# Novel, Highly Potent NBD1 Stabilizing Development Candidates Enable Full ΔF508-CFTR Correction and a Path to Wild-type Function

Poster 289

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## Abstract

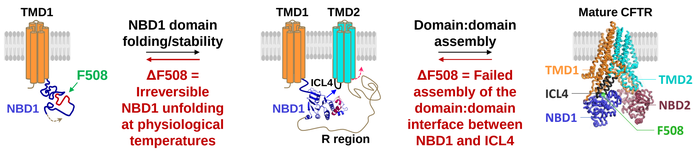
**Background:** ΔF508-CFTR is the most prevalent CFTR mutation in cystic fibrosis. ΔF508-CFTR results in the loss of phenylalanine 508 (F508) within CFTR's first nucleotide binding domain (NBD1). This results in NBD1 destabilization, which contributes centrally to defective ΔF508-CFTR folding, trafficking, half-life, and function [1]. As F508 also participates in the interface of NBD1 with CFTR intracellular loop 4 (ICL4) and transmembrane domain 1 (TMD1), ΔF508 further weakens CFTR domain-domain assembly, adding to its dysfunction [2]. Complete pharmacological correction of ΔF508-CFTR will likely require drugs that both fully stabilize NBD1 and restore normal CFTR domain-domain interactions [2]. Approved CFTR modulators provide clinical benefits to eligible patients through an impact on ΔF508-CFTR. However, approved modulators have no direct impact on NBD1 and do not fully normalize CFTR function in most people with CF [3]. After over a decade of research, our science team discovered SION-638 a first-in-class modulator that directly stabilizes NBD1 in a native conformation. Here we describe previously undisclosed development candidates from a second series of NBD1 stabilizers with significantly improved activity and potency compared to SION-638. Sionna is also developing complementary ICL4- and TMD1-directed modulators to use in combination with NBD1 stabilizers.

**Methods:** In rigorously validated functional and biochemical assays, we demonstrate the activity of a new series of Sionna NBD1 stabilizers, alone and in combination with Sionna ICL4- and TMD1-directed correctors, and with approved CFTR modulators.

**Results:** In the clinically-predictive CFHBE model and other preclinical systems, we demonstrate that highly potent Sionna Series 2 NBD1 stabilizers can fully restore ΔF508-CFTR maturation, trafficking, and function to wild-type levels in double combinations with mechanistically complementary modulators, including Sionna ICL4 or TMD1-directed development candidates, or when added to current standard-of-care modulators.

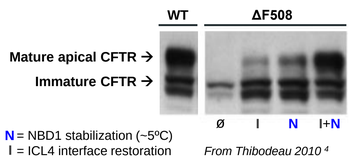
## Introduction

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




- NBD1 destabilization 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




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

*From Thibodeau 2010 [4]*

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

***NBD1 was considered undruggable.[5]***

### A fully-restorative therapy must address ΔF508's domain assembly and NBD1 stability[6]

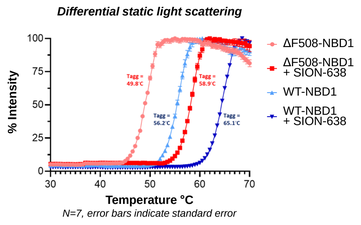
**To address the key drivers of ΔF508-CFTR dysfunction, Sionna is developing first-in-class NBD1 stabilizers plus novel modulators that correct CFTR assembly.**

- We leverage 14 years of effort and investment by Genzyme, Sanofi, CFF, and Sionna.
- We show that NBD1 stabilizers synergize with ICL4- and TMD1 correctors enabling full correction.
- **First-in-class NBD1 stabilizer SION-638 is now in Phase 1.**
- We are advancing additional NBD1 and other modulators to enable full ΔF508-CFTR correction.
- **ICL4-directed corrector SION-109 is progressing to Phase 1 studies**

## NBD1 Stabilizer SION-638 Increases ΔF508-CFTR Function

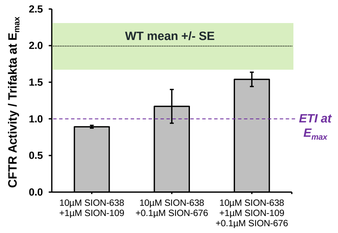
**SION-638 increases ΔF508-NBD1 stability above WT-NBD1 levels and ΔF508-CFTR channel activity to WT levels if combined with complementary modulators.**

**Figure 1.** 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.




*N=7, error bars indicate standard error*

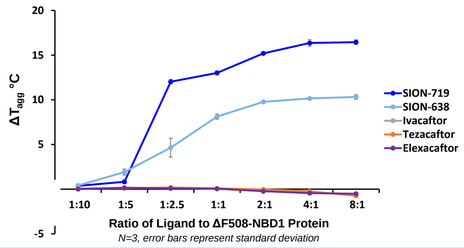
**Figure 2.** ΔF508 CFHBE CFTR current compared to ETI and, for reference, 8 WT donors (green bar is mean +/- SE, 6-8 replicates per donor). All compounds at $E_{max}$.




## SION-719 Stabilizes the NBD1 Domain of CFTR

**Differential static light scattering was used to assess the ability of Development Candidate SION-719 to stabilize NBD1.**

**Figure 3.** SION-719 increases ΔF508-NBD1 stability by more than 16°C, and SION-638 increases ΔF508-NBD1 stability by 10.4°C. Approved modulators elexacaftor (ELX), ivacaftor (IVA), and tezacaftor (TEZ) have no direct impact on NBD1 stability.




*N=3, error bars represent standard deviation*

## SION-719 Improves ΔF508 Maturation and Function

**NBD1 stabilizer SION-719 corrects ΔF508-CFTR maturation and channel function to fully WT levels when combined with complementary CFTR modulators.**

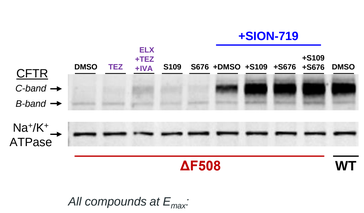



**Figure 4.** Impact of Sionna high potency NBD1 development candidate SION-719 and ICL4-directed development candidate SION-109, on ΔF508-CFTR maturation. Western blot of CFTR expressing CFSMEo- cells. Biological replicates comparing both ΔF508-CFTR and WT-CFTR expressing cells, treated as indicated, 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*).

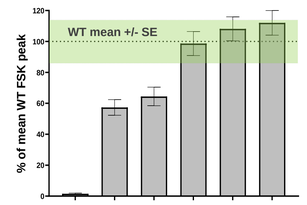
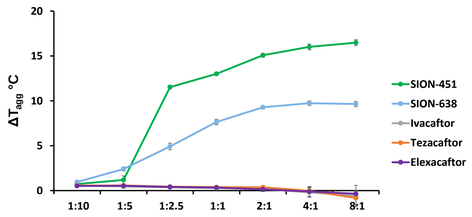



**Figure 5.** CFTR activity of ΔF508-CFTR homozygous CFHBEs treated for 48 hours with SION-719 alone or in combination with ICL4-directed SION-109 or the TMD1-directed modulator SION-676, compared with ETI (ELX/TEZ/IVA) at its $E_{max}$. CFTR-dependent chloride transport (vehicle-subtracted FSK peak) is expressed as a relative percentage of the average response across 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.

## SION-451 Stabilizes the NBD1 Domain of CFTR

**Differential static light scattering was used to assess the ability of Development Candidate SION-451 to stabilize NBD1.**

**Figure 6.** SION-451 increases ΔF508-NBD1 stability by more than 16°C, and SION-638 increases ΔF508-NBD1 stability by 10.4°C. Approved modulators elexacaftor, ivacaftor, and tezacaftor have no direct impact on NBD1 stability.




*N=4, error bars represent standard deviation*

## SION-451 Improves ΔF508 Maturation and Function

**NBD1 stabilizer SION-451 corrects ΔF508-CFTR maturation and channel function to fully WT levels when combined with complementary CFTR modulators.**

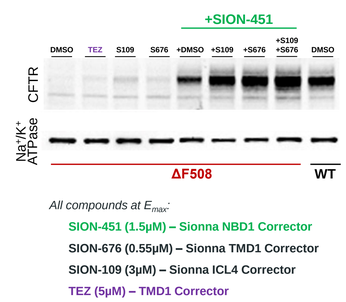



**Figure 7.** Impact of Sionna high potency NBD1 development candidate SION-451, ICL4-directed development candidate SION-109, and/or TMD1-directed candidate SION-676 on ΔF508-CFTR maturation. Western blot of ΔF508-CFTR expressing CFSMEo- cells. The combination of SION-451 with complementary ICL4- or TMD1-directed modulators 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*).

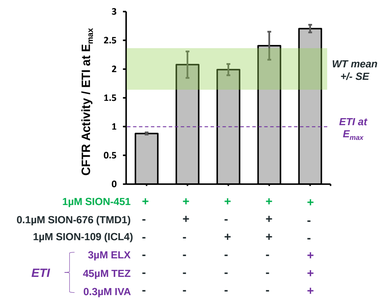
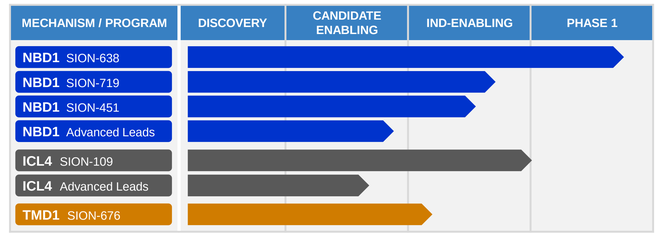



**Figure 8.** CFTR activity of ΔF508-CFTR homozygous CFHBEs in VALI media supplemented with human serum (20% vol/vol) and treated for 48 hours with SION-451 alone or in the combinations indicated. CFTR activity is expressed as a ratio relative to ETI at its $E_{max}$. Grey vertical bars represents the mean +/- standard error of 4 replicates. The green horizontal bar shows mean CFTR activity in 8 non-CF HBE donors. CFTR activity achieved non-CF HBE levels when NBD1 stabilizer SION-719 was combined with ICL4- or TMD1-directed correctors, the combination thereof, or when combined with ETI.

## Sionna Modulators - a Path to Full ΔF508-CFTR Correction

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




## Conclusions

Sionna is advancing small molecule stabilizers of CFTR NBD1, a novel therapeutic class with the potential to fully correct ΔF508 CFTR. These include the highly potent development candidates SION-719 and SION-451, and clinical-stage SION-638. Sionna TMD1- and ICL4-modulators include clinical candidates SION-676 and SION-109, which synergize with NBD1 stabilizers, enabling Sionna combinations with the potential to normalize ΔF508-CFTR function.

## Acknowledgements

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## References

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