Novel CFTR Modulator Combinations Directly Address the ΔF508-CFTR NBD1 Stability Defect and Enable Full CFTR Correction

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Abstract # EPS3.10

Abstract

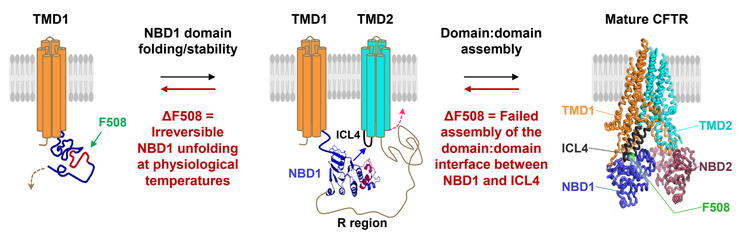
Background: Cystic fibrosis (CF) results from CFTR mutations, the most prevalent being ΔF508. Approved modulators of CFTR increase its function, providing eligible patients with clinical benefits. Despite this, current modulators do not provide most people with CF with normal levels of CFTR function, indicated by the fact that mean sweat chloride levels do not reach the normal range in most eligible patient groups. ΔF508 results in loss of phenylalanine F508 within CFTR's first nucleotide-binding domain (NBD1). ΔF508 causes NBD1 destabilization: a key driver of the impaired folding, trafficking, half-life, and function of ΔF508-CFTR.[1-4] Thus, full ΔF508-CFTR correction may require NBD1 stabilization. F508 also directly participates in the interface of NBD1 and CFTR's fourth intracellular loop (ICL4), an interaction that helps connect NBD1 to CFTR transmembrane domains (TMD), contributing to domain-domain assembly. ΔF508 weakens this interface, adding to ΔF508-CFTR's molecular pathology.[5,6] Consistent with this, suppressor mutations that stabilize NBD1, when combined with others that improve CFTR assembly, can correct ΔF508-CFTR to wild-type (WT) levels.[8-10] Without NBD1 stabilization, correction is significantly less. Drugs that stabilize NBD1 thus have the potential to improve patient health, yet no current drugs fully address this key defect. NBD1 has been considered likely undruggable.[11]

Methods: For the past 14 years, our science team has focused on the discovery of NBD1 stabilizers and complementary modulators to restore WT function to ΔF508-CFTR. Using functional and biochemical assays, we demonstrate the activity of Sionna's NBD1 stabilizers alone and in combination with Sionna's ICL4- and TMD1-directed correctors, and standard-of-care CFTR modulators.

Results: Sionna's NBD1 stabilizers, including SION-638 in phase 1, can restore ΔF508-CFTR maturation, trafficking, and function to WT levels when combined with mechanistically complementary agents, including Sionna's and standard-of-care modulators. Data from the clinically predictive CFHBE model suggest that NBD1 stabilizers enable multiple potential paths to full restoration of CFTR function for most people with CF.

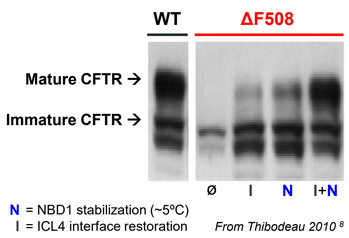
Introduction

ΔF508-CFTR leads to NBD1 instability and defective CFTR domain-domain assembly



- NBD1 destabilization of is a key driver of impaired ΔF508-CFTR folding, trafficking, and function.
- ΔF508 also weakens the NBD1-ICL4 interface, contributing to ΔF508-CFTR dysfunction.

Proof of hypothesis: 2nd-site mutations that stabilize NBD1 and the NBD1-ICL4 interface



Suppressor mutations that stabilize NBD1 and the NBD1-ICL4 interface restore ΔF508-CFTR maturation to WT levels: a roadmap to more effective therapies.

***NBD1 was considered undruggable.*[12]**

A fully-restorative therapy must address ΔF508's domain assembly and NBD1 stability[11]

To address the key drivers of ΔF508-CFTR dysfunction, Sionna is developing first-in-class NBD1 stabilizers and other novel modulators that correct CFTR assembly.

- We leverage 14 years of effort and investment by Genzyme, Sanofi, CFF, and Sionna.
- We show that novel NBD1 stabilizers synergize with both ICL4- and TMD1-directed correctors.
- **SION-638, a first-in-class NBD1 stabilizer, has advanced to Phase 1 studies.**
- Sionna is advancing correctors of complementary MoAs to enable full ΔF508-CFTR correction.
- **IND-enabling studies have been completed for ICL4-directed corrector SION-109.**

SION-638 Binds to the CFTR NBD1 Domain

We identified small molecules with high affinity to NBD1, *e.g.* SION-638, and fully characterized their binding sites and modes.

Compound	WT NBD1 KD (μM)	ΔF508-NBD1 form 1 KD (μM)	ΔF508-NBD1 form 2 KD (μM)
SION-638	0.029	0.040	0.032

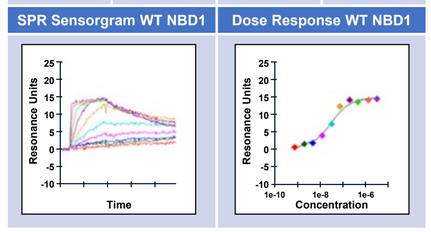


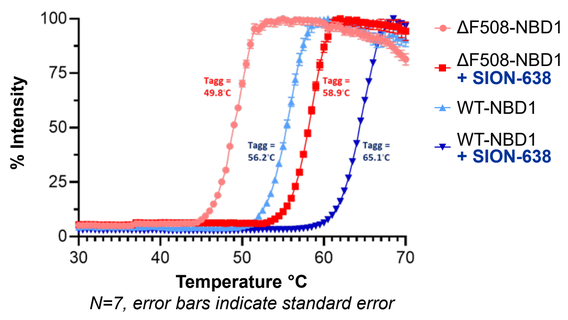
Figure 1. Surface Plasmon Resonance (SPR) was used to evaluate SION-638 binding to human WT NBD1 [(S)389-673] and two ΔF508-NBD1 isoforms. SION-638 demonstrated 1:1 binding across all NBD1 isoforms with KD values ranging from 29nM - 48nM in multiple experiments. Representative SPR sensorgrams and dose-response curves are shown.

NBD1 binding sites were assessed for >80 NBD1 small molecule ligands by protein-observed NMR and high-resolution NBD1 X-ray crystal co-structures were solved for >150 compounds.

SION-638 Stabilizes the NBD1 Domain of CFTR

Ligands like SION-638 increase ΔF508-NBD1 stability above WT-NBD1 levels.

Differential static light scattering was used to assess the ability of SION-638 to stabilize NBD1.



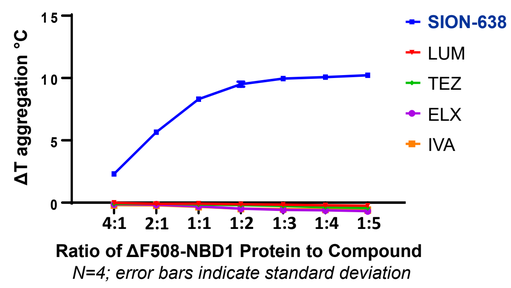
N=7, error bars indicate standard error

Figure 2. SION-638 increases the thermal stability of ΔF508 and WT-NBD1. SION-638-treated ΔF508-NBD1 has greater stability than that of untreated WT-NBD1.

SION-638 Stabilizes the NBD1 Domain of CFTR

Unlike NBD1 ligands like SION-638, SoC modulators do not increase the stability of the isolated ΔF508-NBD1 domain.

Differential static light scattering was used to assess the ability of SION-638 to stabilize NBD1.



N=4; error bars indicate standard deviation

Figure 3. SION-638 increased ΔF508-NBD1 stability by up to 10.4°C. Approved modulators elexacaftor (ELX), ivacaftor (IVA), tezacaftor (TEZ), and lumacaftor (LUM) have no direct impact on NBD1 stability.

SION-638 Improves ΔF508-CFTR Maturation

NBD1 stabilizer SION-638 corrects ΔF508-CFTR maturation and trafficking to fully WT levels when combined with complementary CFTR modulators.

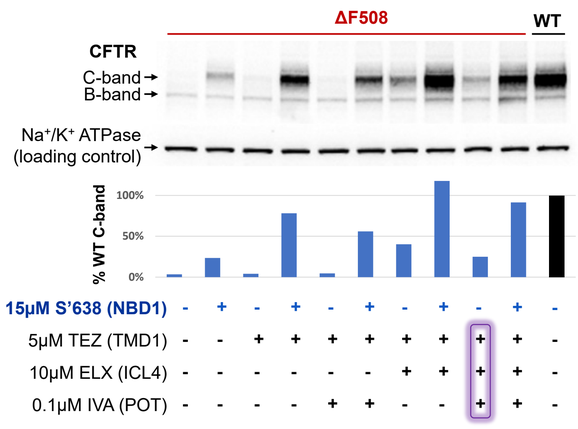


Figure 4. Western blot demonstrating that SION-638 corrects ΔF508-CFTR to WT levels when used in combination with complementary modulators in CFSMEo- cells as evidenced by WT levels of CFTR C band. TEZ binds to CFTR TMD1;[13] based on our work, ELX primarily acts on the ICL4 interface; as shown here, IVA is a CFTR potentiator with a negative impact on ΔF508-CFTR maturation.[14] In all studies, ELX, TEZ and IVA were used at their respective E_{max} concentrations.

SION-638 Improves ΔF508-CFTR Trafficking

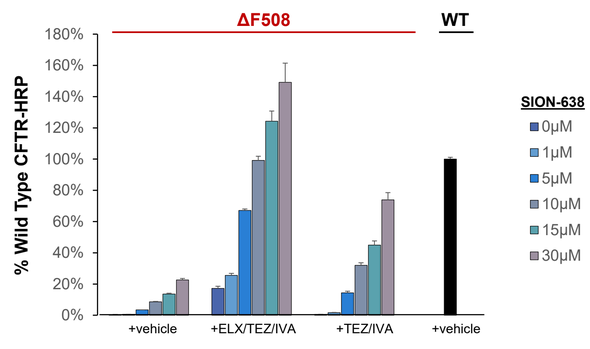


Figure 5. SION-638 increases cell surface ΔF508-CFTR to WT levels when combined with complementary modulators. An ΔF508-CFTR-horseradish peroxidase (HRP) trafficking assay was used to quantitate cell surface CFTR levels, following addition of a cell-impermeable HRP substrate to cells expressing ΔF508-CFTR or WT-CFTR with an HRP reporter inserted into the CFTR 4th extracellular loop. Data represent the mean of three biological replicates, and error bars display SE.

SION-638 Increases ΔF508-CFTR Function

CFTR channel activity in ΔF508 homozygous CFHBEs achieves WT levels when SION-638 is added to approved complementary modulators.

CFTR-dependent chloride transport measured in ΔF508/ΔF508 human bronchial epithelial cells (CFHBE) treated for 48 hours with SION-638 plus ELX/TEZ/IVA at their respective E_{max} concentrations.

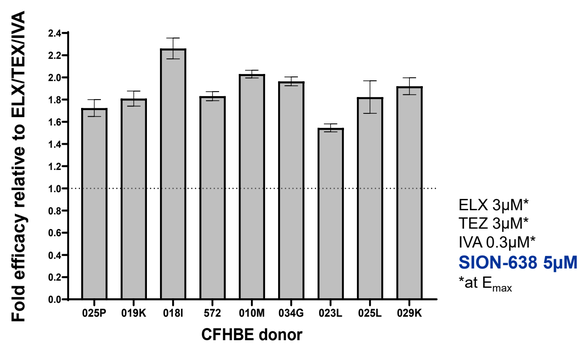


Figure 6. Vehicle-subtracted forskolin (FSK) peak of 9 CFHBE donors treated as shown, compared with ELX/TEZ/IVA alone in the same donor, same experiment. Bars are mean +/- SE of 6-8 replicates.

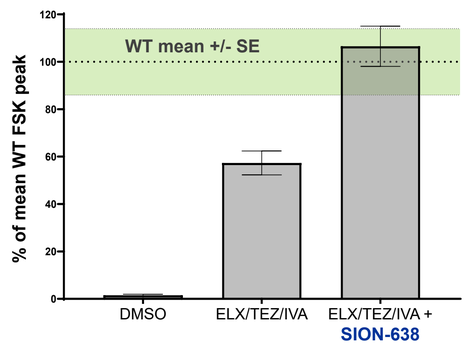
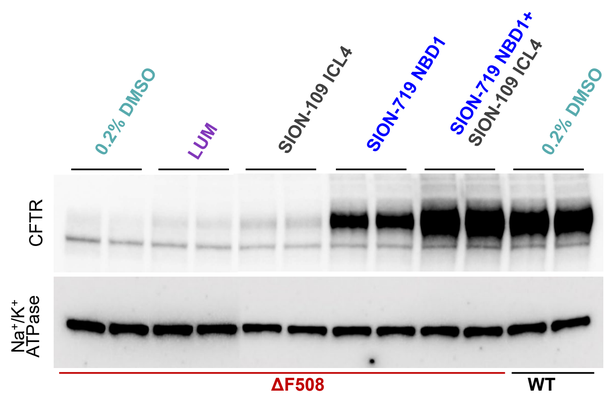


Figure 7. Mean data across donors in figure 6, expressed as FSK response compared to the average FSK response (green bar) across a panel of 8 WT donors (6-8 replicates per, dotted lines are +/- SE).

Advanced Higher Potency Sionna Modulators

Additional Sionna NBD1 stabilizers and complementary modulators provide the basis for novel combination therapies with the potential to be fully restorative.



3μM LUM – TMD1 corrector

3μM SION-109 – Sionna ICL4 corrector

3μM SION-719 – Sionna NBD1 stabilizer

Figure 8. Impact of Sionna high potency NBD1 development candidate SION-719 and ICL4-directed development candidate SION-109, on ΔF508-CFTR maturation. Western blot of CFTR expressing CFSMEo- cells. Biological replicates comparing both ΔF508-CFTR and WT-CFTR expressing cells, treated as indicated, are shown. The combination of SION-719 with ICL-directed SION-109 improves ΔF508-CFTR maturation to levels exceeding WT, further demonstrating the synergy between NBD1 stabilizers and modulators that address ΔF508-CFTR domain-domain assembly defects. ΔF508-CFTR trafficking studies fully recapitulate these results (*not shown*).

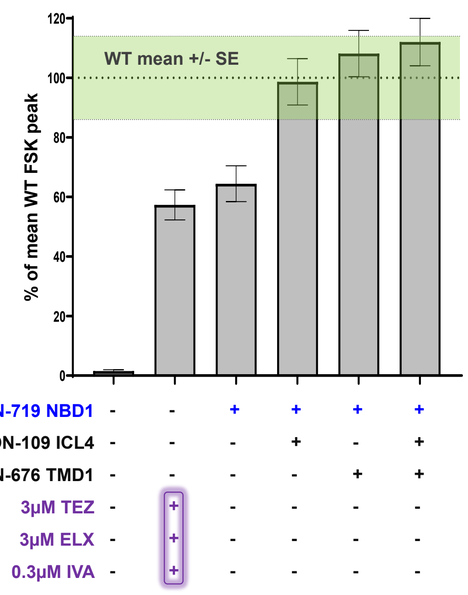


Figure 9. CFTR activity of ΔF508-CFTR homozygous CFHBEs treated for 48 hours with SION-719 alone and in combination with ICL4-directed SION-109 or the Sionna TMD1-directed modulator SION-676, compared with TEZ/IVA/ELX at its E_{max}. CFTR-dependent chloride transport (vehicle-subtracted FSK peak) is expressed as a relative percentage of the average FSK response across a panel of 8 non-CF HBE donors (green horizontal bar). Grey vertical bars represents the mean +/- standard error of 9 CFHBE donors with 6-8 replicates per donor. CFTR activity achieved non-CF HBE levels when NBD1 stabilizer SION-719 was combined with ICL4- or TMD1-directed correctors, or the combination thereof.

Sionna Modulators, a Path to Full Normalization

Anchored by NBD1 stabilizers, our goal is to deliver new therapies of unprecedented efficacy to patients with ΔF508 and other responsive mutations.

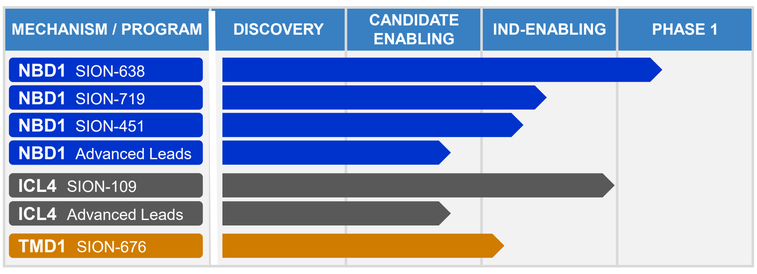


Figure 10. Sionna is advancing small molecules that robustly stabilize CFTR NBD1, a new and mechanistically novel therapeutic class, and TMD1- and ICL4-directed CFTR correctors that complement NBD1 stabilization. Novel Sionna NBD1-containing combinations have the potential to normalize ΔF508-CFTR maturation, trafficking, and function.

Acknowledgements

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