Journal of Cystic Fibrosis 21 (2022) 254–257

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Forskolin induced swelling (FIS) assay in intestinal organoids to guide eligibility for compassionate use treatment in a CF patient with a rare genotype

B.L. Aalbers[a,*], J.E. Brunsveld[b], C.K. van der Ent[b], J.C. van den Eijnden[a], J.M. Beekman[b], H.G.M. Heijerman[a]

[a] Department of pulmonology, UMC Utrecht, The Netherlands
[b] Department of pediatric pulmonology, UMC Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 7 November 2021
Revised 30 December 2021
Accepted 19 January 2022
Available online 31 January 2022

ABSTRACT

Background: Forskolin-induced swelling of patient-derived organoids has been used to measure patient-specific CFTR function and CFTR modulator response. We present a case where CFTR function assessment in intestinal organoids was decisive for a patients' acceptance to a compassionate use program.
Case description: A 56 years old female with cystic fibrosis compound heterozygous for F508del and a rare CFTR allele (c.3717+5G>*T*) experienced rapid clinical deterioration. The forskolin-induced swelling assay on her rectal organoids was used to confirm that the rare mutation is a minimal residual function mutation, and that other CFTR modulators would not likely be effective. Based on these two criteria and her clinical status, she was accepted for compassionate use of elexacaftor/tezacaftor/ivacaftor and showed improvement in all clinical parameters.
Conclusions: This reports describes a first example that intestinal organoids were used to identify a previously unknown CFTR mutation as a minimal function mutation. The individual FIS-based definition of minimal residual function, response to ele/tez/iva and/or lack of response to other CFTR modulating drugs, may thus provide a tool for access to ele/tez/iva treatment for people with rare genotypes.

1. Introduction

Elexacaftor (ele) in combination with tezacaftor (tez) and ivacaftor (iva) is a next generation CFTR modulator treatment and has been approved for treatment of cystic fibrosis in October 2019 in the US and in August 2020 in the EU. Ele/tez/iva is a triple CFTR modulating drug combination with higher efficacy compared to single and dual CFTR modulating drugs in subjects with the F508del/F508del genotype and for F508del compound heterozygous with defined minimal function CFTR mutations [1,2].

Many mutations associated with cystic fibrosis (CF) remain uncharacterized and have not been functionally annotated [3]. Patient-derived intestinal organoids facilitate the measurement of individual CFTR function through the forskolin-induced swelling (FIS) assay, enabling both the assessment of CFTR residual function and the effects of CFTR modulators. [4,5]. This assay measures CFTR-dependent ion and fluid transport into the organoid lumen that causes rapid organoid swelling. We report the case of an individual with CF who has a F508del compound heterozygous CFTR genotype and c.3717+5G>*T* who was originally rejected for compassionate of ele/tez/iva because her rare allele was not characterized and defined as a minimal function allele. Organoids were used for an individual assessment of CFTR function and classified her allele as minimal function, which guided inclusion into the ele/tez/iva compassionate use program.

2. Case description

We present a 56 years old female with cystic fibrosis and a c.1521_1523del(F508del)/c.3717+5G>*T* genotype, who was diagnosed with CF in her first year of life. She has exocrine pancreatic insufficiency as well as CF related diabetes mellitus for which she uses insulin. She has had recurrent airway infections throughout her life, and is chronically infected with Pseudomonas aeruginosa.

In recent years, her clinical condition had deteriorated with frequent pulmonary exacerbations. In the previous year, she received multiple courses of antibiotics and 2 courses of prednisone because of pulmonary exacerbations, two of which required pro-

* Corresponding author.
E-mail address: b.l.aalbers-2@umcutrecht.nl (B.L. Aalbers).

https://doi.org/10.1016/j.jcf.2022.01.008

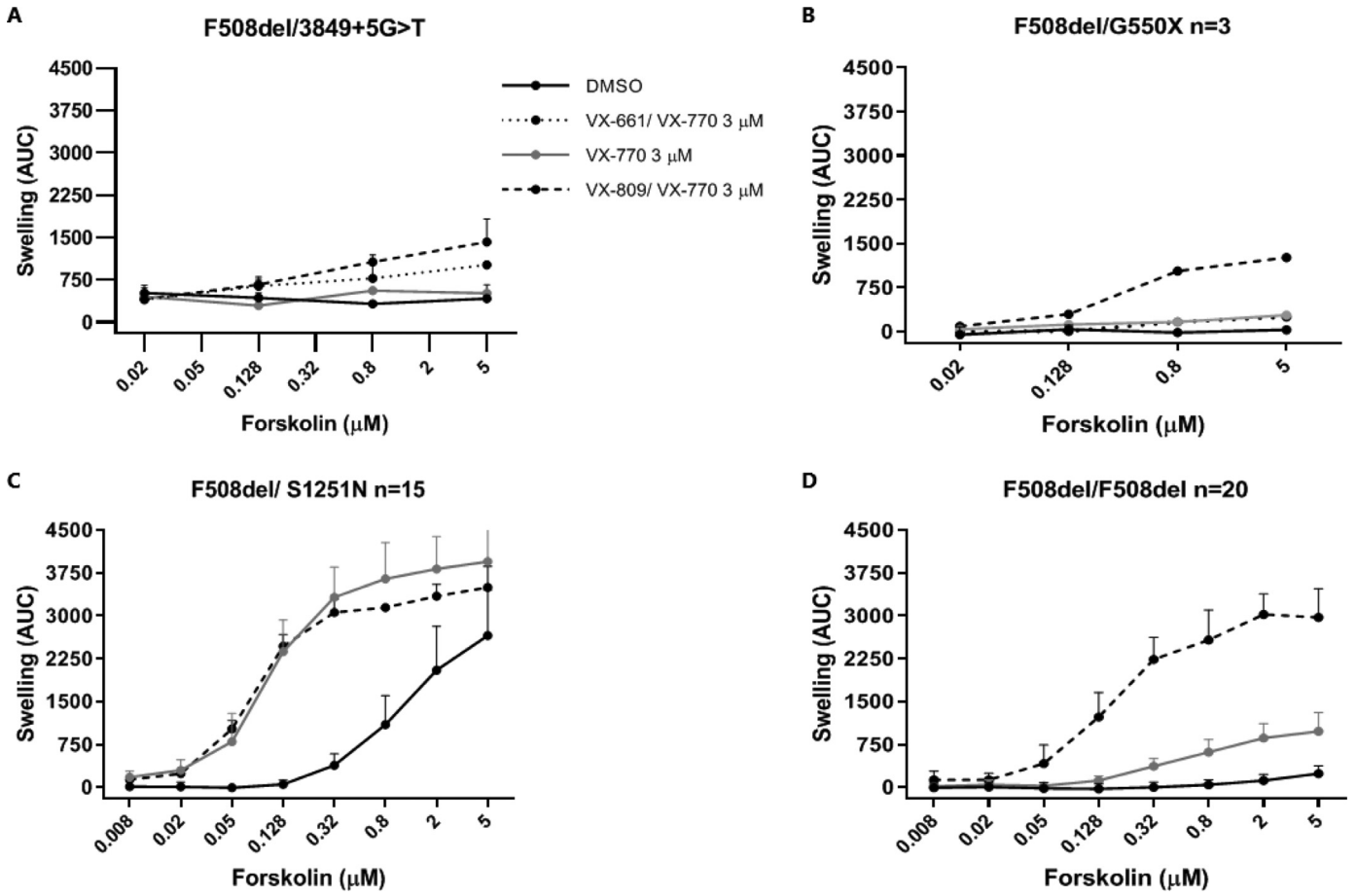

Fig. 1. Graphs of the FIS assay with responses to ivacaftor (VX-770) ivacaftor/lumacaftor (VX-770/VX-809) ivacaftor/tezacaftor (VX-770/VX-661).
A: Our case patients' organoids (2 measurements, individual measurement graphs in supplement)
B: Reference organoids ($N = 3$) derived from patients with F508del/G550X genotype
C: Reference organoids ($N = 15$) derived from patients with F508del/S1251N genotype
D: Reference organoids ($N = 20$) derived from patients with F508del/F508del genotype.

longed hospitalization for intravenous therapy. Her FEV_1 at this point was at 30% of predicted, with an annual average decline of 4% in the previous 3 years. Due to the severely impaired lung function and the frequent exacerbations, the disease had a grave impact on the patients' daily life. Because of the gradual deterioration in clinical condition, screening for lung transplantation was started, and concurrently her eligibility for ele/tez/iva treatment on a compassionate use basis was investigated. A request for inclusion into the compassionate use program was denied as the ultra-rare c.3717+5G>*T* mutation was not designated as minimal residual function mutation.

The individual agreed to provide rectal biopsies for generation of intestinal organoids and CFTR function measurement. An increasing dose of forskolin was used to test organoid swelling, and showed no baseline swelling of her organoids indicative of a minimal residual function genotype and only no (ivacaftor) or limited swelling after lumacaftor/iva or tez/iva incubation, quantitatively comparable to average responses of organoids with one F508del allele combined with a nonsense mutation (G542X) (Fig. 1, 2). A combination of ele/tez/iva was not tested on the organoids, as elexacaftor was not yet available for in vitro testing in our laboratory. When it became available, we encountered an inability to restore living cultures from the patients' biobanked organoids to perform a new FIS assay. We deemed it unethical to request new biopsies from our patient if it would solely be for this purpose.

Based on these results in combination with her clinical status, this patient was allowed to join the compassionate use program and start ele/tez/iva treatment. After only days of treatment, she started coughing less and feeling more energetic. After 6 months of treatment, her FEV_1 had increased to 52% of predicted (+22%), her sweat chloride had dropped to 49 mmol/l (−48 mmol/l) and her chest CT (Fig. 3) showed markedly less mucus plugging, bronchial wall thickening and peribronchial thickening compared to a CT obtained 2 months before the start. Her CFQ-R scores improved not only on the respiratory domain (44 to 83 on the 100 point scale), but also on the subdomains health, vitality, social, physical, role and digestive. In the first 12 months of treatment she had no pulmonary exacerbations. After 18 months of treatment, her FEV_1 had improved further to 57% of predicted, the highest level since at least 16 years.

3. Discussion

We report the first case, to our knowledge, in which organoid FIS was used to argue eligibility for triple CFTR modulating treatment on a compassionate use basis.

One condition for applicability to this case, is that the technique should be able to verify if an ultra-rare mutation of which the consequences are unknown, is a minimal function mutation. The FIS assay provides a CFTR dependent readout for individuals that correlates with other biomarkers of CFTR function, clinical disease severity and CFTR modulator responses [4,6,7] Baseline residual function measurement was clearly absent and different from organoids with residual function alleles (Fig. 1) [4,6].

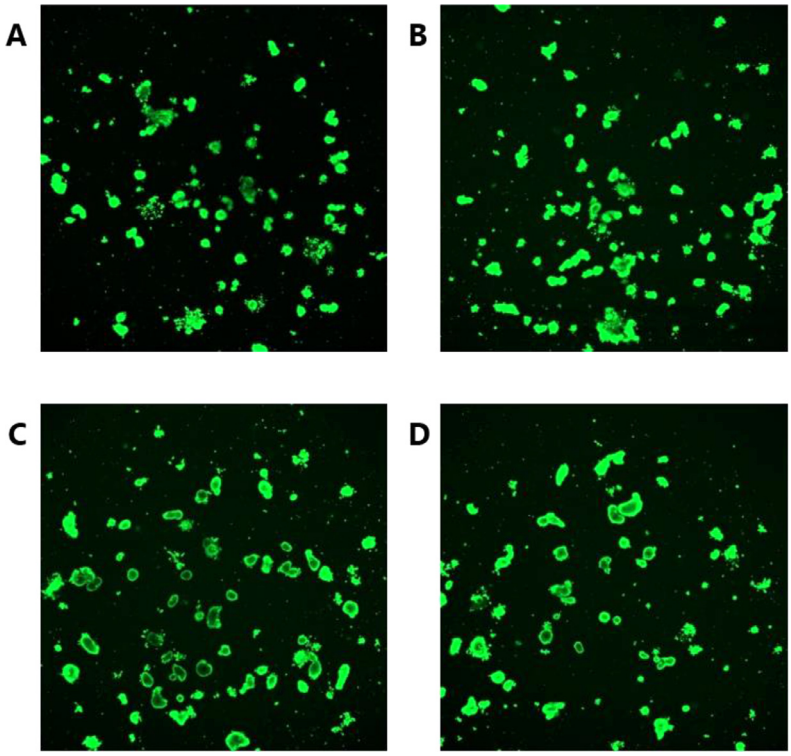

Fig. 2. Images of FIS assay on rectal organoids of our case patient
A: Before stimulation with forskolin
B: DMSO control after maximum forskolin stimulation (5.0uM)
C: VX809/VX770 pretreated, after maximum forskolin stimulation (5.0uM)
D: VX661/VX770 pretreated, after maximum forskolin stimulation (5.0uM).

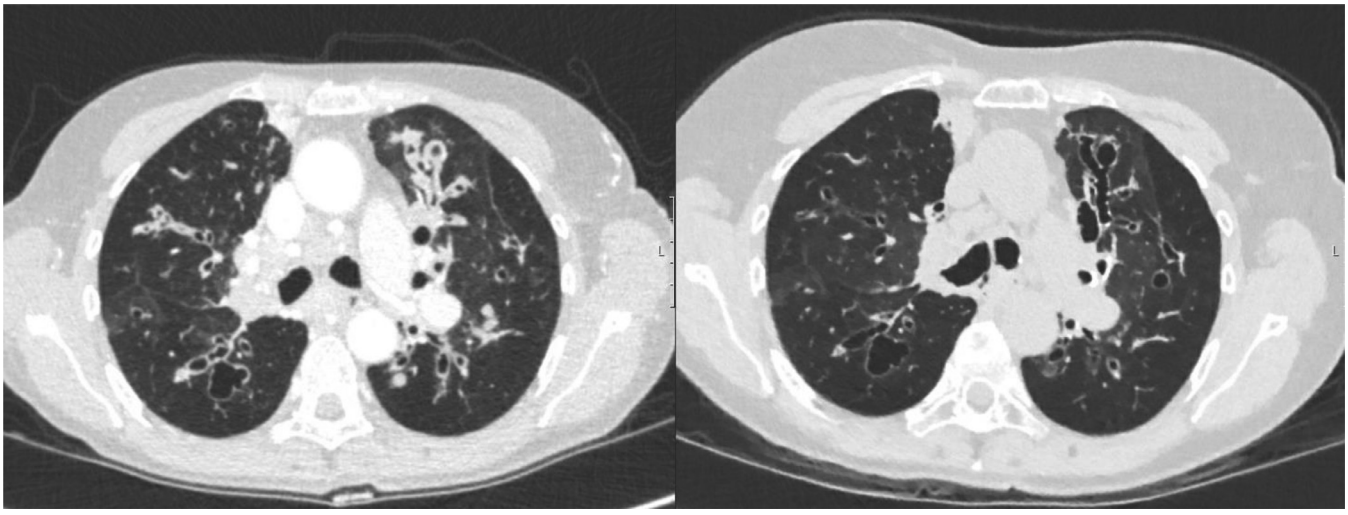

Fig. 3. CT scan images of our patient 1 month before start (left) and 3 months after start of treatment (right).

Strong responses to the then available first generation modulators were also absent. By quantitatively comparing the current measurements to published average organoid responses with defined CFTR genotypes [5], the c.3717+5G>*T* allele would be designated as a non-functional class I splicing mutation.

This report shows that individual CFTR function measurements can be used to classify CFTR genotypes as minimal function genotypes. This is different from the currently used CFTR allele-specific typing by Fisher Rat Thyroid cells that quantifies CFTR function upon introducing mutations in a defined genetic context. This system does not integrate individual genetic variability within the CFTR gene and other modifiers that could impact on individual function, and is also not equipped to assess splice mutations in a direct and individual manner as was studied here. We anticipate that the methodology used here that is based on individual CFTR function assessment in organoids could help more people with CF and rare, uncharacterized mutation to get access to critical treatment.

An issue that should be addressed in the future, is the current lack of a cut-off value for the definition of minimal function based on the FIS assay or any other measure. In our patients' case it was very clear that residual function was close to zero in the grown

organoids. However, the thresholds that discriminate between individuals having minimal residual function or 'more-than-minimal' remain unclear for organoid FIS or any other individual CFTR function test or clinical feature. This unclarity hampers the implementation of the FIS test or other criteria (e.g. based on pancreatic insufficiency or sweat chloride concentration) in facilitating access to ele-tez-iva for individuals with uncharacterized CFTR genotypes.

4. Conclusion

We show a first application of the FIS assay on patient-derived organoids to define minimal CFTR residual function of genotypes and associated alleles in the context of eligibility for compassionate use of ele/tez/iva. This individual-based approach may provide a timely solution for assessment of drug eligibility for people with CF who have rare genotypes.

Declaration of competing interest

HGM Heijerman has provided assistance to Vertex pharmaceuticals, the manufacturer of lumacaftor/ivacaftor, as member of the advisory board, speaker and investigator in clinical studies.; BL Aalbers has assisted in clinical trials for Vertex as a sub-investigator.; CK van der Ent reports grants from Vertex, outside the submitted work.; JM Beekman and CK van der Ent are inventors on patent(s) related to organoid swelling, and received royalties from 2017 onwards.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2022.01.008.

CRediT authorship contribution statement

B.L. Aalbers: Conceptualization, Methodology, Investigation, Writing – original draft, Visualization. **J.E. Brunsveld:** Visualization, Investigation. **C.K. van der Ent:** Resources, Writing – review & editing. **J.C. van den Eijnden:** Visualization, Writing – review & editing. **J.M. Beekman:** Resources, Supervision, Methodology, Writing – review & editing. **H.G.M. Heijerman:** Conceptualization, Supervision, Methodology, Writing – review & editing.

References

[1] Middleton PG, Mall MA, Dřevínek P, et al. Elexacaftor-Tezacaftor-Ivacaftor for cystic fibrosis with a single Phe508del allele. N Engl J Med 2019 Nov 7;381(19):1809–19.
[2] Heijerman HGM, McKone EF, Downey DG, et al. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. Lancet 2019;394:1940–8.
[3] Farrell PM, White TB, Ren CL, et al. Diagnosis of cystic fibrosis: consensus guidelines from the cystic fibrosis foundation. J Pediatr 2017 Feb;181S:S4–S15.e1 10.1016.
[4] Dekkers JF, Wiegerinck CL, de Jonge HR, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. Nat Med 2013;19(7):939–45 10.1038.
[5] Dekkers JF, Berkers G, Kruisselbrink E, et al. Characterizing responses to CFTR–modulating drugs using rectal organoids derived from subjects with cystic fibrosis. Sci Transl Med 2016 Jun 22;8(344):344ra84.
[6] de Winter-de Groot KM, Berkers G, Marck-van der Wilt REP, et al. Forskolin-induced swelling of intestinal organoids correlates with disease severity in adults with cystic fibrosis and homozygous F508del mutations. J Cyst Fibros 2020;19(4):614–19 10.1016.
[7] Berkers G, van Maurik P, Vonk AM, et al. Rectal organoids enable personalized treatment of cystic fibrosis. Cell Rep 2019;26(7):1701–1708.e3.