, ivacaftor was initially approved for people with CF having at least one copy of the G551D mutation, the most common variant leading to gating mutations (*i.e.* class III mutations) [8]. Subsequently, approval for ivacaftor has been expanded to include other similar gating mutations, but in total these variants encompass only about 8% of people with CF [9]. More recently, ivacaftor has been studied in combination with additional CFTR modulators, which has led to expansion of its use to patients who are homozygous for F508del (lumacaftor/ivacaftor and tezacaftor/ivacaftor) and more recently to patients who have at least one copy of F508del (elexacaftor/tezacaftor/ivacaftor; Trikafta) [6].

For people with CF who are eligible for treatment with CFTR modulators, the treatments represent a new era of hope. Unfortunately, at the time of writing this editorial, not all people with CF are eligible for these therapies. A recent study by McGarry and McColley [10] from the USA evaluated the eligibility of people with CF to receive CTFR modulator therapy based upon CFTR genotype. They reported that 92% of non-Hispanic White patients were eligible for current treatments compared to 76% of Hispanic patients, 70% of Black patients, and 81% of patients who identified as other races. Furthermore, the patients not eligible for CFTR modulators also displayed lower lung function compared to the rest of the cohort [10]. These findings are multifactorial in that minority populations are less likely to have the F508del mutation and are more likely to have other rare variants that are underrepresented in clinical trials. Current models of clinical trials have clearly benefited people with CF who have the most common mutations and have undeniably improved their care. However, we are left with the problem of how to study patients with less common variants for which traditional clinical trials would be underpowered. There is precedent for the evaluation of tissue to study the efficacy of CFTR modulators, using *in vitro* models such as intestinal epithelial organoid cultures [11]. Indeed, ivacaftor approval has been extended to include additional residual function mutations based on *in vitro* data [12].

In the present issue of the *European Respiratory Journal*, Sette *et al.* [13] report findings from their study of nasal epithelial cells (NECs) obtained from people with CF using patient-derived conditionally reprogramed cells. In this study, the authors note that Trikafta is now approved for the treatment of people with CF having one copy of F508del but acknowledge that there are several genotypes that are not currently eligible for this treatment. The authors go on to describe a series of experiments that are designed to evaluate an individual's response to a given CFTR modulator using *in vitro* model systems derived from primary cells, otherwise known as theratyping [14]. Primary airway epithelial cells have been used in *in vitro* model systems to model diseases including asthma, COPD, CF, cigarette smoke, *etc*. for many years [15]. However, a significant limitation to their use is that primary cells become senescent over repeated passages *ex vivo*, limiting the number of cells available for use in experiments [16]. In order to overcome this limitation, the authors take advantage of a recent technical advancement using a culture reprograming condition (CRC) [17]. By using the CRC methodology, the authors can proliferate the primary cells obtained from a donor and generate large stocks of cells to use in subsequent experiments. In the first series of data shown in the article, the CRC-derived cells are characterised and demonstrate epithelial-specific cellular markers. The CRC cells are then differentiated at an air–liquid interface (ALI), demonstrating that the cells acquire an organotypic NEC morphology producing both mucin and cilia. Additionally, the CRC-derived cells are shown after they have been differentiated into organoids. After establishing that the CRC-derived NECs can generate both the ALI and organoid cultures, the authors proceed to investigate these model systems using NECs derived from people with CF. First using the ALI cultures, CFTR expression was evaluated and found to be most highly expressed in the NECs differentiated at an ALI. Molecular characterisation of the ALI cultures by immunoblotting demonstrated the presence of wild-type CFTR as well as the misfolded variant CFTR protein. Treatment of the NECs with tezacaftor/elexacaftor and lumacaftor/elexacaftor *in vitro* led to quantifiable increases of the wild-type CFTR protein in F508del homozygous NECs. Importantly, cells derived from other donors with one copy of F508del and an insertion mutation also demonstrated increased production of wild-type CFTR. To ascertain whether the increased expression of CFTR resulted in improved CFTR function, the authors evaluated NECs in the organoid model using a forskolin-induced swelling protocol and a fluid absorption model in the ALI cultures. These studies demonstrate a correlation between increased wild-type CFTR expression and increased CFTR function following treatment with CFTR modulators.

In their article, Sette *et al.* [13] provide a convincing proof-of-concept study that primary NECs obtained from people with CF can be used to "theratype" an individual's response to a CFTR modulator or group of CFTR modulators *in vitro*. While additional validation may be needed to correlate *in vitro* responses with clinical responses, these studies hold significant promise for the personalisation of CFTR modulator therapies. In addition to providing a valuable pre-clinical model system to study future CFTR modulators as they become available, the use of *in vitro* theratyping could also open the door to study existing CFTR modulators to treat people with CF who have CFTR variants for which CFTR modulators are not yet approved and for whom clinical trials are not practical.

Conflict of interest: S.R. Reeves has nothing to disclose.

Support statement: This work was supported by the National Heart, Lung, and Blood Institute (grant: K08HL135266). Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Dickinson KM, Collaco JM. Cystic fibrosis. *Pediatr Rev* 2021; 42: 55–67.
2 Shteinberg M, Haq IJ, Polineni D, *et al.* Cystic fibrosis. *Lancet* 2021; 397: 2195–2211.
3 McKone EF, Ariti C, Jackson A, *et al.* Survival estimates in European cystic fibrosis patients and the impact of socioeconomic factors: a retrospective registry cohort study. *Eur Respir J* 2021; 58: 2002288.
4 Sharma J, Keeling KM, Rowe SM. Pharmacological approaches for targeting cystic fibrosis nonsense mutations. *Eur J Med Chem* 2020; 200: 112436.
5 Amaral MD. Novel personalized therapies for cystic fibrosis: treating the basic defect in all patients. *J Intern Med* 2015; 277: 155–166.
6 Goetz DM, Savant AP. Review of CFTR modulators 2020. *Pediatr Pulmonol* 2021; in press [https://doi.org/10.1002/ppul.25627].
7 Yu W, Kim Chiaw P, Bear CE. Probing conformational rescue induced by a chemical corrector of F508del-cystic fibrosis transmembrane conductance regulator (CFTR) mutant. *J Biol Chem* 2011; 286: 24714–24725.
8 Ramsey BW, Davies J, McElvaney NG, *et al.* A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011; 365: 1663–1672.
9 Harutyunyan M, Huang Y, Mun KS, *et al.* Personalized medicine in CF: from modulator development to therapy for cystic fibrosis patients with rare CFTR mutations. *Am J Physiol Lung Cell Mol Physiol* 2018; 314: L529–L543.
10 McGarry ME, McColley SA. Cystic fibrosis patients of minority race and ethnicity less likely eligible for CFTR modulators based on CFTR genotype. *Pediatr Pulmonol* 2021; 56: 1496–1503.
11 Furstova E, Dousova T, Beranek J, *et al.* Response to elexacaftor/tezacaftor/ivacaftor in intestinal organoids derived from people with cystic fibrosis. *J Cyst Fibros* 2021; in press [https://doi.org/10.1016/j.jcf.2021.07.006].
12 Van Goor F, Yu H, Burton B, *et al.* Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J Cyst Fibros* 2014; 13: 29–36.
13 Sette G, Lo Cicero S, Blaconà G, *et al.* Theratyping cystic fibrosis *in vitro* in ALI culture and organoid models generated from patient-derived nasal epithelial conditionally reprogrammed stem cells. *Eur Respir J* 2021; 58: 2100908.
14 Crawford KJ, Downey DG. Theratyping in cystic fibrosis. *Curr Opin Pulm Med* 2018; 24: 612–617.
15 Baldassi D, Gabold B, Merkel O. Air-liquid interface cultures of the healthy and diseased human respiratory tract: promises, challenges and future directions. *Adv Nanobiomed Res* 2021; 1: 2000111.
16 Reeves SR, Barrow KA, White MP, *et al.* Stability of gene expression by primary bronchial epithelial cells over increasing passage number. *BMC Pulm Med* 2018; 18: 91.
17 Sette G, Salvati V, Giordani I, *et al.* Conditionally reprogrammed cells (CRC) methodology does not allow the *in vitro* expansion of patient-derived primary and metastatic lung cancer cells. *Int J Cancer* 2018; 143: 88–99.