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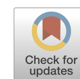
ARTICLE

CFTR rescue in W1282X cystic fibrosis patient-derived intestinal organoids (PDIOs) mediated by translational readthrough-inducing drugs (TRIDs)



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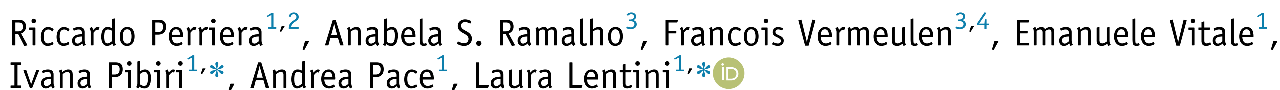

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ABSTRACT

Purpose: Pathogenic variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene result in dysfunctions of the CFTR protein, leading to cystic fibrosis (CF). This genetic disorder is characterized by severe symptoms in the respiratory and digestive systems.Currently, highly effective CFTR modulator treatments, such as the Elexacaftor-Tezacaftor-Ivacaftor combination, may represent the primary therapeutic option for approximately 82% of people with cystic fibrosis who have at least 1 F508del variant. However, the remaining 18% with rare *CFTR* variants, including nonsense variants, often lack access to these therapies. Nonsense variants lead to nonfunctional CFTR proteins and contribute to more severe CF symptoms. Research efforts focus on understanding the effects of these variants on disease severity and response to treatment.
Methods: This study utilizes patient-derived intestinal organoids to evaluate the recovery of CFTR function in cells with nonsense variants.
Results: Specifically, we tested 3 translational readthrough-inducing molecules: NV848, NV914, and NV930. Our studies highlighted the positive effect of NV848 on patient organoid swelling, improving CFTR channel function, whereas NV914 and NV930 did not induce organoid swelling, similar to PTC124 treatment.
Conclusion: In conclusion, this study highlights the potential of translational readthrough-inducing molecules to restore CFTR function in cells with nonsense variants. By leveraging patient-derived intestinal organoids, our findings showed that NV848, in combination with Elexacaftor-Tezacaftor-Ivacaftor and the nonsense-mediated mRNA decay inhibitor NMDI14, enhances CFTR activity. This contributes to the development of personalized therapies for individuals with rare *CFTR* variants, addressing a critical unmet need in cystic fibrosis treatment.

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Introduction

In vitro research has recently shifted toward more complex systems, surpassing the classic cell-based monolayer models (2D cell cultures) in the study of human biology and diseases. However, it still represents a fundamental step. The evolution of 2D cell cultures to 3-dimensional (3D) in vitro organoid cultures, which mimic the structure and physiology of several organs in vivo and consider the patient's genetic background, is a significant step forward.[1,2] Organoids are 3D, organ-like structures derived from stem cells, including human induced pluripotent stem cells or tissue-derived adult multipotent stem cells. These "miniature organs" exhibit cell differentiation, self-organization, and functionality similar to the corresponding organs in the human body.[3] The organ-like architecture and functionality of organoids offer a unique system for studying organ physiology and pathology.[4,5]

One of the key advantages of organoids is their ability to recapitulate the complexity and heterogeneity found in real organs while maintaining the genetic background of the person from which they are derived.[6] Patient-derived intestinal organoids (PDIOs) derived from crypts present in rectal biopsies primarily consist of multipotent adult epithelial stem cells with high expression of cystic fibrosis transmembrane conductance regulator gene (*CFTR*) (HGNC:1884), which is reduced after cell differentiation.[7] This in vitro 3D model appears as a sphere, with different cellular types constituting its surface, including multipotent and progenitor cells, enterocytes, goblet cells, and Paneth cells interacting in a physical matrix composed of Matrigel (70%). At the same time, the inner part is composed of the lumen of the organoid, which consists of the apical side of the cells, in which *CFTR* is expressed, and the outer part, or basolateral side, of the cells' layer. PDIOs are an innovative in vitro system for studying intestinal function, intestinal-related diseases, and drug discovery screening.[7]

Cystic fibrosis (CF) is an autosomal recessive genetic disorder caused by variants in the *CFTR* gene, which is expressed on the apical side of epithelial cells and functions as an anion-selective channel.[8] The CFTR protein regulates ion transport, specifically Cl- and HCO3-. It maintains water flow through the plasma membranes of numerous secretory epithelial cells, including those in the airways, intestines, pancreas, kidneys, sweat glands, and male reproductive tract.[9] The absence or alteration of CFTR function leads to dehydration of the mucus layer secreted in several organs' airways and the ducts. This abnormal condition results in nutrient absorption defects, inflammation, duct obstruction, and tissue damage, primarily in the pancreas, lungs, and intestines.[10]

Nowadays, highly effective CFTR modulator treatments, such as Elexacaftor-lumacaftor-ivacaftor (ETI), may represent the primary therapeutic option for approximately 82% of people with CF who carry at least one F508del variant [NC_000007.14: g.117559593_117559595del NM_000492.4:c.1521_1523del NP_000483.3:p.Phe508-del].[11] However, the remaining 18% have rare *CFTR* variants that are not eligible for ETI in most countries because of genotype incompatibility, including nonsense variants (corresponding to around 8% of all *CFTR* alleles).[12]

Nonsense variants introduce a premature termination codon (PTC) instead of a canonical amino acid codon into the *CFTR* mRNA sequence.[13] The consequence is the production of a truncated and nonfunctional polypeptide that is signaled for degradation. However, this does not occur in most cases because the mRNAs harboring the PTCs are prematurely degraded by the nonsense-mediated mRNA decay pathway (NMD), a cellular quality control system.[14]

A strategy to restore the correct translation of nonsense-related *CFTR* mRNAs involves the misreading promotion of the PTC by translational readthrough.[15] The translational readthrough is a mechanism in which a near-cognate transfer RNA is inserted into the A site of the ribosome instead of release factors during the transition of the ribosome on the premature translation termination (PTC). In this case, the resulting protein is full-length and will not be degraded.

Strategies to counteract nonsense variants via NMD inhibition aim to stabilize nonsense-containing mRNAs, allowing for the translation of partially functional proteins. Small-molecule inhibitors, eg, Amlexanox[16] and NMDI14,[17] targeting NMD components, such as UPF1, are key to this approach and can be combined with readthrough agents to enhance protein synthesis from mutated genes. However, NMD inhibition poses risks because it may accumulate defective mRNAs, producing truncated or dysfunctional proteins because of its central role in mRNA quality control.[18]

Several translational readthrough-inducing drugs (TRIDs) have been identified (eg, aminoglycoside antibiotics, PTC124, ELX-02) during the last decades. Still, none of these have been able to reach defined outcomes in CF clinical trials.[19-21]

Recently, 3 new TRIDs were identified and tested in nonsense-related diseases by 2D in vitro systems with promising results. These compounds comprise an oxadiazole core and different substituents for each molecule: NV848, NV914, and NV930 (NV molecules).[22,23] Considering the limits of the pharmacological approach to suppress nonsense variants, this encouraging evidence must be verified in more complex CF experimental systems to confirm the readthrough efficacy of the NV848, NV914, and NV930.

Table 1 Molecule preparation

Compound	Solvent	Stock Concentration	Final Concentration	Manufacturer	Catalog Number
ELX-02	H_2O	8mM	80 mM	MedChem Express (MCE)	HY-114231
PTC124	DMSO	100 mM	10 mM	MedChem Express (MCE)	HY-14832R
NV848	H_2O	100 mM	12-24-48	a	a
NV914	DMSO	100 mM	12 mM	a	a
NV930	DMSO	100 mM	12-24-48 mM	a	a
VX-445 (Elexacaftor)	DMSO	10 mM	3 mM	Selleckchem	S8851
VX-661 (Tezacaftor)	DMSO	20 mM	3 mM	Selleckchem	S7059
VX-770 (Ivacaftor)	DMSO	20 mM	3 mM	Selleckchem	S1144
NMDI14	DMSO	6.25 mM	0.625 mM	MedChem Express (MCE)	HY111374

[a]All compound stocks were stored at −20 °C.

CFTR is highly expressed in colon tissue, and PDIOs are an excellent in vitro system for studying the functionality of the channel through specific assays, such as the "swelling assay."[24] The forskolin-induced swelling (FIS) assay allows the quantification of the CFTR function in PDIOs after treatment with specific compounds to restore the function of the mutated protein channel.[25,26] A positive FIS assay result using CF patients-derived organoids could predict the clinical response to treatment in that patient.[27] This technique's use of patient-specific samples is a significant advantage, particularly for analyzing rare CF genotypes and implementing personalized clinical approaches.[28] PDIOs are among the most reliable in vitro models for assessing CFTR protein functionality.[29]

This work utilized PDIOs derived from patients carrying *CFTR* nonsense variants to assess the rescue of CFTR activity after treatment with NV molecules. Two different and prevalent nonsense variants were selected for the study: NC_000007.14:g.117199644C>A NM_000492.4:c.1624G >T NP_000483.3:p.Gly542Ter (G542X) and NC_000007. 14:g.1172 27834G>A NM_000492.4:c.3846G>A NP_00 0483.3:p.Trp1282Ter (W1282X).

The intestinal organoids were treated with NV848, NV914, or NV930, and the FIS assay was performed to quantify the rescue of CFTR activity. Furthermore, the CFTR modulator compounds Elexacaftor, Tezacaftor, and Ivacaftor were combined with NV molecules to improve the CFTR functionality and protein stability.[11,30] Finally, the NMD pathway was inhibited with NMDI14 to test the possible synergy with the translational readthrough mediated by NV molecules.

Materials and Methods

Compounds

NV848, NV914, and NV930 were prepared as reported in the literature (patents: WO2019101709A1 [IT], US20210002238A1 [US], and EP3713934B1 [EU][31]) or purchased as indicated in Table 1. Compounds were dissolved in DMSO (dimethyl sulfoxide, Sigma-Aldrich) or sterile water (H_2O) at the indicated stock concentrations.

Human intestinal organoids

Samples of rectal mucosa tissues from people with CF were obtained by suction biopsy during a routine clinic visit at the UZ Leuven University Hospital (Herestraat 49, 3000 Leuven, Belgium). This study was approved by the University Hospital Leuven Ethics Review Board (S56329). All patients/parents gave written informed consent and/or assent. The biopsies were stored in ADF+++ and kept in ice until crypt isolation (see Vonk et al[32] for details).[25]

Crypts were isolated from the rectal biopsies and subsequently mixed with 50% matrigel (diluted in cold ADF+++) and plated on 24-well plates; human organoid expansion medium (HM: A83-01 500 nM, SB202190 p38 inhibitor 10 μM, EGF 50 ng/ml, Nicotinamide 10 mM, N-Acetylcysteine 1.25 mM, B27 supplement 2%, Wnt3A conditioned medium 50%, fcNoggin conditioned medium 10%, RspoI conditioned medium 20%, ADF+++ 20%) was added after the solidification of matrigel (0.5 ml per well). The medium was changed every day. Organoids were split by mechanical disruption after 7 days in culture. Two different *CFTR* genotypes were selected for the study: $CFTR^{G542X/G542X}$ and $CFTR^{W1282X/del2,3}$ NC_000007.14:g. 117199644C>A NM_000492.4:c.1624G>T NP_000483.3: p.Gly542Ter (G542X/G542X) and NC_000007.14:g.11749 8309_117519392del NM_000492.4:c.54-5944_273+10250 del (del2,3/W1282X).

Organoids CFTR activity by FIS assay

Briefly, organoids (between the 4th and 20th passages) were seeded onto 96-well plates in 4 μL of 50% Matrigel drops. Each drop, containing 15 to 60 organoids, was covered with 50 μL of HM supplemented with the compounds to be incubated for 48 hours. The compounds to be incubated for 24 hours were supplemented the next day.

Green calcein (Invitrogen) was added to stain the organoids (0.02 mg/μL) on the day of the

FIS assay experiment. Subsequently, forskolin (5 μM) was added to the organoids to stimulate CFTR activity, which was immediately analyzed by confocal live-cell microscopy (LSM800, 5× objective; Zeiss). Every 10 minutes (from 0 to 60 minutes, t0-t60), a picture was taken of each 96 wells. The total organoid area (*xy* plane) per well and time point was automatically quantified using Zen Blue analysis software (Zeiss) and normalized to the area at t_0.

To test the rescue of CFTR function by CFTR modulator compounds, the organoids were preincubated for 48 hours with 3 μM Elexacaftor (VX-445; Selleckchem) and 3 μM Tezacaftor (VX-661; Selleckchem). Additionally, NV compounds (NV848, NV914, and NV930) were applied at various final concentrations (12, 24, or 48 μM NV848, NV930, and 12 μM NV914) for 48 hours, either individually or in combination with VX-445 and VX-661. In a separate experiment, the NMD inhibitor (NMDI14; final concentration of 0.625 μM; MedChemExpress) was combined with the NV compounds and/or ETI. 3 μM Ivacaftor (VX-770; Selleckchem) was added as a CFTR potentiator in combination with forskolin. Within each organoid experiment, every test condition was assessed in duplicate. For each organoid genotype, 2 independent experiments were performed on separate days. Reported values correspond to the average area under the curve (AUC) calculated from plots representing the mean percentage of organoids swelling from t0 to t60 (60 min) and the standard error of the mean from the 2 independent experiments.

The CFTR function was determined by measuring the organoid swelling after adding forskolin.

Data analysis

The experiments were performed at least in duplicate ($n = 2$, experimental replicates) and using 2 technical replicates for each analysis. All data are expressed as mean values ± standard error of the mean (SEM). The students' *t* tests performed statistical analysis and 1- and 2-way ANOVA when appropriate, using GraphPad Prism software version 7.0.0 for Windows. A probability value (*P*) of less than .05 was regarded as significant and indicated in relevant graphs as 1 symbol (*) for $P < .05$, 2 symbols (**) for $P < .01$, 3 symbols (***) for $P < .001$, and 4 symbols (****) for $P < .0001$.

Variant validator

Variants were validated on: https://variantvalidator.org/

Results

Intestinal organoids derived from patients carrying *CFTR* nonsense variants (*CFTR*$^{G542X/G542X}$ and *CFTR*$^{W1282X/dele2,3}$) were used to evaluate CFTR protein activity after treatment with NV molecules (NV848, NV914, and NV930) and in combination with CFTR modulators.

Data show 2 different treatment responses. In *CFTR*$^{G542X/G542X}$ organoids (Figures 1A and 2), the aminoglycoside ELX-02 (positive control) alone and in combination with ETI show a higher increase in the organoids' area (43.6%) compared with the same treatment in *CFTR*$^{W1282X/dele2,3}$ organoids (15.3%) (Figures 1B and 3).

Regarding TRIDs-treated samples, no swelling was detected in *CFTR*$^{G542X/G542X}$ organoids (Figure 1A). However, a visible swelling appeared in *CFTR*$^{W1282X/dele2,3}$ organoids (Figures 1B and 3) using TRIDs in combination with CFTR modulators. In both genotype samples and all experimental conditions, PTC124 did not induce organoid swelling.

FIS assays were repeated in *CFTR*$^{G542X/G542X}$ and *CFTR*$^{W1282X/dele2,3}$ organoids under the same conditions (Figures 4A, B, 5, and 6), using the NMD inhibitor NMDI14 (Figures 4A, B, 5, and 6).

Regarding ELX-02 treatment, data indicate no significant change in AUC (0.85%) between the experiment without NMDI14 (Figure 1A) and with the addition of the NMD inhibitor (Figure 4A) alongside ETI treatment. NV molecules treatment did not induce organoid swelling in the presence of NMDI14 (Figure 4A) in *CFTR*$^{G542X/G542X}$ organoids.

On the contrary, by inhibiting the NMD pathway through NMDI14 in *CFTR*$^{W1282X/dele2,3}$ organoids, it is possible to detect an increase in AUC in samples treated with TRIDs and CFTR modulators (Figures 4B and 6), compared with the FIS assay without NMDI14 (Figure 1B).

Finally, the percentage of the increasing area during FIS assays in *CFTR*$^{W1282X/dele2,3}$ organoids evidenced the difference between the CFTR rescue mediated by the most potent NV molecule (NV848) and PTC124 treatment in combination with ETI (Figure 7A and B). The same result was visible using the NMD inhibitor NMDI14 (Figure 7C and D).

Discussion

The heterogeneity among *CFTR* variants complicates the identification of a unique CF treatment. In alignment with the principles of precision medicine, which emphasize tailored treatments by identifying a targeted therapy for each specific variant, this study provides essential information about CFTR activity rescue in advanced in vitro systems related to nonsense variants.

This work aimed to study the effect of the 3 NV molecules in CF human organoids harboring a *CFTR* nonsense variant. PDIOs represent an important preclinical in vitro system for estimating a molecule's efficacy in rescuing CFTR activity. Our recent work has demonstrated *CFTR* expression and biochemical maturation in the CF mouse model used to assess protein rescue,[33] thereby supporting its relevance for functional evaluation. Based on this validation, this study focused

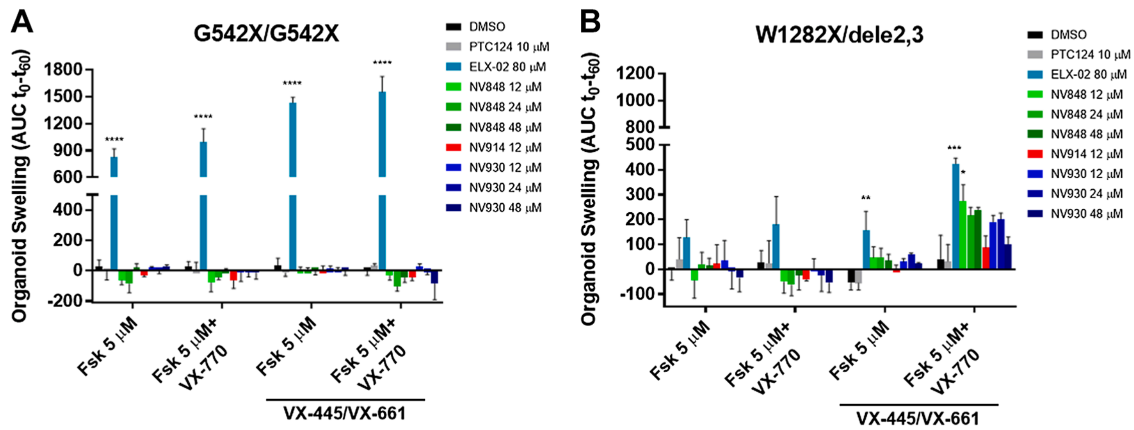

Figure 1 **Measure of the organoid swelling induced by NV molecules alone or in combination with modulators.** *CFTR*$^{G542X/G542X}$ (A) and *CFTR*$^{W1282X/dele2,3}$(B) organoids were treated for 48 hours with DMSO (negative control), PTC124, ELX-02 (positive control), NV848, NV914, and NV930 at indicated concentrations. CFTR correctors (VX-445 Elexacaftor and VX-661 Tezacaftor; 3 μM) and CFTR potentiator (VX-770 Ivacaftor; 3 μM) were added to increase CFTR activity after readthrough rescue. Samples were analyzed and compared with each DMSO negative control (DMSO). Probability value (*P*): 1 symbol (*) for $P < .05$, 2 symbols (**) for $P < .01$, 3 symbols (***) for $P < .001$, and 4 symbols (****) for $P < .0001$. The experiment was performed in duplicate.

on investigating the pharmacological response of CFTR to TRID compounds using functional assays.

NV molecules were tested in 2 different organoid genotypes (G542X/G542X and W1282X/dele2,3) in combination with CFTR modulators, either alone or in conjunction with ETI, and with or without an NMD pathway inhibitor compound.

In all conditions, NV848, NV914, and NV930 did not work in *CFTR*$^{G542X/G542X}$ organoids; however, the combination of each NV molecule with CFTR modulators partially restored CFTR activity in *CFTR*$^{W1282X/dele2,3}$ organoids, especially when using NV848.

Moreover, treatment with NV848, NV914, and NV930, in combination with CFTR modulators and an NMD inhibitor (NMDI14), enhanced the effect of NV molecules in *CFTR*$^{W1282X/dele2,3}$ organoids.

Interestingly, the commercial readthrough agent PTC124 (Ataluren) was ineffective in *CFTR*$^{W1282X/dele2,3}$ organoids, unlike the NV848 treatment, which increases channel activity. The PTC124 compound is the only readthrough agent used in clinics to treat other nonsense-related pathologies (eg, Duchenne muscular dystrophy).[34] NV848 has been designed by optimization of the lead PTC124; regrettably, PTC124 does not work in the rescue of CFTR protein functionality in nonsense-related organoids test, whereas results obtained in CF nonsense-related organoids by treatment with NV848 are interesting (Figure 3). The 3 different concentrations of NV848 (12, 24, and 48 μM) produced similar positive effects as previously observed in FRT *CFTR* ^{W1282X} cells,[23] on organoid swelling, contrasting with the limited impact observed with PTC124 treatment (10 μM; the highest nontoxic concentration), thus highlighting the ability of NV848 to enhance CFTR activity. Notably, the copy of the allele harboring the *CFTR* exon 2-3 deletion [NC_000007.14:g.117498309_117519392del NM_000492.4:c.54-5944_273+10250del] does not yield an increase in functional CFTR protein. Neither NV914 (12 μM; the highest nontoxic concentration) nor NV930 (12, 24,

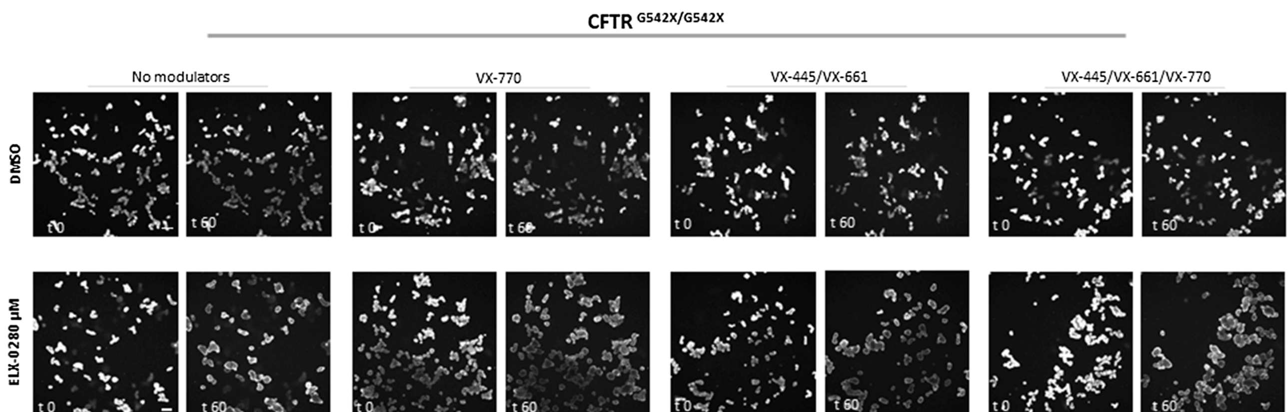

Figure 2 **Representative images of statistically significant** ***CFTR***$^{G542X/G542X}$ **samples and negative controls (DMSO) during the forskolin-induced swelling (FIS) assay.** The organoid area was measured at baseline (t0) and after 60 minutes of forskolin (5 μM) stimulation (t60). Scale bar: 200 μm.

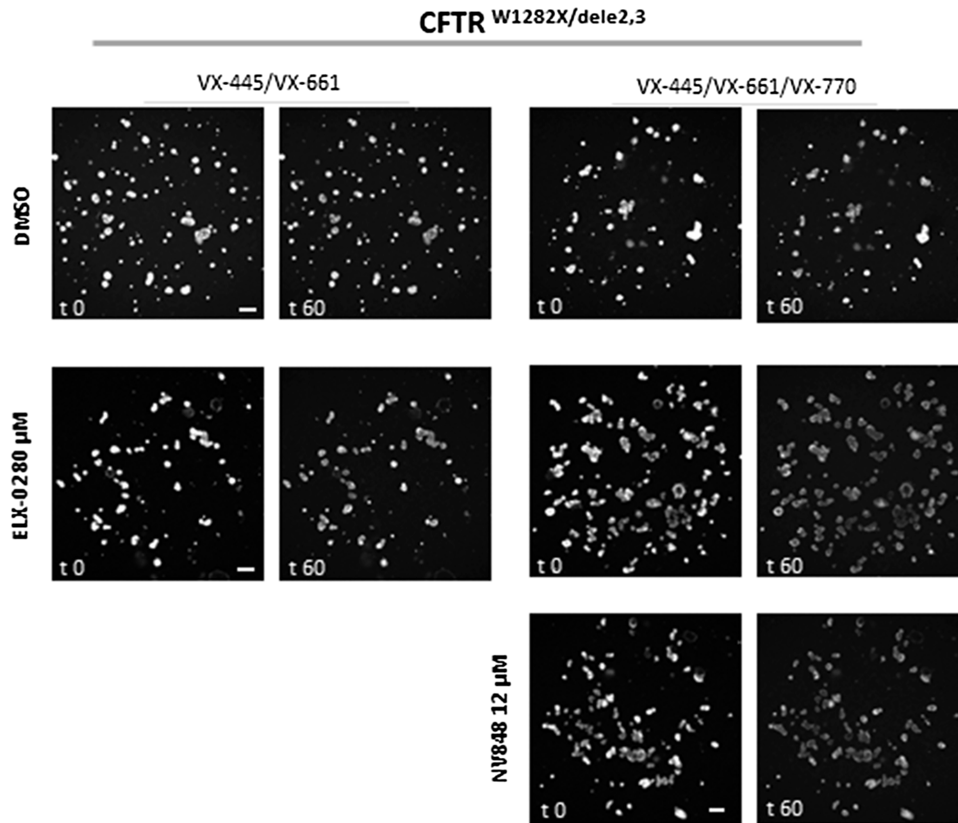

Figure 3 **Representative images of statistically significant *CFTR*$^{W1282X/dele2,3}$ samples and negative controls (DMSO) during the forskolin inducing swelling (FIS) assay.** The organoid area was measured at baseline (t0) and after 60 minutes of forskolin (5 μM) stimulation (t60). Scale bar: 200 μm.

or 48 μM) induced organoid swelling, similar to the lack of response to PTC124 treatment.

Additionally, the readthrough activity of NV848 synergizes with NMD pathway inhibition, which degrades typically nonsense mRNAs and thereby limits the NV848 effectiveness. CFTR activity increases when organoids are treated with NV848 and ETI in combination with the NMD inhibitor NMDI14. NMDI14 disrupts the interaction between UPF1 and SMG7, 2 key protein factors in the NMD pathway.[17,35]

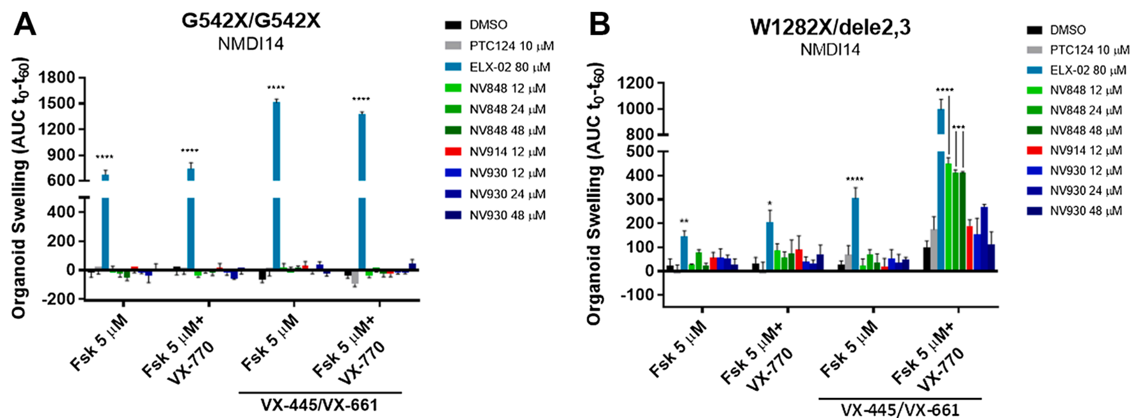

Figure 4 **Measure of the organoid swelling induced by NV molecules alone or in combination with modulators in presence of NMD inhibitor.** *CFTR*$^{G542X/G542X}$ (A) and *CFTR*$^{W1282X/dele2,3}$(B) organoids were treated for 48 hours with DMSO (negative control), PTC124, ELX-02 (positive control), NV848, NV914, and NV930 at indicated concentrations. All samples were treated with NMD inhibitor NMDI14 (0.625 μM). CFTR correctors (VX-445 Elexacaftor and VX-661 Tezacaftor; 3 μM) and CFTR potentiator (VX-770 Ivacaftor; 3 μM) were added to increase CFTR activity after readthrough rescue. Samples were analyzed and compared with each DMSO negative control (DMSO). Probability value (*P*): 1 symbol (*) for $P < .05$, 2 symbols (**) for $P < .01$, 3 symbols (***) for $P < .001$, and 4 symbols (****) for $P < .0001$. Experiment was performed in duplicate.

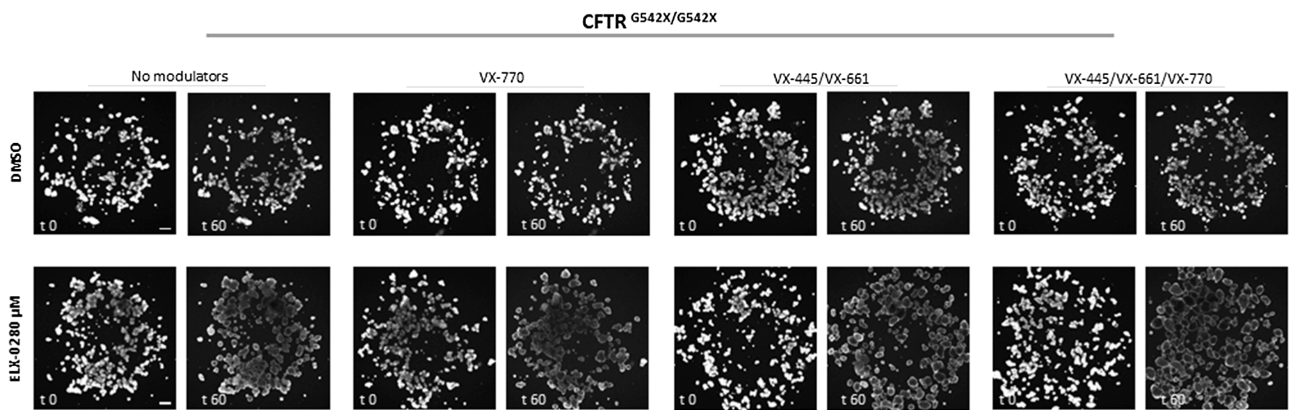

Figure 5 Representative images of statistically significant *CFTR*$^{G542X/G542X}$ samples and negative controls (DMSO) during the forskolin-induced swelling (FIS) assay. All samples were treated with NMDI14 (0.625 μM). The organoid area was measured at baseline (t0) and after 60 minutes of forskolin (5 μM) stimulation (t60). Scale bar: 200 μm.

The findings obtained in this work added new evidence regarding the study of small molecules capable of restoring the functionality of CFTR in CF nonsense-related in vitro systems.

The synergistic effect of the added NMD inhibitor underscores the necessity for an increased mRNA substrate to support readthrough promoter activity, which may explain the lack of efficacy in cases in which the nonsense codon occurs too early in the sequence. Furthermore, the observed synergy with CFTR modulators suggests that readthrough likely converts a nonsense variant into a missense variant. This highlights the critical importance of using robust models to evaluate the functionality of the rescued protein following readthrough treatment.[36]

The identification of new effective compounds against *CFTR* nonsense variants remains one of the most promising avenues in the pharmacological field, especially given the significantly promising compound (ELX-02), which, despite positive in vitro 3D results, failed a recent clinical trial (ClinicalTrials.gov Identifier: NCT04135495). NV molecules exhibit a chemical structure distinct from aminoglycoside ELX-02 and have demonstrated good tolerability in mouse animal models.[37,38] In addition, in vitro studies on the readthrough side effects of NV molecules on natural termination codons are very promising,[39] increasing expectations for these 3 molecules in treating patients with nonsense variants.

Moreover, it is essential to highlight that the outcome of the preclinical development stage of these prospective drugs

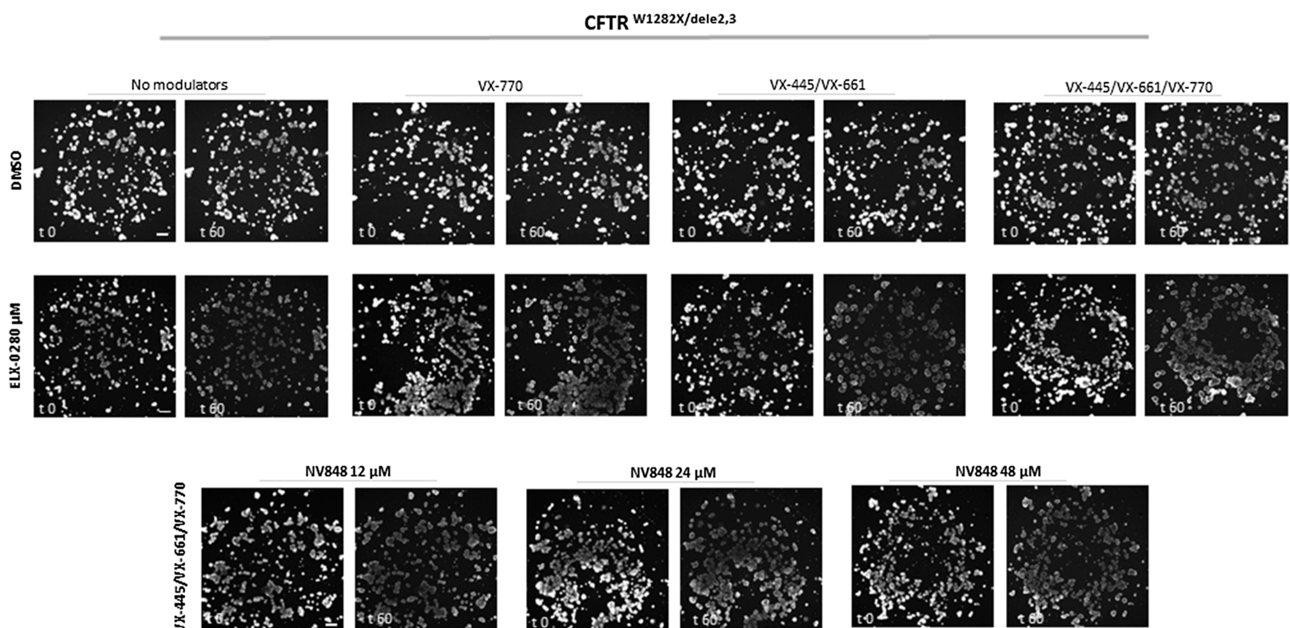

Figure 6 Representative images of statistically significant *CFTR*$^{W1282X/dele2,3}$ samples and negative controls (DMSO) during the forskolin-induced swelling (FIS) assay. All samples were treated with NMDI14 (0.625 μM). Organoid area was measured at baseline (t0) and after 60 minutes of forskolin (5 μM) stimulation (t60). Scale bar: 200 μm.

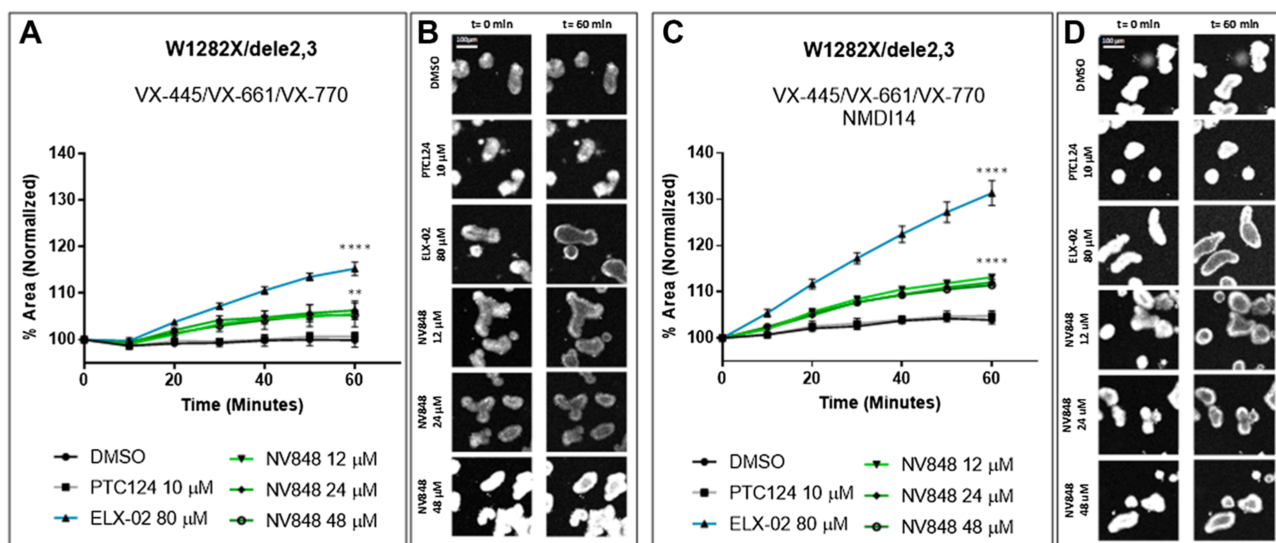

Figure 7 **Measure of the percetage of organoid area variation induced by NV848 molecule.** (A-C) Percentage of total *CFTR*$^{W1282X/dele2,3}$ organoids area (1 hour of FIS), 48 hours after treatment with DMSO (negative control), PTC124, ELX-02 (positive control), and NV848 at indicated concentrations in combination with CFTR modulators (VX-445 Elexacaftor; VX-661 Tezacaftor; VX-770 Ivacaftor; 3 μM). NMD pathway inhibitor NMDI14 (0.625 μM) increased TRIDs' efficacy (C). Samples were analyzed and compared with each PTC124 treatment (10 μM). (B-D) Representative confocal images (calcein staining) of human intestinal organoids (W1282X/dele2,3) during FIS assay (0 min to 60 min). Probability value (P): 2 symbols (**) for $P < .01$ and 4 symbols (****) for $P < .0001$. Scale bar: 100 μm. The experiment was performed in duplicate.

would benefit several patients affected by various genetic diseases due to nonsense variants, thereby enlarging the cohort of patients and being particularly helpful, especially for ultrarare diseases still orphaned of a cure.

A limitation of this study is the lack of comparative data on PDIOs carrying compound heterozygous genotypes (ie, a PTC and a rescuable missense variant), which would allow a direct comparison between the efficacy of TRIDs combined with CFTR modulators versus CFTR modulators alone. Although such an approach would further clarify the therapeutic contribution of TRID compounds, appropriate patient-derived models were not available at the time of this study. Future work will aim to include PDIOs with broader genotypic representation to address this critical aspect.

In conclusion, the results support the potential of NV molecules, particularly NV848, as candidates for further preclinical investigation in the context of *CFTR* nonsense mutations, while emphasizing the need for additional studies to better define their efficacy and therapeutic relevance.

Data Availability

All data described in this study can be obtained from the authors upon request.

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Author Contributions

Conceptualization: L.L., R.P., A.S.R.; Methodology: R.P., A.S.R., F.V.; Data Curation: R.P., A.S.R., I.P., L.L., A.P.; Investigation: R.P., A.S.R., F.V.; Formal Analysis: R.P., A.S.R.; Validation: L.L., R.P., A.S.R., I.P.; Resources: L.L., I.P.; Writing-original draft: L.L., R.P., A.S.R., I.P.; Writing-review and editing: L.L., R.P., A.S.R., I.P., F.V., E.V., A.P.; Supervision: L.L., I.P., A.S.R. All authors reviewed and approved the manuscript before submission.

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Ethics Declaration

This project was approved by the University Hospital Leuven Ethics Review Board (S56329).

All patients and parents gave written informed consent and/or assent.

Conflict of Interest

Ivana Pibiri, Laura Lentini, and Andrea Pace have patent licenses to WO2019101709. All other authors declare no conflicts of interest.

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