Original Article

# Antisense oligonucleotide splicing modulation as a novel Cystic Fibrosis therapeutic approach for the W1282X nonsense mutation

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ABSTRACT

Background: Antisense oligonucleotide- based drugs for splicing modulation were recently approved for various genetic diseases with unmet need. Here we aimed to generate skipping over exon 23 of the CFTR transcript, to eliminate the W1282X nonsense mutation and avoid RNA degradation induced by the nonsense mediated mRNA decay mechanism, allowing production of partially active CFTR proteins lacking exon 23.
Methods: ~80 ASOs were screened in 16HBEge W1282X cells. ASO candidates showing significant exon skipping were assessed for their W1282X allele selectivity and the increase of CFTR protein maturation and function. The effect of a highly potent ASO candidates was further analyzed in well differentiated primary human nasal epithelial cells, derived from a W1282X homozygous patient.
Results: ASO screening led to identification of several ASOs that significantly decrease the level of CFTR transcripts including exon 23. These ASOs resulted in significant levels of mature CFTR protein and together with modulators restore the channel function following free uptake into these cells. Importantly, a highly potent lead ASOs, efficiently delivered by free uptake, was able to increase the level of transcripts lacking exon 23 and restore the CFTR function in cells from a W1282X homozygote patient.
Conclusion: The highly efficient exon 23 skipping induced by free uptake of the lead ASO and the resulting levels of mature CFTR protein exhibiting channel function in the presence of modulators, demonstrate the ASO therapeutic potential benefit for CF patients carrying the W1282X mutation with the objective to advance the lead candidate SPL23–2 to proof-of-concept clinical study.

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## 1. Introduction

Cystic Fibrosis (CF) disease is caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, with >2000 variants in the CFTR gene identified of which >400 are known to cause disease [1]. Recent development of CFTR modulators to increase CFTR quantity and function have been approved for some CFTR mutations [2]. However, the approved CFTR modulators are not effective for all patients with CF [3] leaving an unmet need for drug development for those patients with non-responsive mutations. These mutations include nonsense mutations.

The CFTR protein is composed of two membrane-spanning domains (MSD1 and MSD2; two cytosolic nucleotide binding domains (NBD1 and NBD2) and a regulatory domain (RD) [4]. NBD2 is the last domain to be translated [4,5] and correct inter-domain assembly is essential to form a stable CFTR unit that satisfies quality control in the endoplasmic reticulum [6]. CFTR proteins truncated at their C-terminus, and completely lacking NBD2, are ultimately transported to the cell surface where they form characteristic CFTR chloride channels with low opening probability; therefore NBD2 is not essential for CFTR maturation

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but the protein is only partially functional [7] and the addition of CFTR modulators improves the function of these truncated proteins [8].

This study focuses on a minimal function class I nonsense mutation, W1282X, one of the ten most prevalent CFTR mutations and is associated with a severe form of the disease [1,9]. The W1282X nonsense mutation generates a premature termination codon (PTC) and thus the transcripts are subjected to degradation by the nonsense-mediated mRNA decay (NMD) mechanism [10–12], which detects and degrades such transcripts, preventing the synthesis of truncated proteins [10,11]. NBD2 is translated from mid-exon 22 to the beginning of exon 26, with the W1282X mutation located in exon 23. As this exon is "in frame", it can be removed without causing a frameshift. This project aimed to generate skipping over exon 23 of the CFTR transcript, in order to eliminate the PTC created by the W1282X mutation, avoid RNA degradation induced by the NMD mechanism, and allow residual active CFTR protein albeit deleted for exon 23.

Antisense oligonucleotides (ASOs) are small synthetic nucleic acid molecules able to bind specific sequences within target RNA molecules. ASOs are used for a variety of applications including splicing modulation by enhancing retention or skipping of a specific exon. The use of ASOs is highly specific allowing manipulation of only the targeted exon.

In this study we designed ASOs targeted to skip over exon 23. Screening of these ASOs in W1282X cells identified significantly decreased CFTR transcripts that included exon 23 and increased CFTR transcripts lacking this exon. The proteins translated from the exon 23-skipped variants are mature. Importantly, we demonstrate that lead ASOs (SPL23–2 and SPL23–3), together with CFTR modulators, restore CFTR channel function in primary human nasal epithelia cells from a patient homozygous for W1282X. These results highlight the potential of skipping over exon 23 as a potential therapy for CF patients carrying the W1282X mutation, with the aim to advance SPL23–2 into clinical studies.

## 2. Materials and methods

### 2.1. Cell lines

The 16HBEge CFTR W1282X and F508del cell lines [13]. were used throughout the ASO screen and selection studies.16HBE cell lines were grown in Minimum Essential Medium Eagle (MEM) supplement with 10% FBS, 1% Glutamine, 1% Penicillin/streptomycin on plates coated with COLLAGEN I BOVINE (GIBCO), Fibronectin (SIGMA) and Bovine serum albumin (BSA). 1 μM VX-445 (Elexacaftor) and 3 μM VX-661 (Tezacaftor) were added for 48 h (Selleckchem).

### 2.2. ASO synthesis and transfections

The ASOs were synthesized with the 2′-O-Methoxyethyl (2′-O-MOE) sugar modification on the background of a full phosphorothioate backbone (2′-O-MOE/PS), which confers increased nuclease resistance and high affinity to the target RNA [14]. For the ASO screen, 16HBE cells were transfected with 10 nM of each of the ASOs and RNA was extracted after 24 h. For protein evaluation, 16HBE cells were transfected every 24 h for 2 days with 10 nM of each ASO and total protein was extracted following 48 h from the first transfection. Lipofectamin 2000 Transfection Reagent (Thermo Fischer scientific) was used for transfecting the various ASOs.

### 2.3. ASO free uptake

For functional analysis 16HBE cells were seeded at a density of $15 \times 10^6$ cells onto Transwell 24-well filter inserts (Corning 3470) pre coated with Bovine collagen I, Fibronectin and BSA. Cells were grown in Liquid-liquid Interface (LLI) in MEM supplement with 10% FBS, 1%

Glutamine, and 1% Penicillin/streptomycin. Naked ASO were added to the cell culture media for 7 days. After a total of 7 days, 16HBE cells typically formed electrically tight epithelia with transepithelial resistance (Rt) > 150Ω*cm$^2$.

### 2.4. RNA analysis of splicing pattern

Total RNA from the cell lines was extracted using the RNeasy mini-Kit (Qiagen). Total RNA from HNEs was extracted using the QIA shredder and RNeasy micro-Kit (Qiagen). Complementary DNA (cDNA) synthesis was performed using the High-Capacity cDNA kit (Applied Biosystems). Exon 23 skipping was analyzed by (1) RT-PCR using Platinum™ SuperFi™ Green PCR Master Mix (Invitrogen). PCR Primers: Fwd - 5′-TCACAGCAAAATACACAGAAGGT-3′ (exon 22) and Rev - 5′-CTTGTGGCCATGGCTTAGGA-3′ (exon 25). (2) RT-qPCR was performed using specific TaqMan master Mix (Applied Biosystems). The expression level was normalized to the transcript levels of GUSb. For statistical analysis of the ASO effect, paired *t*-test was performed on the delta Ct values of each ASO relative the delta Ct values of the control ASO.

### 2.5. Primary human nasal epithelial (HNE) cell sampling and culture

Bilateral nasal brushing was performed to a CF patient homozygous for the W1282X mutation. The brushes were immediately immersed in PBS. Freshly isolated HNE cells were expanded on flasks using conditionally reprogramming media [15]. The initial expansion phase was maintained for two passages. Then cells were seeded on porous filters (as described in [16]

### 2.6. Ussing chamber measurements

The short-circuit current (Isc) was measured using a protocol detailed previously [16] except for the use of 10 μM CFTR inhibitor CFTRinh172.

### 2.7. Western Blot

Protein extracts were prepared, and Western Blot was performed as previously described using mouse anti CFTR 596 (CFF) and rabbit anti Calnexin (Sigma) antibodies [16]. Quantification was performed using Image Studio software. All bands were normalized to calnexin levels.

### 2.8. Statistics

Statistical analyses were performed with GraphPad Prism version 9.0 or Microsoft Excel software. Differences between groups were determined by *t*-test. For all statistical tests, P values less than 0.05 were considered significant.

### 2.9. Study approval

The study was approved by the Hadassah IRB committee, all experiments were performed in accordance with the guidelines and regulations described by the Declaration of Helsinki in Israel, and the patient signed informed consent.

## 3. Results

I. ASO design and screening for the identification of lead ASO candidates

In order to design ASOs as therapeutic candidates, an algorithm was developed that allows the selection of ASO position, length and sequence composition, to predict ASO potency, ensure uniformity in target coverage, and avoid immunogenicity. Using this algorithm, we de-

signed 79 ASOs that align to exon 23 of the *CFTR* pre-mRNA (156 nucleotides, nt) and its flanking intronic sequences (70 nt upstream and downstream of the exon) (Supplementary Figure S1). The ASOs were designed at an optimal length range (18–22 nt) for efficient *in-vivo* delivery.

Following transfection of 16HBEge W1282X cells with each ASO (or control ASO) RNA was extracted and the CFTR splicing pattern was analyzed by RT-PCR. The results showed that 50% (39/79) of the tested ASOs led to significant skipping (>50%) over exon 23 (Figure S2). The effect of these ASOs was further ranked by RT-qPCR analysis (Fig. 1). Sixteen ASOs leading to >50% exon 23 skipping were chosen for further evaluation of their effect on the CFTR protein and their specificity to the W1282X allele. An additional ASO, SPL23–46, was also chosen for further analyses, since although it led to <50% transcripts lacking exon 23 (Figure S2D), it had a very efficient effect in the RT-PCR analysis (Fig. 1). As expected, exon 23 skipping led to an overall increased CFTR transcripts (Figure S3).

II. The effect of the lead ASO candidates on CFTR protein production and maturation

The ability of the 17 selected ASOs (SPL23 2,3,27–35, 40 and 43–47) to impact production of mature, fully glycosylated [17], CFTR proteins, was analyzed in 16HBEge W1282X cells. The ASOs effect were analyzed with and without CFTR correctors (VX-661 and VX-445). As can be seen in Fig. 2 and Figure S4, after treatment with a control ASO, there are undetectable levels of full-length (including exon 23) mature proteins. Following treatment with the ASO candidates, the CFTR protein missing exon 23 was clearly observed (Fig. 2A). As expected, this CFTR protein has a lower molecular weight than wildtype (WT) (Band B' and C').

The level of the mature CFTR proteins differed following treatment with the various ASOs. Mature CFTR protein (Band C') following treatment of all ASOs was semi-quantified and the percentage of band C' compared to Band C of 16HBE14o- WT was calculated (Fig. 2B and C). As can be seen, the range of Band C' levels is between 0.6% and 43% of WT without correctors (Fig. 2B) and between 1.5% and 49% of WT with correctors (Fig. 2C). These 17 selected ASOs significantly decreased the level of CFTR transcripts with exon 23 (Fig. 1) and thereby bypassed the W1282X mutation that subjects the transcripts and truncated proteins to degradation.
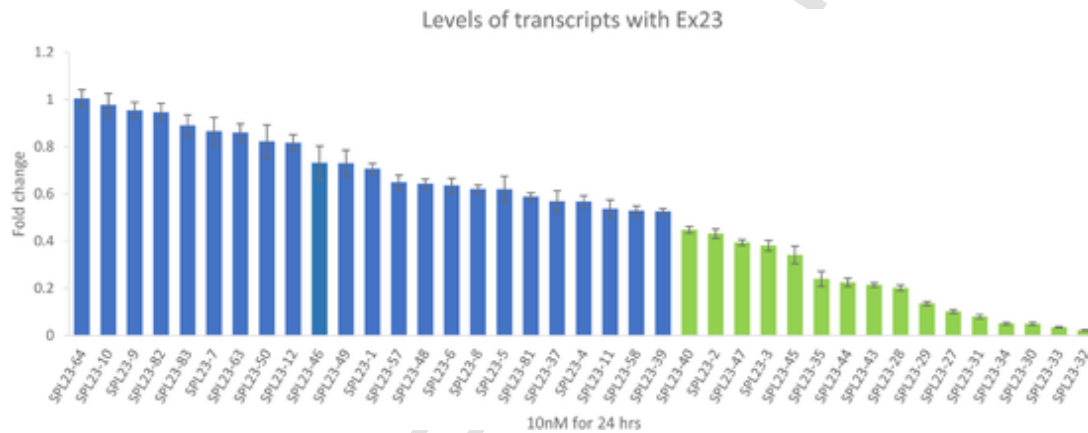


**Fig. 1. CFTR transcripts levels in 16HBEge W1282X cells following ASO transfection (**10 nM). The values shown are the average fold change (mean ± SEM) from 3 to 4 independent experiments relative to cells treated with a control ASO. The 16 ASOs that reduced the levels of transcripts with Ex23 to less than 50% are marked in green. ASOs leading to >50% transcripts with Ex23 are marked in blue.
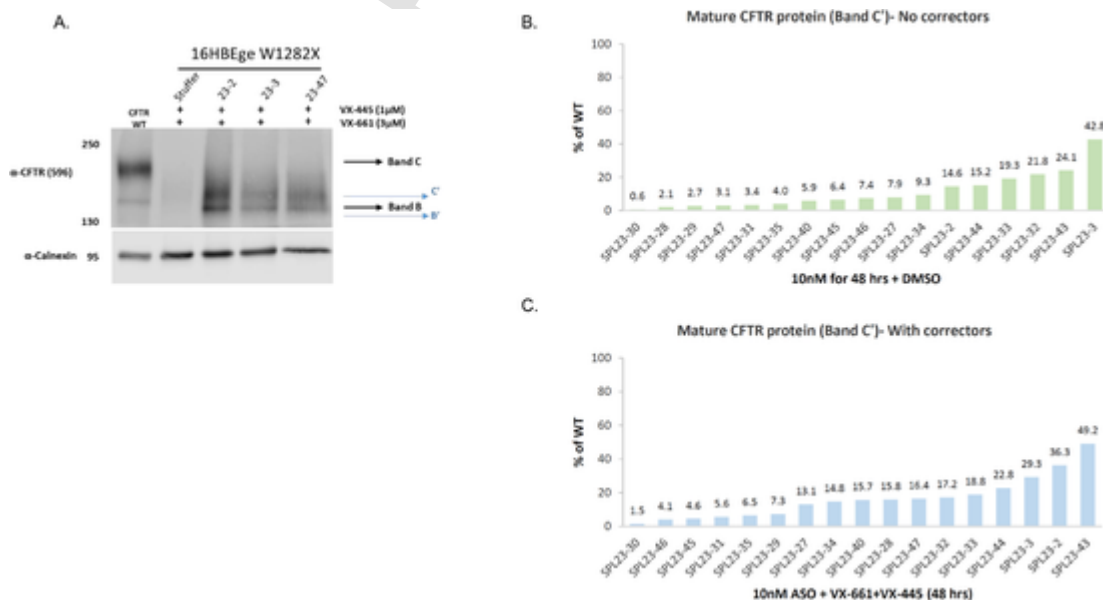


**Fig. 2. ASOs targeted to skip over exon 23 resulted in generation of mature CFTR protein in16HBEge W1282X cells**. The additive effect of the ASOs and correctors was analyzed. 16HBE14o-WT cells were used as a control.**A.** A representative Western blot image of 3 representative ASOs. **B-C** The levels of 17 ASOs C' bands were quantified relative to C band in WT cells. All bands were normalized to calnexin levels without correctors (B) and with correctors (C) .

### III. The specificity of ASO candidates to the W1282X allele

The W1282X mutation is the result of one nucleotide substitution (c.3846G > A), generating a UGA stop codon. Therefore, ASOs designed to target the mutation site have one missense relative to the WT sequence. One missense in short oligonucleotides was previously shown to significantly compromise the strength of target binding [18]. However, ASOs targeted to additional areas along the exon and the flanking intronic sequences might also show different affinity between the WT and mutant sequence, due to potential secondary structures of the mRNA. To verify the specificity of the candidate ASOs, we compared the effect of these ASOs on exon 23 skipping in 16HBE14o- WT cells and 16HBEge F508del cells to their effect in 16HBEge W1282X cells. The specificity is important for heterozygous patients, to ensure a minimal effect on the CFTR activity from the non-W1282X allele, thus was analyzed in WT and F508del cells. As shown in Figure S5, six of the tested ASOs (SPL23–2, −3, −28, −35, −40 and −46) exhibited clear specificity for transcripts carrying the W1282X mutation. The levels of exon 23 skipping, as indicated by the percentage of transcripts without exon 23 (Figure S5B), were significantly lower in WT and F508del cells than the levels seen in the mutant 16HBEge W1282X cells. All specific ASOs led to ≤30% skipping in both non-W1282X alleles, except for SPL23–35, which led to significant skipping in F508del cells but not in WT alleles. The other ASOs were non-specific and therefore showed a similar effect on exon 23 skipping in all three cell lines (data not shown). SPL23–47, showing high levels of exon 23 skipping for the three cell lines, is used as an example of a non-specific ASO in Figure S5.

The specificity of these six lead ASOs (SPL23–2, −3, −28, −35, −40 and −46) to the W1282X allele was further tested by analyzing the effect of the ASOs on CFTR protein maturation in 16HBEge F508del and 16HBE14o- WT cells by Western blot analysis. ASO specificity was de-termined by the lack of exon 23 skipping (lack of Band B' and C' in these cells). As seen in Figure S6A, treatment of 16HBE14o-WT cells with ASOs SPL23–28, −35, −40 and −46 did not lead to visible Band B' or C', while SPL23–2 and −3 led to a low level of only Band B' in comparison to SPL23–47, which served as a control for a non-specific ASO. No Band B' or C' were detected for all ASOs tested in F508del cells, apart from the positive control SPL23- 47 (Figure S6B). As ASO SPL23–35 was inferior in its performance to other tested ASOs, it was excluded from further analysis.

### IV. The effect of the lead ASO candidates on CFTR function in 16HBEge W1282X cells

The next stage of ASO screening and selection was analyzing the effect of the five candidate ASOs on CFTR activity in 16HBEge W1282X cells, using Isc measurements following ASO free uptake by the target cells. We first determined the baseline CFTR activity in the WT parental cells (16HBE14o−) to serve as a reference point for calculating the effect of CFTR activity following ASO-treatment in 16HBEge W1282X cells. The level of CFTR activity is indicated by the response of the specific CFTR inhibitor (Inh172). A representative measurement of WT cells can be seen in Fig. 3A. The average level of CFTR activation in WT cells as measured by $\Delta IscCFTRinh172$ is $52 \pm 8.7 \mu A/cm2$.

We analyzed the effect of SPL23–2, −3, −28, −40 and −46 and the control ASO, treated by free uptake, together with VX661, VX445 and VX770, (elexacaftor-tezacaftor-ivacaftor, ETI) on the CFTR function in 16HBEge W1282X. Fig. 3B shows representative traces of electrophysiological responses in Ussing chambers in 16HBEge W1282X cells following treatment with 20 μM of control ASO or SPL23–2. The effect of the ASOs on CFTR activation is presented in Fig. 3C. As can be seen, SPL23–2 and SPL23–3 show the highest effect on CFTR activity, which was dose dependent (Fig. 3D). In contrast, the effect of SPL23–28 and SPL23–46 is very low. Since treatment with SPL23–40 led to very low
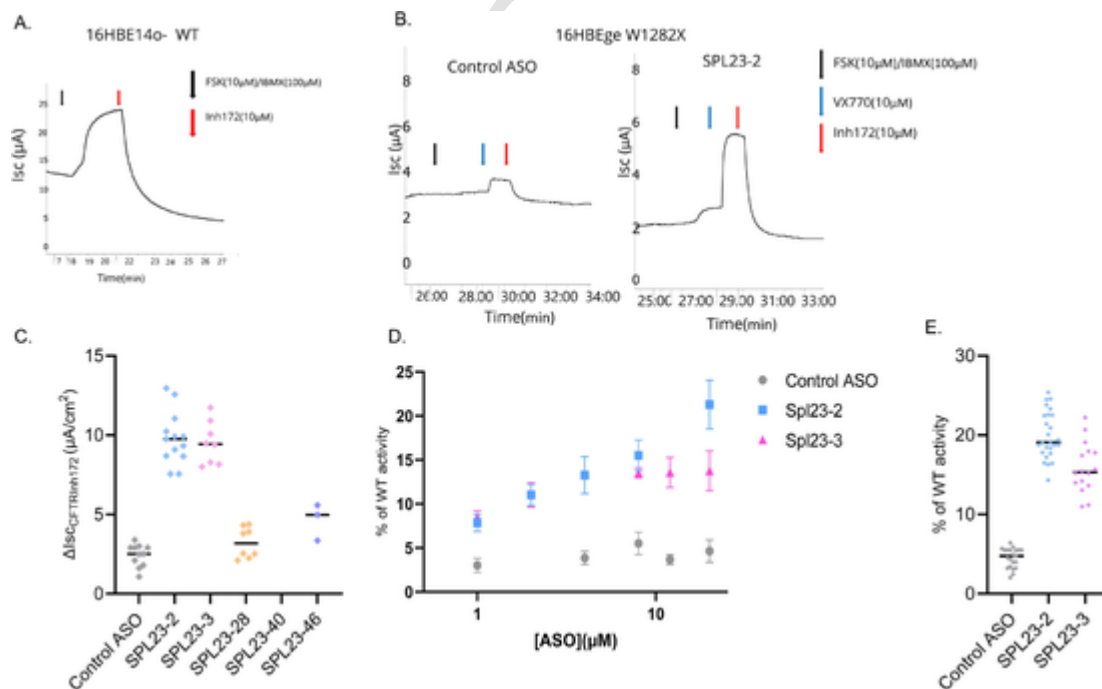


Fig. 3. Freeuptakeof Lead ASO can did a testogether with trikaftarescue CFTR functionin 16HB Ege W1282X cells.A Representative trace of electrophysiological responses in Ussing chambers in 16 HBE 14o WT cells. B. Representative trace of electrophysiological responses in Ussing chambers in 16HBEge W1282X cells following free uptake of 20μM control ASO (Left panel) and SPL 23-2 (Right panel) and trikafta. C. The effect of free uptake 20μM of the 5 lead candidate ASOs and trikafta in 16HBEge W1282X cells is presented as median of the absolute ΔIscCFTRinh172 values calculated from 3-13 filters from at least 2 biological experiments D Dose response curves of CFTR activation presented as values of % of WT calculated from values of ΔIscCFTRinh172 in 16HBEge W1282X cell following treatment with 6 different concentrations 1-20μM) of SPL23-2 ,SPL23-3 and Control ASO together with trikafta The values for each concentration are calculated from 2-14 filters derived from at least 2 biological experiments. E. Values of % of WT were calculated from values of ΔIscCFTRinh172 in 16HBEge W1282X cells following treatment with 20μM of SPL23-2, SPL23-3 and Control ASO and trikafta. The values are calculated from 16-27 filters treated with these lead ASOs derived from 4-8 biological experiments.

resistance, the effect of this ASO on CFTR function was not measured. The effect of SPL23–2 and SPL23–3 was calculated and presented as percentage of WT level and, leading to median ASO effect of 15% of WT for SPL23–3 and 19% for SPL23–2 (Fig. 3E).

The specificity of ASOs was further analyzed in 16HBE 14o-WT cells, with higher ASO concentration, which showed a minimal to undetectable exon skipping (Figure S7A and B) and no band B' or C' (Figure S7C).

**V. The effect of candidate ASOs on exon 23 skipping and CFTR protein production and maturation in 16HBEge W1282X cells**

We analyzed the effect of the lead candidates on exon 23 skipping. RNA was extracted from the same cell filters following the functional measurements and the levels of transcripts without exon 23 were measured relative to control ASO. Exon 23 skipping levels are in accordance with the functional analysis (Figs. 4A and B); the levels of exon 23 skipping are higher in SPL23–2 and SPL23–3 than in the other three ASOs (SPL23–28, −40, and −46). In addition, a dose dependent response in the transcript level was observed (Fig. 4C and D).

We analyzed CFTR protein production in 16HBEge W1282X cells grown on filters and treated with the five candidate ASOs. Treatment with control ASO leads to almost non-detectable levels of CFTR protein even in the presence of correctors; however, ASO treatment together with VX-661 and VX-445 leads to the production and maturation of sig-

nificant levels of CFTR protein. SPL23–2 and SPL23–3 resulted in the highest levels of CFTR protein (Fig. 4E and F).

**VI. The effect of the ASOs SPL23–2 and SPL 23–3 on CFTR function and exon 23 skipping in primary HNE cells from CF patient homozygous for the W1282X mutation**

We further tested the effect of the lead ASO candidates on CFTR activity in primary HNE cells using Isc experiments, as these cellular systems serve as a pre-clinical model able to predict patient-specific respiratory improvement [15]. The ASOs were introduced into the cells by free uptake for 14–15 days. The analysis of HNEs shows a low baseline CFTR activity following addition of ETI. Treatment with SPL23–2 and SPL-23–3 together with ETI led to a 2-fold increase of CFTR activity relative to control ASO, showing the high potency of these ASOs; SPL23–2 provided slightly higher CFTR activity levels as compared to SPL23–3 (Fig. 5A and B).

We further analyzed the effect of these candidates on exon 23 skipping in HNE cells from the subject. RNA was extracted from the same cells following the functional measurements demonstrating that ASO treatment generates CFTR transcripts without exon 23 (Fig. 5C). In parallel, we analyzed CFTR protein production in these cells treated with the candidate ASOs and ETI. Treatment with a control ASO and ETI leads to low levels of the CFTR protein, treatment with the lead ASOs together with VX-661 and VX-445 leads to the production and maturation of significant levels of CFTR protein (Fig. 5D).
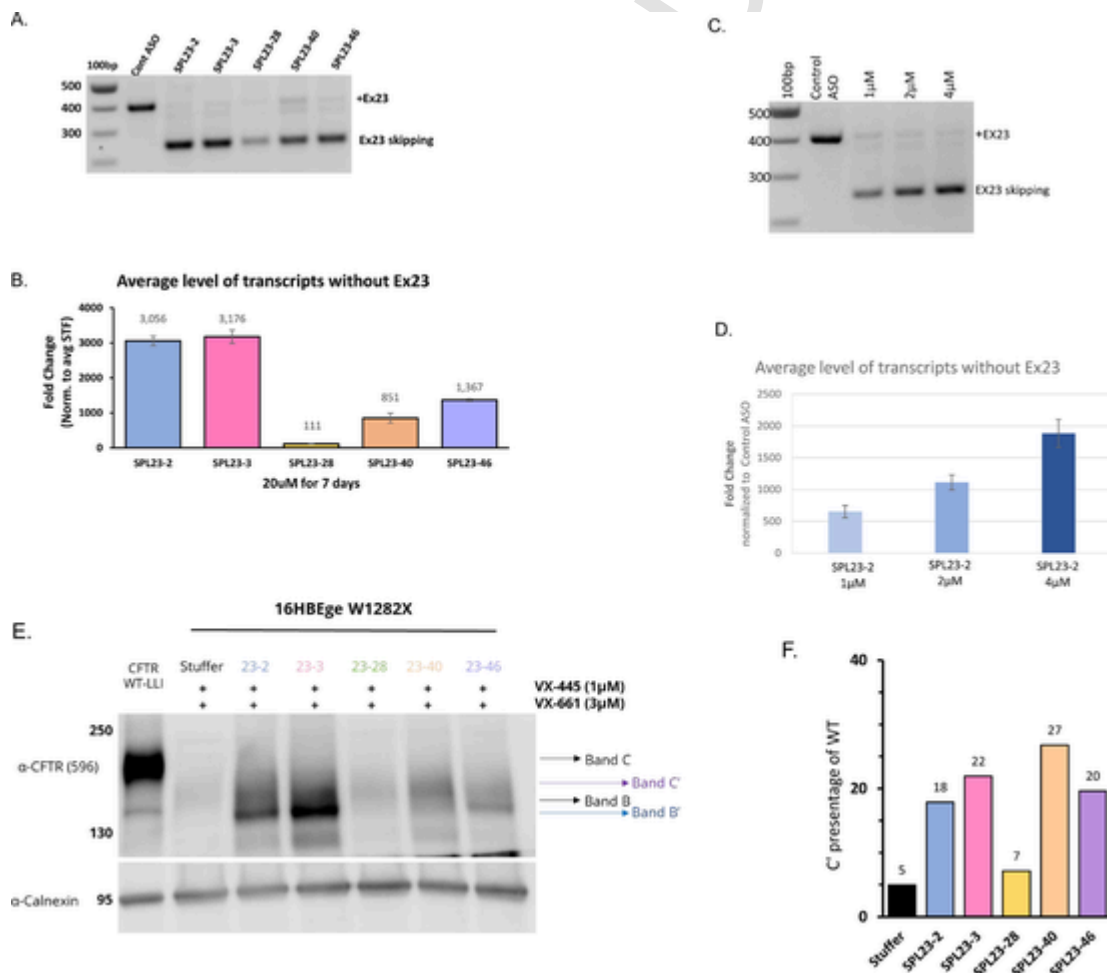


**Fig. 4. Splicing modulation by the lead ASOs in 16HBEge W1282X cells.A. and B**. RNA was extracted from 16HBEge W1282X cells ($n$ = 4–9 filters for each condition form at least 2 independent experiments) following the functional measurements presented in Fig. 3. **A.** Representative RT-PCR examples following treatment with the indicated ASOs. **B** . The effect of the ASOs on the level of CFTR transcripts without exon 23 as measured by RT-qPCR. **C. and D.**16HBE-W1282X cells were treated with 1,2,4 μM SPL23–2 or control ASORNA was extracted from these cells after Ussingmeasurements (6–8 filters). **C.** Representative RT-PCR examples following treatment with the indicated ASOs. **D** . The effect of the ASOs on the level of CFTR transcripts without exon 23 as measured by RT-qPCR. **E.** Protein extracts were prepared and analyzed by immunoblotting. **F.** The levels of ASOs C' Bands were quantified relative to C band in WT cells.
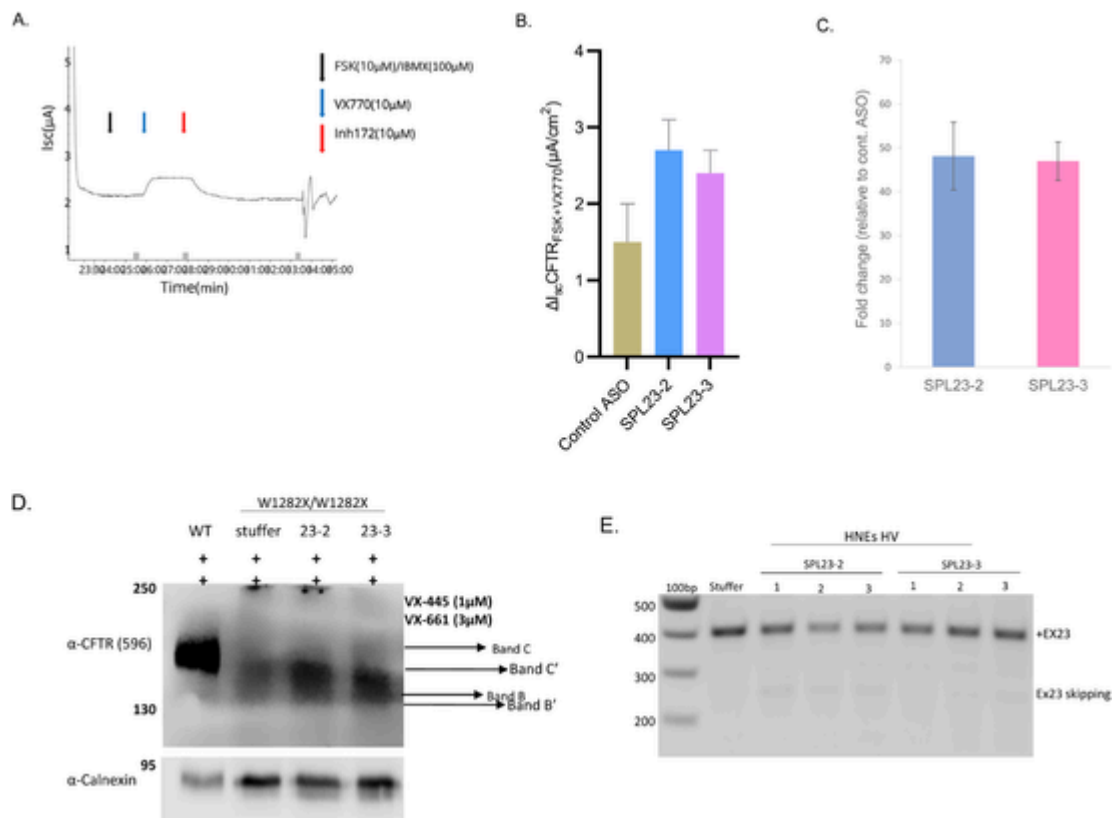
**Fig. 5. Lead ASO candidates rescue CFTR function by splicing modulation in primary HNE cells derived froma patient homozygous for the W1282X mutation.** HNE cells were treated with 10 μM ASO by free uptake for 14–15 days A. A representative trace of electrophysiological responses in Ussingchambers. B. The effect of the candidate ASOs is presented as median of the absolute ΔIscCFTRFSK + VX770 values calculated from 2 to 4 filters. **C.** RNA was extracted from the HNE W1282X cells following the functional measurements. The effect of the ASOs on the level of CFTR transcripts without exon 23 was measured. **D.** Protein extracts were prepared and analyzed by immunoblotting. **E** HNE cells from a healthy volunteer (HV) were treated with 10 μM ASO by free uptake for 14 days RNA was extracted and the effect of the ASOs on the level of CFTR transcripts was analyzed.

Finally, we analyzed the specificity of these lead ASO candidates to the W1282X by analyzing their effect on exon 23 skipping by free uptake in HNE cells derived from a healthy non-CF volunteer. The effect of the ASOs on exon 23 skipping is minimal in WT cells (Fig. 5E) further confirming the specificity of the ASOs to the W1282X sequence.

## 4. Discussion

We have demonstrated robust methodological analyses ("ASO walk") of ASOs aiming to skip over an exon harboring a severe CFTR mutation to produce functional proteins. We screened several consecutive ASOs aligned to CFTR exon 23 carrying the W1282X mutation and its flanking intronic sequences, thereby targeting the intronic and exonic sequence elements intended to permit skipping exon 23. The results identified selected ASOs that significantly decreased the level of CFTR transcripts with exon 23 (Fig. 1) and thereby bypassed the W1282X mutation. As a result, a significant level of CFTR proteins lacking exon 23 was produced and matured (Fig. 2). In addition, these two candidate ASOs (SPL23–2 and SPL23–3) combined with ETI led to a considerable CFTR activation, with SPL23–2 reaching activation of ~20% of the level in the isogeneic 16HBE14o- WT (Fig. 3) and a considerable improvement in HNEs (Fig. 5). Response to treatment in HNE*s* serves as a good predictor for drug development in CF [15,19] . Altogether, these results indicate that the ASO mode of action (MoA) underlying the CFTR functional correction is the generation of transcripts lacking exon 23. CFTR proteins generated from these transcripts are the molecular basis for the functional response measured by the Isc experiments following the addition of the CFTR modulators. The analysis in

HNEs suggests that SPL23–2 is superior to its counterpart lead ASO SPL23–3, as reflected by functional analysis following cell free uptake.

An important consideration for this type of therapy is the ASO specificity to the W1282X allele. We thus analyzed their effect on RNA and protein in non-W1282X cell lines. Although some ASO candidates were non-specific and showed a similar effect on exon 23 skipping in other non-W1282X cell lines, we identified candidate ASOs that showed specificity for the W1282X sequence both on RNA and protein levels (Figures S5, S6 and 5E). This includes SPL23–2 and SPL23–3 that overlap the W1282X mutation sequence and thus have one mismatch to the non-W1282X alleles, shown previously to significantly compromise the strength of target binding [18]. The CFTR protein produced through this ASO MoA is truncated and NBD2 is partially absent. Previous studies have shown that NBD2 is not essential for maturation and stability of the CFTR proteins, yet proteins lacking NBD2 form characteristic CFTR channels with lower open probability [6–8]. Our results show that CFTR proteins lacking only part of the NBD2 (exon 23) remain functional (Figs. 3 and 5), opening the way for development of ASO-based therapies for patients carrying mutations in NBD2.

ASOs as therapeutic agents are becoming more widespread as gene and chemical modifications enhance their properties that make them attractive candidates for drug development [20]. Their potential has been demonstrated in other human genetic diseases, such as Spinal Muscular Atrophy (SMA), The ASO-based drug (SPINRAZA-FDA Approval, 2016), showing significant improvement in motor function milestones in SMA infants. ASO-based therapy is also used for Duchenne muscular dystrophy gene (Exondys 51, Eteplirsen) [21,22]. The significant effect of these drugs demonstrates the great potential of

the splice switching ASO-based therapeutic approach for the treatment of genetic diseases.

In summary, the results presented here highlight the potential therapeutic and clinical benefit of ASO-based exon 23 skipping SPL23–2, supplemented by CFTR modulators, for CF patients carrying the W1282X nonsense mutation.

## Declaration of competing interest

Batsheva Kerem has equity in SpliSense and is paid for consultancy. All other authors have no financial conflict of interest.

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## Author contributions

YSO listed first because of her extensive contribution to the project conception and design, experiments, data analyses and manuscript writing. OBA contributed to project design, ASO design, conducted experiments and data analyses and she also contributed extensively to the functional experiments; EOG contributed to the project design, ASOs design and data analyses; RME, MRS, TB,CDS and MO contributed to experiments performance; OL and CEB contributed to project conception; MCC contributed to collecting epithelial cells from patients; EK contributed to the design of the project, the recruitment of patients and nasal epithelial cells scraping as well as data analyses and discussion; BK contributed to the conception and design of the project, data analysis and interpretation, obtained financial support, manuscript writing and final approval of the paper. All authors contributed and edited the manuscript.

## Credit author statement

**Yifat S. Oren:** Conceptualization, Methodology, Investigation, Formal analysis, Validation, Writing – Original draft, Writing - review and editing, Visualization. **Ofra Barchad-Avitzur:** Conceptualization, Methodology, Investigation, Validation, Formal analysis, Software, Writing-review and editing, Visualization. **Efrat Ozeri-Galai:** Conceptualization, Methodology, Validation, Supervision. **Renana M. Elgrabli, Meital R. Schirelman, Tehilla Blinder, Chava D. Stampfer, Merav Ordan** Formal analysis, Investigation. **Onofrio Laselva**: Conceptualization **Christine E Bear:** Conceptualization. **Malena Cohen-Cymberknoh:** Resources. **Eitan Kerem:** Conceptualization, Methodology, Resources, Supervision, Writing - review and editing. **Batsheva Kerem:** Conceptualization, Methodology, Validation, Writing – Original draft, Writing - review and editing, Supervision, Funding acquisition.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcf.2021.12.012.

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