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ORIGINAL ARTICLE

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Elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis and rare mutations

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Abstract

Introduction: The triple combination of elexacaftor/tezacaftor/ivacaftor (ETI) has dramatically improved the outcome of people with Cystic Fibrosis (pwCF) with at least one F508del mutation. However, carriers of rare cystic fibrosis transmembrane conductance regulator (CFTR) variants are not candidates for this innovative treatment.

Methods: In this observational study, we report the results of the compassionate use of ETI in 10 pwCF carriers of rare mutations after 2 months of treatment. Rectal organoids and short-term cultures of nasal epithelium obtained from rectal suction biopsies and nasal brushing were obtained from four subjects.

Results: After 2 months of ETI, all patients (4 males, mean age 30.1 ± 13.3 years) showed a significant increase of FEV_1% predicted values [+8.0 (3.5–12.7) %, $p < 0.010$], body mass index [+0.85 (0–1.22) kg/m^2, $p < 0.020$] and cystic fibrosis questionnaire-revised [+19.5 (6.3–29.2) points, $p < 0.009$]. A significant decrease of sweat chloride concentration [−11.2 (−1.7 to −34.0) mmol/L, $p < 0.020$] and exacerbations [−1.5 (−2 to −1), $p < 0.008$] was also recorded. Overall, 7 out of 10 participants were considered full responders. All patients reported cough disappearance ($n = 3$) or reduction ($n = 7$). Long-term oxygen was discontinued in two out of three patients and one also stopped noninvasive ventilation and was removed from the lung transplantation waiting list.

Conclusions: Despite the limited number of cases, our results support the use of CFTR modulators in patients with rare CFTR variants that are not currently approved for ETI in Europe.

KEYWORDS

cell culture, cystic fibrosis, elexacaftor/tezacaftor/ivacaftor, ETI, modulator, organoid, organoids, rare mutations, rare variants, theratyping

1 | INTRODUCTION

Cystic fibrosis (CF) is a life-limiting autosomal recessive genetic disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein.[1] Depending on the genetic mutation, the dysfunction of CFTR can result in absent or decreased protein production, misfolding that can lead to altered functionality, reduced stability and rapid degradation or a combination of these defects. To date, the CF Mutation Database (CFMD)[2] reports more than 2000 *CFTR* mutations; in the Italian

Registry nearly 70% of patients have at least one copy of the Phe508del CFTR mutation.[3]

Over the last decade, CFTRm modulators (CFTRm) have been developed and dramatically improve the outcome of most patients with CF. CFTRm are molecules that either correct protein misfolding and misprocessing, increasing the amount of mature CFTR protein on cell surface (correctors) or improve channel gating enhancing the transport of chloride and bicarbonate (potentiators).[4] The triple combination of the two CFTRm elexacaftor and tezacaftor with the potentiator ivacaftor, known as elexacaftor/tezacaftor/ivacaftor (ETI) was approved in 2019 in the United States (US) by the Food and Drug Administration (FDA) and in 2020 in Europe by the European Medicines Agency (EMA) for patients with at least one F508del mutation. This combination has been shown to improve lung function, nutritional status and quality of life, reduce the frequency of pulmonary exacerbations and decrease sweat chloride concentration.[5–7] Furthermore, there is evidence that ETI can also modify lung structure and morphology, particularly by reduction of mucus plugging and wall thickening/bronchiectasis.[8,9]

Very recently, FDA extended the use of ETI to 177 rare variants based on unpublished data from in vitro studies showing partial rescue of CFTR function in Fischer Rat Thyroid (FRT) cells expressing these mutations.[10] However, not all CF patients are currently candidates for this innovative treatment, as many carrying rare CFTR variants are not included among those eligible for ETI or other modulators. Increasing data have been published on the effectiveness of ETI treatment and other modulators on rare CFTR mutations in vivo[11–16] and in vitro after exposure to ETI, both in human nasal epithelial cell (HNEC) cultures[13,16–18] and patient-derived intestinal organoids (PDIOs)[16,19–21] (see Supporting Information S1: Table S1).

In this manuscript, we report the results of the compassionate use of ETI in 10 people with CF (pwCF) with rare mutations not eligible for ETI and provide evidence of the predictive power of theratyping in selected cases.

2 | METHODS

From January to October 2023, 10 pwCF with mutations other than F508del accessed the ETI off-label therapeutical program. ETI was granted and fully reimbursed by the Italian National Healthcare System following a request by referral physicians to the Regional Health Authority. ETI was obtained following evidence of CFTR rescue in in vitro assays performed on rectal organoids in 3 cases (patient 5, HIT Europe study, Ospedale Gianna Gaslini, Genova, Italy; patients 3 and 4, Università Verona, Verona, Italy) and HNEC culture in one patient (patient 1, Ospedale Gianna Gaslini, Genova, Italy). Rectal organoids and nasal epithelial-cell culture methodologies are reported in detail in the Supporting Information S1.

In these patients, we collected demographics (age, gender) and clinical data [CFTR mutation, lung function values measured by spirometry, body mass index (BMI), sweat chloride concentration, number of pulmonary exacerbations, quality of life by cystic fibrosis questionnaire-revised (CFQ-R), and presence/intensity of cough] before and after 2 months of ETI. Presence and intensity of cough were classified as disappeared, decreased or unchanged. According to previous cut-off values,[15] patients with a reduction in sweat chloride ≥20 mmol/L and/or an increase in forced expiratory volume in the first second (FEV_1% predicted) ≥10 percentage points following ETI were considered as responders.

Written informed consent was obtained from all patients included in the study or their parents. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the University Hospital of Parma (407/2022/OSS/AOUPR).

2.1 | Statistical analysis

The normality of distribution was assessed by Kolmogorov-Smirnov test. Normally distributed variables were reported as mean and SD, non-normally distributed variables were reported as median and interquartile range (IQR). Because almost all variables (e.g. FEV_1) did not have a normal distribution, for the before-after ETI comparison we used Wilcoxon signed-rank test.

A two-tailed p-value of <0.05 was considered statistically significant. Data analysis was performed using GraphPad Prism 5 (GraphPad software version 5.00 for Windows, GraphPad Software, www.graphpad.com).

3 | RESULTS

3.1 | Population

Ten patients (four males) were included in the study. Mean age (SD) at time of starting ETI therapy was 30.1 (13.3) years. Before ETI initiation, two patients had been on modulator therapy with either lumacaftor/ivacaftor (n = 1) or ivacaftor (n = 1). Characteristics of the population are described in Table 1. None of the patients included in this study was eligible for ETI in Europe but two (patient 4 and 10) had an FDA-approved CFTR variant (R347P and R1066H).

3.2 | Theratyping of selected cases

We developed rectal organoids and short-term cultures of nasal epithelium obtained from rectal suction biopsies and nasal brushing in 4 subjects. We measured the short-circuit current.

(Isc) response of patients of untreated and ETI-treated epithelium grown on Transwell filters. Data on PDIOs indicate a significant improvement of CFTR-specific currents for the cells carrying the R347P/R347P (patient 4), mirrored by increased CFTR maturation as determined by western blot analysis. Conversely, the L227R/L227R

cells (patient 3) display a virtually undetectable current not modified by ETI treatment. No evidence of mature CFTR was recorded by western blot analysis before and after treatment (Figures 1 and 2).

TABLE 1 Characteristics of patients included in the study.

Number of patients	10
Males/females	4/6
Age, years	30.1 (13.3)
Patients < 18 years, *n* (%)	3 (30)
At least one CFTR variant approved by FDA, *n* (%)	2 (20)
Pancreatic insufficiency, *n* (%)	7 (70)
Diabetes, *n* (%)	1 (10)
FEV_1% predicted	45.2 (17.8)
BMI, kg/m^2	20.2 (5.5)
Sweat chloride concentration, mmo/L[a]	79.4 (25.8)
Exacerbations in the past 2 months treated with:	
– Oral antibiotics	2 (2–3)
– IV antibiotics	1 (0–1)
Enteral tube feeding, *n* (%)	2 (20)
Patients treated with previous modulator, *n* (%)	2 (20)
Long-term oxygen therapy, *n* (%)	3 (30)
Long-term NIV, *n* (%)	1 (10)
On waiting list for lung transplantation, *n* (%)	1 (10)

Note. Data are presented as SD. Numbers of exacerbations during the previous 2 months are expressed as IQR.

Abbreviations: BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator; FEV_1, forced expiratory volume in the first second; IQR, median; IV, intravenous; NIV, noninvasive ventilation; SD, mean.

[a]data missing for patient n.10.

Patient n 3 started lumacaftor/ivacaftor 2 years before ETI as compassionate use, based on previous results of PDIOs which showed an improvement of Isc response after incubation with lumacaftor/ivacaftor.

After stimulation with tezacaftor/ivacaftor the organoids of cells carrying the G542X/3120+1kbdel16kb genotype (patient 5) showed a lower swelling response than the concomitant swelling response in a F508del/F508del control subject.

Analysis of CFTR-mediated chloride secretion was performed, by means of Isc measurements in Ussing chamber, on nasal epithelia derived from patient 1 carrying 2183AA > G and 3892delTT variants in trans, both causing the lack of or reduced expression of functional CFTR. Stimulation with a cAMP analogue produced a negligible Isc current, approximately equal to 1 μA (for comparison CFTR-mediated current in epithelia derived from non-CF subjects ranges between 20 and 30 μA). Treatment with ETI did not result in any functional recovery of CFTR (Figure 3).

3.3 | Effects of ETI

At baseline, 3 patients were on long-term oxygen. Of these, one required noninvasive ventilation (NIV) and was enlisted for lung transplantation. Median (IQR) number of exacerbations during the 2 months before ETI was 2 (2–3) requiring at least one hospitalization for 7 out of 10 patients. After 2 months of ETI, all patients showed a significant increase of FEV_1% predicted [median (IQR): +8.0 (3.5–12.7) percentage points ($p < 0.010$)], BMI [+0.85 (0–1.22) kg/m^2 ($p < 0.020$)] and CFQ-R [+ 19.5 (6.3–29.2) points ($p < 0.009$)]. A significant decrease of sweat chloride concentration [median (IQR) −11.2 (−1.7 to −34.0) mmol/L ($p < 0.020$)] and exacerbations number [−1.5 (−2 to −1) ($p < 0.008$)] were also recorded.

Comparison of FEV_1, BMI, CFQ-R, sweat chloride concentration and exacerbations number before and after ETI are reported in Table 2.

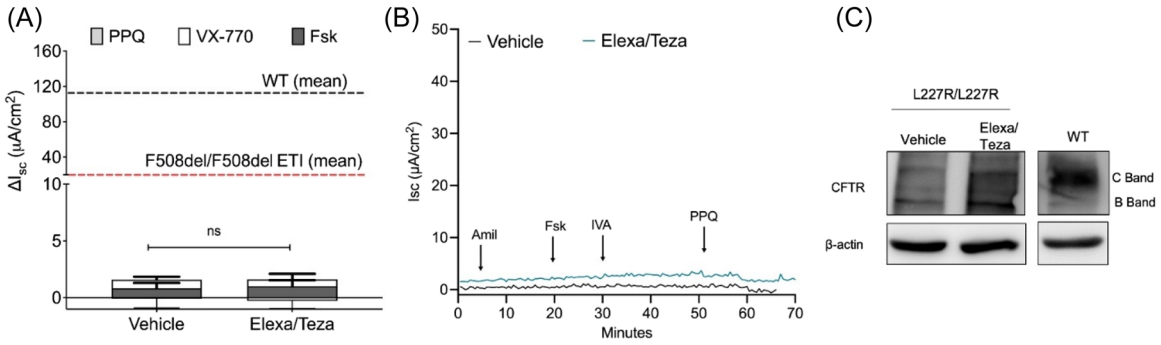

FIGURE 1 Representative Ussing chamber recordings of transepithelial current measurements in colonoids with the L227R/L227R genotype. Arrows indicate the addition of compounds: 100 μM apical amiloride (Amil), apical and basal 10 μM forskolin (Fsk), apical and basal 0.3 μM VX-770 (IVA) and apical and basal addition of 20 μM cystic fibrosis transmembrane conductance regulator (CFTR)-inhibition PPQ-102 (PPQ). (B) Representative tracings showing no evidence of detectable response to the agonists. Values were normalized by the surface area of the 2D filters. (C) Western blot analysis of CFTR and β-actin (loading control) expression in human rectal organoids after 24-h pretreatment with vehicle (DMSO 0,1%) and Teza (3 μM) together with Elexa (3 μM). C band: mature, complex-glycosylated CFTR; B band: immature, core-glycosylated CFTR. WT-CFTR was used as a reference. Note the absence of detectable bands associated with fully processed CFTR.

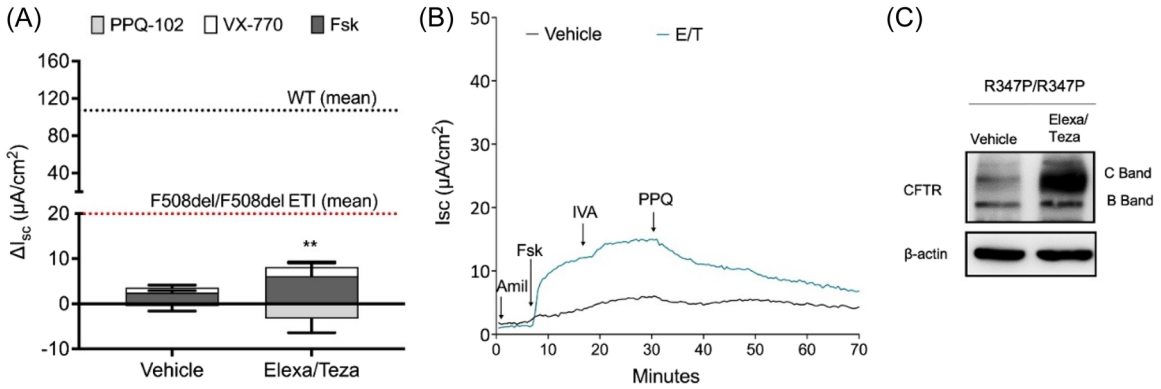

FIGURE 2 Representative Ussing chamber recordings of transepithelial current measurements in colonoids with the R347P/R347P genotype. Arrows indicate the addition of compounds: 100 μM apical amiloride (Amil), apical and basal 10 μM forskolin (Fsk), apical and basal 0.3 μM VX-770 (IVA) and apical and basal addition of 20 μM cystic fibrosis transmembrane conductance regulator (CFTR)-inhibition PPQ-102 (PPQ). (B) Representative tracings showing a significant response to the agonists. Values were normalized by the surface area of the 2D filters. (C) Western blot analysis of CFTR and β-actin (loading control) expression in human rectal organoids. (C) Western blot analysis of CFTR and β-actin (loading control) expression in colonoids after 24-h pretreatment with vehicle (DMSO 0,1%) and Teza(3 μM) together with Elexa (3 μM). C band: mature, complex-glycosylated CFTR; B band: immature, core-glycosylated CFTR.

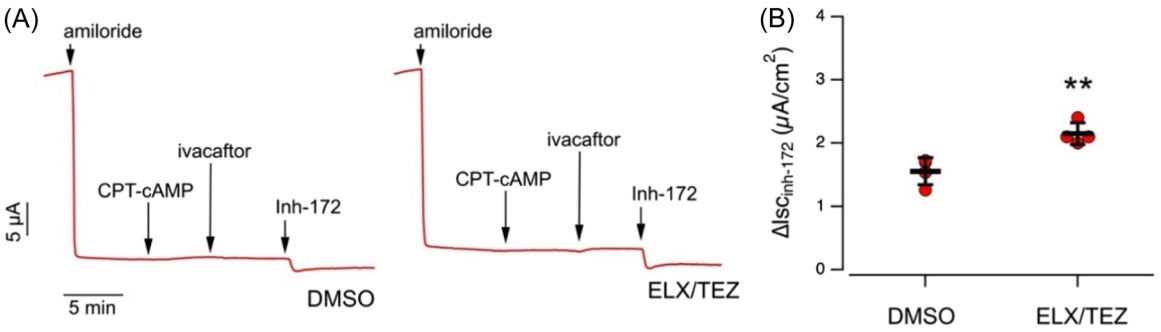

FIGURE 3 Functional evaluation of cystic fibrosis transmembrane conductance regulator (CFTR) activity and rescue by combined corrector treatment on nasal epithelia derived from the 2183AA > G/3892delTT CF patient. Representative traces of the effect of vehicle (DMSO), or the combinations of Elexa (ELX, 3 μM) with Teza (TEZ, 10 μM), on patient-derived nasal epithelial cells with the short-circuit current technique. During the recordings, the epithelia were sequentially treated (as indicated by downward arrows) with amiloride (10 μM; added on the apical side), CPT-cAMP (100 μM; added on both apical and basolateral sides), ivacaftor (1 μM; apical side) and the CFTR inhibitor-172 (inh-172; 20 μM; apical side). (B) Scatter dot plot showing the summary of results obtained from experiments described in (A).

TABLE 2 Comparison of FEV_1% predicted, BMI, sweat chloride concentration, CFQ-R and number of pulmonary exacerbations 2 months before and after treatment with ETI. Data are presented as median (IQR).

	Before ETI	After ETI	*p*
FEV_1% predicted	41 (25–52.5)	53.5 (29–77)	*<0.01*
BMI, kg/m^2	19.5 (16.5–21.6)	20.5 (16.8–22.3)	*<0.02*
Sweat chloride concentration, mmol/L	79.7 (69.1–105.8)	81.4 (39.3–84.6)	*<0.02*
CFQ-R	61.1 (43.0–75)	83.3 (75.0–88.9)	*<0.009*
Number of exacerbations in the past 2 months	2.0 (2.0–3.0)	0.5 (0–1.2)	*<0.008*

Abbreviations: BMI, body mass index; CFQ-R, cystic fibrosis questionnaire; ETI, elexacaftor/tezacaftor/ivacaftor; FEV_1, forced expiratory volume in the first second.

Four out 10 patients (patients 2, 7, 8 and 9) showed ≥10% increase in FEV_1 and 4 (patients 1, 4, 6 and 7) had ≥20 mmol/L decrease in sweat chloride concentration. Overall, 7 out of 10 participants were considered as responders. Two patients (patient 3 and patient 5) had a response below the chosen cut off in both lung function and sweat chloride concentration.

All patients reported cough disappearance ($n = 3$) or reduction ($n = 7$). Long-term oxygen was discontinued in 2 out of 3 patients; among these patients, one also stopped NIV and was removed from the lung transplantation waiting list. Regarding the safety profile of ETI, no adverse effect leading to ETI discontinuation was reported. Cutaneous rash occurred in one patient (patient n.2) and abdominal pain with diarrhoea in another patient (patient n.10).

Table 3 reports data describing clinical and theratyping response.

4 | DISCUSSION

In the present study, we showed that in pwCF with rare mutations not eligible for modulator therapy, ETI treatment resulted in an overall significant improvement in lung function, BMI, sweat chloride concentration, quality of life, cough and pulmonary exacerbations in the majority of cases. Notably, 7 out of 10 patients were responders to ETI treatment: 4 patients showed significant improvement (≥10%) in FEV_1 and 4 patients had significant improvement (≥20 mmol/L) in sweat chloride concentration.

All but two patients carried variants that are not currently approved by the FDA for ETI. The variants approved by FDA but not by EMA are R347P and R1066H, carried both in homozygous state by patients n.4 and n.10, respectively.

Since the majority of patients were heterozygous for CFTR variants, identifying which variant was responsive to ETI was not possible.

Nevertheless, excluding non-sense variants or variants without sufficient data in CFTR registries, on the basis of clinical, functional, and biological data as a whole, we hypothesize that variants 2183 AA > G, 4022insT, R347P, N1303K, 3849 + 10 kb C > T, 2789 + 5 G > A, W1282X, and R1066H were responsive to ETI.

Patient 4 was homozygous for R347P, a missense mutation included in class II, III and IV causing misfolding, gating defect and decreased conductance which results in reduced activity of less than 30% compared to the wild-type. Data obtained from PDIOs showed a significant increase in induced currents and mature CFTR following in vitro treatment with ETI combination (Figure 2), demonstrating the efficacy of ETI in this variant. Clinical data were consistent with in vitro results and showed a significant reduction in sweat chloride concentration (−35.3 mMol/L) and in pulmonary exacerbations and an increase in respiratory function (FEV_1 + 9%).

Patient 10 was homozygous for missense mutation R1066H, a residual function mutation often associated with pancreatic sufficiency. Based on in vitro studies, FDA has recently approved R1066H for ETI treatment. After ETI initiation, this patient had a significant improvement in lung function (FEV_1 + 9%); sweat chloride data were not available.

ETI effectiveness may not be limited to FDA-approved variants. Interestingly, 2 patients carrying the N1303K mutation showed different response to ETI. Patient n.6 was classified as a responder based on significant decrease in sweat chloride concentration (−33 mmol/L) without change in lung function.

In contrast, patient n.9, genotype N1303K/1248 + 1 G > C, was classified as responder based on a significant increase in FEV_1 (+15%), but not in sweat chloride concentration. Previous studies have reported good response to ETI in nasal epithelial cultures and rectal organoids from N1303K homozygotes[13,14,17,19,20] or heterozygotes.[11,13,18–20] Similar results were described in vivo,[14–16,22] with a distinction in those who carried N1303K together with non-sense variants like G542X or G550X, who had no improvement.[20] A recent study reported a general improvement in lung function but not in sweat chloride concentration after ETI in patients carrying N1303K in homozygosity or associated with nonsense variants.[14]

In patient 7, genotype G452X/3849 + 10 kb C > T, ETI demonstrated significant improvement in lung function and sweat chloride concentration. Patients with G452X/3849 + 10 kb C > T variant may carry variable amounts of abnormally spliced CFTR mRNA, leading to variability in phenotypic severity,[23] and which would presumably translate to CFTR modulator response heterogeneity.

Patient n.7 carrying variant W1282X associated with 2789 + 5 G > A showed significant increase in FEV_1 and BMI but no change in sweat chloride concentration. PwCF carrying W1282X variant have been described as nonresponder to ETI[13,15,20] but experiments reported conflicting results. In the patient's derived organoids, Ensick et al.[19] demonstrated increase in CFTR trafficking, density and function, while Lo Cicero et al.[21] showed no response. In this patient, the response to ETI may be due to the coexistence of 2789 + 5 G > A, a splicing variant that is able to produce an appreciable amount of functional CFTR. 2789 + 5 G > A variant is included between those with residual function, probably responder to potentiators. It is of note that our patient before ETI was successfully treated with ivacaftor.

In our study, we provided the first evidence of the effects of ETI in rare variants 2183AA > G/3892delTT (patient 1) and 4022insT/W1282G (patient 2). Patient 1 was a 15-years-old girl with severe phenotype with respiratory insufficiency requiring continuous oxygen therapy and with gastrostomy and enteral feeding due to very poor nutritional status (BMI < 15). This girl had the variant 2183AA > G, a frameshift mutation that strongly affects CFTR synthesis and is classified as a class I variant. The second variant, 3892delTT, is a very rare variant not reported in any CFTR database. The response to modulators on nasal cells of this patient was very poor, although statistically significant (Figure 3). Nevertheless, ETI was started before having the results of these assays due to the worsening clinical conditions of the patient. Surprisingly, after 2 months of ETI treatment, we recorded a significant reduction in sweat chloride concentration and number of exacerbations, increase in BMI and disappearance of cough.

Patient 2 (4022insT/W1282G) had improvement in lung function and BMI but no change in sweat chloride concentration. Of note,

TABLE 3 Individual values at baseline and after 2 months of modulating therapy with ETI in the study population. In bold: patients with positive response of FEV_1 and/or sweat chloride.

	Gender, age in years	CFTR variants	CFTR variants	Modulator before ETI	FEV_1% predicted		BMI, kg/m^2		Sweat chloride concentration, mmol/L		Cough intensity	*In vitro* response
					Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*		
1	F, 15.6	2183AA > G	3892delTT	N	25	29	14.7	15.2	112.8	43.8	Disappeared	No[a]
2	F, 52.6	4022insT	W1282G	N	45	77	19.3	20.3	90.2	81.4	Disappeared	na
3	M, 15	L227R	L227R	LUM/IVA	33	35	15.5	16.8	75.2	84.2	Decreased	No[b]
4	M, 16.2	R347P	R347P	N	46	55	16.8	16.8	74.3	39	Decreased	Yes[b]
5	F, 32	3120 + 1Kb del16Kb	G542X	N	33	40	17.3	17.3	98.7	87.5	Decreased	No[b]
6	F, 20.9	N1303K	G542X	N	81	77	19.7	20.9	114.9	82.1	Decreased	na
7	F, 28	3849 + 10KB C > T	G542X	N	41	54	33.1	35.3	63.9	39.1	Decreased	na
8	F, 35.7	2789 + 5 G > A	W1282X	IVA	41	53	19.9	20.6	49.8	39.6	Decreased	na
9	M, 38.2	N1303K	1248 + 1 G > C	N	35	50	20.2	21.2	79.7	85.1	Decreased	na
10	M, 46.7	R1066H	R1066H	N	72	81	25.7	25.6	35	na	Disappeared	na

Abbreviations: BMI, body mass index; CFTR, cystic fibrosis transmembrane conductance regulator; ETI, elexacaftor/tezacaftor/ivacaftor; F, female; FEV_1, forced expiratory volume in the first second; HNEC, human nasal epithelial culture; IVA, Ivacaftor; LUM/IVA, Lumacaftor/Ivacaftor; M, male; N, no; na, not available; PDIO, patient derived intestinal organoid.

[a]Data on HNEC.

[b]Data on PDIO.

patient 2 has been withdrawn from NIV and removed from the lung transplant waiting list.

Two patients out of 10 (patients 3 and 5) had no response with the chosen cut off,[15] neither in lung function nor in sweat chloride concentration. Patient 3 was homozygous for the very rare CFTR variant L227R, a position that resides in the transmembrane domain 1 (TMD1) that constitutes the channel pore, together with TMD2. Hence, the L227R/L227R genotype is expected to be associated with minimal CFTR function, which is confirmed by the clinical history of a severe phenotype, including pancreatic insufficiency. Data on PDIOs for cells of patient n. 3 (L227R/L227R) displayed a virtually undetectable current not modified by ETI treatment. Incubation with lumacaftor/ivacaftor showed a partial correction in ISc and in forskolin-induced swelling (FIS) assay. Consequently, we treated patient n. 3 with lumacaftor/ivacaftor for 2 years with success from a clinical point of view in terms of less hospitalizations due to exacerbations. However, because of the progression of pulmonary disease, we stopped lumacator/ivacaftor and switched to ETI since no other therapeutic options were available before lung transplantation.

Patient 5 was heterozygous for G542X with 3120 + 1Kb del16Kb; the first is a nonsense variant and the second consists in a large deletion; consequentially, they are both variants with a predicted minimal function. Intestinal organoids of patient 5 after stimulation with tezacaftor/ivacaftor showed a lower swelling response than the average F508del/F508del organoids. Therefore, because of poor clinical conditions of patient 5, we decided to start an experimental treatment with ETI. Both patients (3 and 5), despite the classification as non-responders to ETI, showed reduction in respiratory symptoms and in pulmonary exacerbations, suggesting that even a minor effect on CFTR might have positive clinical consequences that might not be recorded by a limited number of tissue-specific assays.

Regardless of the response to ETI, all patients were offered to continue ETI treatment.

The main limitations of this study are the small number of patients, the retrospective nature of the study and the fact that only four patients have in vitro data to demonstrate the activity of the modulator on CFTR activity. In addition, we did not provide any evidence of improvement of structural lung disease since chest magnetic resonance imaging (MRI) or computed tomography (CT) scans were not performed before and after ETI treatment.

In conclusion, we described the effects of ETI treatment in 10 CF patients with rare CFTR variants, not eligible for modulator therapy. Despite the limited number of cases, the response to CFTRm is not entirely predictable using in vitro tools, particularly when dealing with small, but clinically meaningful responses. Seven out of 10 cases can be classified as responders to therapy based on widely accepted outcomes. The results of the remaining 3 cases highlight the complexity of the disease with the potential discrepancy between biomarkers and clinical outcomes in specific cases and suggest the need for future studies to better understand the criteria to define CF patients as responders to ETI, independently of preclinical investigations carried out in cell-based models.

AUTHOR CONTRIBUTIONS

Valentina Fainardi: Writing—original draft; writing—review and editing; formal analysis; data curation. **Federico Cresta**: Investigation; writing—review and editing; methodology; data curation. **Claudio Sorio**: Investigation; writing—review and editing; methodology; formal analysis; data curation. **Paola Melotti**: Data curation; writing—review and editing; methodology; investigation. **Emanuela Pesce**: Methodology; investigation; data curation. **Michela Deolmi**: Investigation; data curation. **Francesco Longo**: Investigation; data curation. **Kleinfelder Karina**: Investigation; methodology; formal analysis. **Susanna Esposito**: Writing—review and editing; supervision. **Giovanna Pisi**: Conceptualization; investigation; writing—original draft; writing—review and editing; supervision; data curation; formal analysis; methodology.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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