Clinical Stage CFTR NBD1 Stabilizers SION-719 and SION-451 Synergize with Galicaftor (SION-2222) or SION-109 to Enable Full Correction of ΔF508-CFTR

G. Hurlbut[1], S. Altmann[1], S. Bercury[1], J. Foley[1], O. Hurlbut-Lesk[1], Z. Gao[1], A. Hunnicutt[1], J. Liao[1], M. Munson[1], D. Stepp[2], G. Topalov[1].

[1]Sionna Therapeutics, Waltham MA, USA, [2]Sanofi, Waltham MA, USA

ECFS 2025

Abstract # WS19.01

Abstract

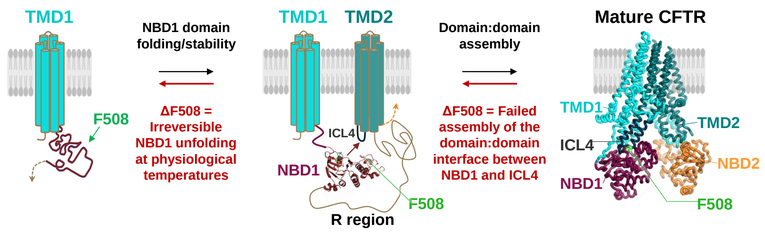
Objectives: Approved CFTR modulators have significantly improved outcomes for people with CF (pwCF) [1], but do not restore normal CFTR function to most patients. ΔF508, the most prevalent CF mutation, causes a loss of F508 within CFTR's first Nucleotide Binding Domain (NBD1). This destabilizes NBD1, impairing CFTR folding and weakening NBD1's interface with CFTR transmembrane domains [2-5]. In preclinical studies, combining NBD1-stabilizing CFTR suppressor mutations with others that improve CFTR assembly has been shown to correct ΔF508 to wild-type (WT) levels. Without NBD1 stabilization, correction of ΔF508 in these studies was significantly reduced [6,7]. NBD1 stabilizing drugs have the potential to improve treatment, but no approved modulator directly stabilizes NBD1 [8]. With the goal to improve ΔF508-CFTR function and deliver meaningful clinical benefit to pwCF, Sionna has advanced two potent first-in-class small molecule NBD1 stabilizers, SION-719 and SION-451, into Phase 1 clinical trials. Here, we use preclinical models to assess combinations of NBD1 stabilizers with complementary Sionna CFTR modulators, including SION-2222 (galicaftor), which demonstrated clinical activity in Phase 2 studies, and SION-109, which has completed Phase 1.

Methods: In functional and biochemical preclinical studies, we assessed clinical-stage NBD1 stabilizers SION-451 and SION-719 in combination with SION-2222, SION-109 and other complementary modulators in our pipeline.

Results: We show that Sionna NBD1 stabilizers, in dual combinations with SION-109 or SION-2222, restored ΔF508-CFTR activity to WT levels when administered at E_{max} in CF models.

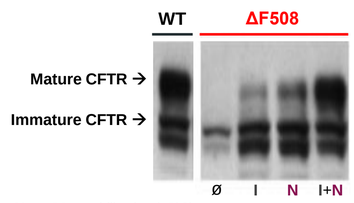
Introduction

ΔF508-CFTR Leads to NBD1 Instability and Defective CFTR Domain-Domain Assembly



- NBD1 instability is a key driver of ΔF508-CFTR folding, trafficking, and ion channel function defects. [2-5]
- ΔF508 also impairs CFTR domain-domain assembly, adding to ΔF508-CFTR dysfunction. [6,7]

Proof of Hypothesis: 2nd-site Mutations that Stabilize NBD1 or Domain-Domain Assembly Can Together Correct ΔF508-CFTR



N = NBD1 stabilization (~5ºC)
I = ICL4 interface restoration

From Thibodeau 2010 [3]

Suppressor mutations that stabilize NBD1 combined with those that improve CFTR domain-domain assembly (e.g. NBD1-ICL4 interface stabilizers) correct ΔF508-CFTR maturation to WT levels.

Full Pharmacological ΔF508-CFTR Restoration May Require Addressing Both Domain Assembly and NBD1 Stability Defects[6,7]

To address the key drivers of ΔF508-CFTR dysfunction, Sionna's goal is to develop first-in-class NBD1 stabilizers and other novel modulators that can correct CFTR assembly.

- Historically, NBD1 has been considered undruggable.[9]
- We leverage 15 years of our work (Genzyme, Sanofi, and Sionna).
- NBD1 stabilizers SION-719 and SION-451 have completed Phase 1 SAD & MAD trials in healthy volunteers.
- Sionna is also advancing clinical-stage complementary modulators to combine with NBD1 stabilizers.
- Such complementary modulators include:
  - SION-2222 (galicaftor) - Phase 2 stage TMD1-directed corrector, with demonstrated clinical activity.
  - SION-109 - ICL4-directed corrector with a completed Phase 1 trial.

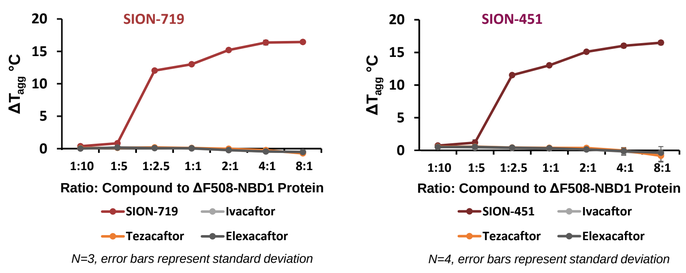
Sionna Clinical-stage NBD1 Stabilizers Directly Bind to and Stabilize CFTR NBD1

Sionna SION-719 and SION-451 Bind Directly to CFTR NBD1.

Table 1. Surface Plasmon Resonance (SPR) was used to evaluate binding to human ΔF508-NBD1 protein. SION-719 and SION-451 display high affinity 1:1 NBD1 binding. In contrast, no NBD1 binding was detected for approved modulators ELX, TEZ and IVA.

	NBD1 Affinity
Compound	ΔF508-NBD1 K_D (μM)
SION-719	0.0043
SION-451	0.0024
ELX	No binding detected
TEZ	No binding detected
IVA	No binding detected

Differential Static Light Scattering to Assess the Ability of SION-719 and SION-451 to Stabilize Isolated CFTR NBD1 Protein.



N=3, error bars represent standard deviation

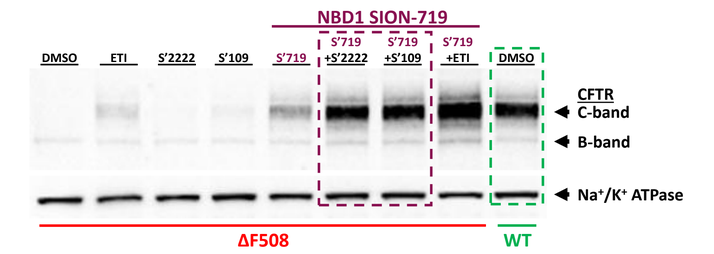
N=4, error bars represent standard deviation

Figure 1 and 2. SION-719 and SION-451 robustly increase ΔF508-NBD1 stability in thermal denaturation studies. In contrast, elexacaftor (ELX), tezacaftor (TEZ), and ivacaftor (IVA), which represent all approved CFTR modulator mechanistic classes, have no impact on isolated NBD1 stability.

NBD1 binding sites and binding modes of Sionna stabilizers have been extensively characterized. Over the course of our programs, high-resolution X-ray crystal co-structures were solved for >150 Sionna NBD1 stabilizers, clearly demonstrating direct interaction.

NBD1 Dual Combos Improve ΔF508-CFTR Maturation to WT CFTR Levels at E_{max}

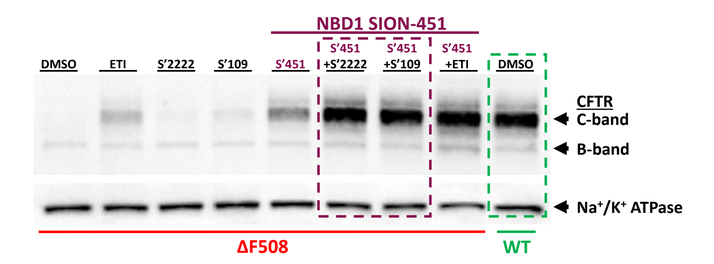
NBD1 Stabilizer SION-719 Corrects ΔF508-CFTR Maturation to WT Levels when Combined at E_{max} with SION-2222 or SION-109.



ETI at E_{max}:
- ELX (10μM) – approved ICL4 Corrector
- TEZ (5μM) – approved TMD1 Corrector
- IVA (0.1μM) – approved Potentiator

SION-719 (1.5μM) – Sionna NBD1 Stabilizer
SION-2222 (5μM) – Sionna TMD1 Corrector
SION-109 (3μM) – Sionna ICL4 Corrector

NBD1 Stabilizer SION-451 Corrects ΔF508-CFTR Maturation to WT Levels when Combined at E_{max} with SION-2222 or SION-109.



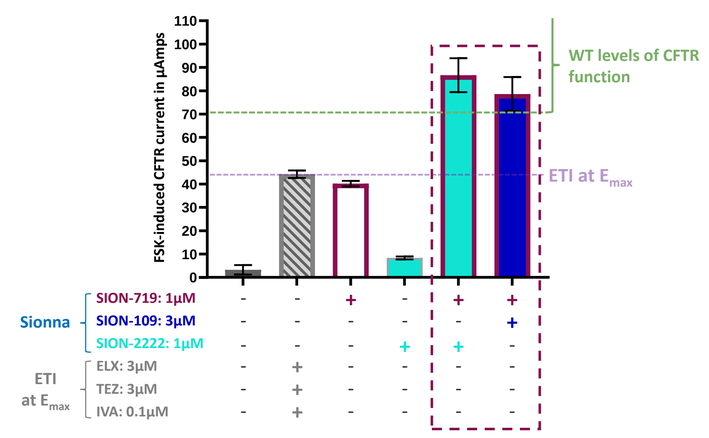
ETI at E_{max}:
- ELX (10μM) – approved ICL4 Corrector
- TEZ (5μM) – approved TMD1 Corrector
- IVA (0.1μM) – approved Potentiator

SION-451 (1.5μM) – Sionna NBD1 Stabilizer
SION-2222 (5μM) – Sionna TMD1 Corrector
SION-109 (3μM) – Sionna ICL4 Corrector

Figures 3 and 4. Impact of Sionna clinical-stage NBD1 stabilizers SION-719 or SION-451 on ΔF508-CFTR maturation in combination with TMD1-directed SION-2222 or ICL4-directed SION-109. Western blots of CFTR expressing CFSMEo- cells, comparing both ΔF508-CFTR and WT-CFTR, treated as indicated. NBD1 stabilizers plus either ICL4- or TMD1-directed correctors can improve ΔF508-CFTR maturation to WT levels, demonstrating the synergy between NBD1 stabilizers and clinical-stage modulators that address ΔF508-CFTR domain-domain assembly defects. ETI components were used at their respective E_{max} concentrations in this context.

NBD1 Dual Combos Improve ΔF508-CFTR Function to WT CFTR Levels at E_{max}

SION-719 Combinations with SION-2222 and SION-109 were Highly Active in the CFHBE (VALI) Model at E_{max}.



SION-451 Combinations with SION-2222 and SION-109 were Highly Active in the CFHBE (VALI) Model at E_{max}.

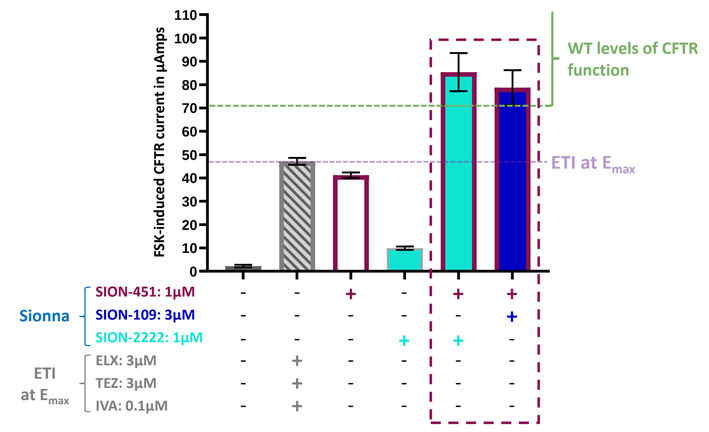
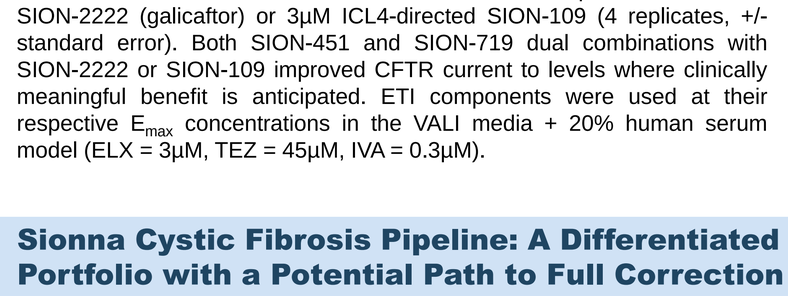


Figure 5 and 6. CFHBE VALI model, vertical bars represent the mean forskolin-induced (FSK) CFTR current in response to indicated treatments (8 replicates, +/- standard error) in a representative ΔF508/ΔF508 CFHBE donor. At E_{max}, SION-719 or SION-451 dual combinations with SION-2222 (galicaftor) or SION-109 improved CFTR current to levels within the range observed separately in a panel of 8 non-CF HBE donors. ETI components were used at their respective E_{max} concentrations in this context.

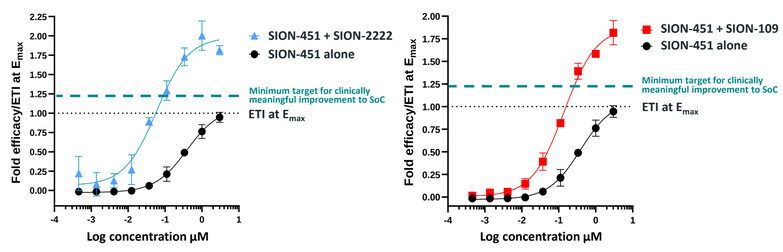
NBD1 Dual Combos Show Potential for Clinically Meaningful Improvement Below E_{max}

In the CFHBE VALI + 20% (v/v) human serum translation model, NBD1 stabilizers, when combined with SION-2222 or SION-109, correct ΔF508-CFTR function to levels anticipated to provide clinically meaningful benefit at exposures achieved in Phase 1.

Dose Response of NBD1 Stabilizer SION-719 Combined with TMD1-directed SION-2222 or ICL4-directed SION-109.



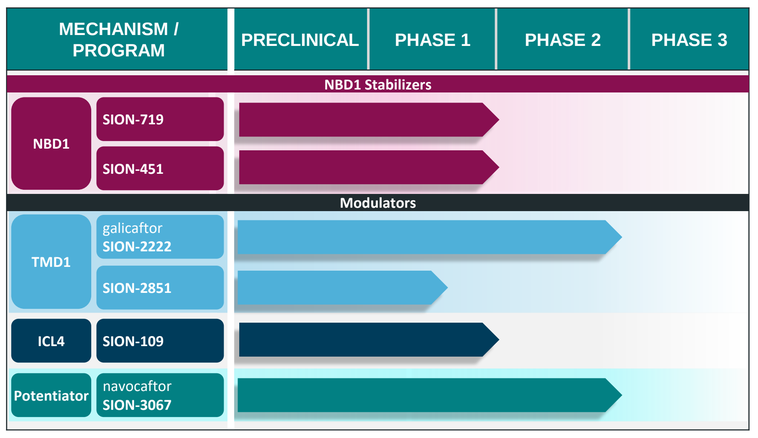
Dose Response of NBD1 Stabilizer SION-451 Combined with TMD1-directed SION-2222 or ICL4-directed SION-109.



Figures 7 and 8. CFTR activity of ΔF508/ΔF508 CFHBE from a representative donor treated for 24 hours with increasing concentrations of SION-719 or SION-451 alone or in combination with 3μM TMD1-directed SION-2222 (galicaftor) or 3μM ICL4-directed SION-109 (4 replicates, +/- standard error). Both SION-451 and SION-719 dual combinations with SION-2222 or SION-109 improved CFTR current to levels where clinically meaningful benefit is anticipated. ETI components were used at their respective E_{max} concentrations in the VALI media + 20% human serum model (ELX = 3μM, TEZ = 45μM, IVA = 0.3μM).

Sionna Cystic Fibrosis Pipeline: A Differentiated Portfolio with a Potential Path to Full Correction

Anchored by novel NBD1 stabilizers, our goal is to deliver differentiated medicines that enable more people with CF to achieve normal CFTR function



Conclusions

Sionna is advancing a portfolio of clinical-stage novel NBD1 stabilizers and complementary correctors with a goal to provide innovative options that have the potential, based on preclinical data, to dramatically improve clinical outcomes and quality of life for people with CF.

Acknowledgements

Work supported by Sionna Therapeutics and the Cystic Fibrosis Foundation, past support from Genzyme and Sanofi. Thanks to Dr. Thibodeau for use of the introductory suppressor mutation figure.

References

1. Konstan M, et al. CFTR modulator-induced sweat chloride changes across the Cystic Fibrosis Population from the CHEC-SC study: 2022 update. Journal of Cystic Fibrosis, Vol 21, Sup 2, S154.
2. Du K, *et al*. The ΔF508 cystic fibrosis mutation impairs domain-domain interactions and arrests post-translational folding of CFTR. Nat Struct Mol Biol 2005; 12(1):17-25.
3. Thibodeau PH, *et al.* The cystic fibrosis-causing mutation ΔF508 affects multiple steps in cystic fibrosis transmembrane conductance regulator biogenesis. J Biol Chem. 2010 Nov 12; 285(46):35825-35.
4. Protasevich I, *et al*. Thermal unfolding studies show the disease causing F508del mutation in CFTR thermodynamically destabilizes nucleotide-binding domain 1. Protein Sci 2010; 19(10):1917-31.
5. Hoelen H, *et al*. The primary folding defect and rescue of ΔF508 CFTR emerge during translation of the mutant domain. PLoS One 2010; 5(11):e15458
6. Rabeh WM, *et al.* Correction of both NBD1 energetics and domain interface is required to restore ΔF508 CFTR folding and function. Cell 2012;148(1–2):150–63
7. Okiyoneda T, *et al*. Mechanism-based corrector combination restores ΔF508-CFTR folding and function. Nat Chem Biol 2013; 9(7):444-54.
8. Hurlbut G, *et al*. Beyond SION-638: a novel series of highly potent stabilizers of NBD1 enables full correction of ΔF508-CFTR in a dual combination with Sionna CFTR modulators and a path to wild-type function. Journal of Cystic Fibrosis, Volume 22, S145.
9. Hall J, *et al.* Binding screen for cystic fibrosis transmembrane conductance regulator correctors finds new chemical matter and yields insights into cystic fibrosis therapeutic strategy. Protein Sci 2016; 25(2):360-373.

www.sionnatx.com