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Editorial

Take it personally: how personal we reach when we are so different from each other?

When the CFTR gene was cloned almost 30 years ago, at least 7 CFTR mutations were expected according to the haplotypes [1]. The first identified mutation was the F508del. It was further shown that homozygous patients to this mutation had different phenotype compared to heterozygotes or those who did not carry this mutation [1–3]. Then, mutations with a milder and atypical phenotype were reported [4]. Over 2000 CFTR sequence variants were reported to the CFTR1 mutation database (http://www.genet.sickkids.on.ca/), and nearly 300 are known to cause the CF disease (https://www.cftr2.org/). CFTR gene mutations were classified according to the molecular mechanisms by which they cause CFTR protein dysfunction [5], and it was thought that drugs can be developed as class specific. *In vitro* cellular models were used to test the effect of many compounds on the mutated CFTR. The first breakthrough was the development of Kalydeco (ivacaftor), a potentiator drug for class III gating mutations. Subsequently, the FDA approved prescription of Kalydeco to patients carrying a list of residual function mutations, based on their effect on CFTR function *in vitro*, in non-human cells! The next drug, Orkambi, combining the potentiator (lumacaftor) with a corrector (ivacaftor) is indicated for patients homozygous for the class II F508del mutation. *In vitro* studies showed a lesser and variable response to Orkambi in other class II mutations [6,7]. This led to the novel concept of "theratypes", which groups CFTR variants according to their effect on the CFTR protein and their response to corrector and/or potentiator compounds [8]. This requires investing resources to test the effect of each drug on a specific mutation or even on a specific patient.

Furthermore, variable response to Kalydeco and Orkambi among patients carrying the same genotype was shown. Since these drugs are extremely expensive, it could be argued that only patients that show clinical benefit should take them. Therefore, *in-vitro* cellular models and genetic tools are needed to predict patient-specific clinical outcomes.

Several approaches were taken to meet this need. In this JCF issue, Clancy et al. report on a workshop of international experts organized by the CFF which discussed the use of preclinical models to examine the nature of CF-causing variants in CFTR, focusing on CFTR theratyping and the role of *in vitro* CFTR modulators testing to predict *in vivo* response to modulators [9]. The cellular models discussed include Human Bronchial Epithelial cells (HBEs), Human nasal epithelial cells (HNEs), gastrointestinal and respiratory organoids and iPSCs. All these systems allow testing of drug response *in vitro*, on the endogenous CFTR alleles in their native context in different human cell systems. The paper by Clancy et al., discusses the advantages and limitations of each of these models. While the HBEs are the gold standard for successful translation of drug development based on currently approved drugs, their main limitation is the need to obtain them by bronchoscopy from bronchial epithelial cells or from explanted lungs after transplantation. In addition, their growth in culture is restricted. This becomes critical in cases of attempts to develop drugs for rare CFTR mutations. Therefore many scientists turned to work with HNEs as a drug response predicting models [10–13]. These cells are easier to obtain from patients by nasal brushing, however, their expansion in cultures is limited.

Further studying the potential use of organoids and iPSCs as patient-derived model systems is highly important, as they offer a limitless supply of donor cells based on their progenitor cells. iPSCs also have a unique potential advantage, as they can in principle be differentiated into any CF relevant epithelia that are inaccessible otherwise (such as pancreatic cells). The current limitation is that the differentiation protocols are still in development. It is worth noting that iPSCs may suffer from aneuploidy, further exposing the cells to genomic instability [14] and thus careful karyotyping is required.

The variation in clinical response of patients carrying the same mutation may result from genetic differences, highlighting the need for comprehensive genetic model systems and clinical data. Indeed, in this issue Eckford et al. [15] report on the CF Canada-Sick Kids Program in Individual CF Therapy (CFIT), a first of its kind, generating a comprehensive resource containing patient-specific cell cultures and data from 100 CF individuals that will enable modeling of therapeutic responses. They are collecting nasal epithelial cells, generating matched gene expression data obtained by RNA sequencing from the primary nasal tissue, whole genome sequencing of blood derived DNA from each participant, induced pluripotent stem

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cells (iPSCs), and CRISPR-edited isogenic control iPSC lines. All will be correlated with clinical data and response to CFTR modulator therapies. These tools will focus on assessing patient specific responses that predict individual outcomes to current and emerging modulators targeting F508del-CFTR and facilitate therapy discovery for rare CF causing mutations.

The current CFTR modifiers, although showing benefit to patients with CF, are far from curing CF. Chronic infection and excessive neutrophilic inflammation persist, which cause progression of lung damage. Currently no efficient anti-inflammatory drug is available. *In vitro* studies lack a robust model to study neutrophil function, since neutrophils do not multiply and have a short life span. In this issue, Jennings et al. [16] generated a homozygous F508del-CF promyelocytic cell line by taking advantage of the CRISPR/Cas9 gene-editing technology, from which unlimited CF neutrophil cells can be differentiated. The derived cells show defective CFTR presentation, deficient phagosomal hypochlorous acid (HOCl) production and compromised microbial killing, suggesting that this human CF promyelocytic cell line might have important implications in CF basic research and drug screening, as well as in studying drug effects on neutrophil function.

These three papers describe cellular systems that can be or are already used to predict response to therapy *in vivo*. More studies are needed to correlate the response in the different *in vitro* systems to clinical response, since other genes, most of them unknown yet, can modify the response *in vivo*. The SLC29A6 gene was suggest to modify response to Kalydeco by modulating the airway response to CFTR directed therapies [17]. The cost of the CFTR modifier therapies, and the possible need to take several in combination in order to achieve full CFTR correction of function, emphasizes the need for various, complementary cellular systems and clinical data that may together enlarge the basis for predicting CFTR drug response in a patient-specific manner.

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