NOVEL MODULATOR COMBINATIONS ADDRESS THE NBD1 STABILITY DEFECT CENTRAL TO ΔF508-CFTR DYSFUNCTION AND ENABLE FULL CORRECTION

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PRESENTER DISCLOSURE SLIDE

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The following relationships exist related to this presentation:

Sionna Therapeutics: co-founder, employee and shareholder
Longwood Capital: consultant

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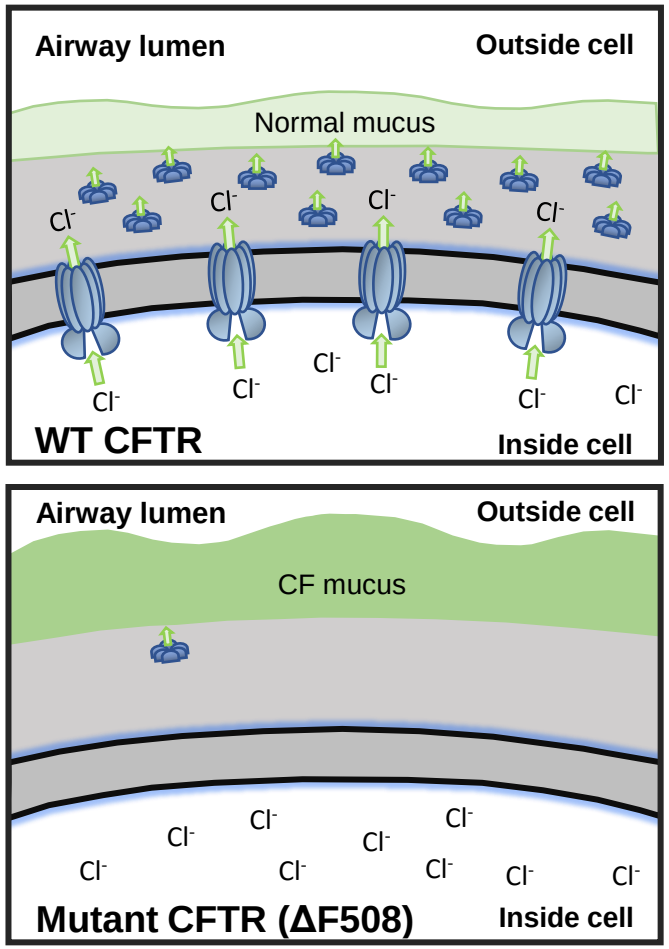
AN OVERVIEW OF CYSTIC FIBROSIS (CF)

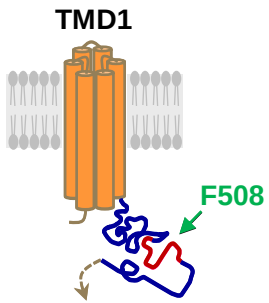
- CF is a serious and potentially fatal genetic disease.
- CF is caused by Cystic Fibrosis Transmembrane conductance Regulator (CFTR) loss of function mutations (LoF).
- CFTR LoF results in general exocrine gland dysfunction, decreased lung mucociliary clearance.
- >100,000 persons with CF worldwide and ~90% harbor the ΔF508-CFTR mutation (1).
- Pharmacological restoration of CFTR function is disease-modifying.
- For most patients, current therapies do not restore CFTR function to wild-type (WT) levels (2,3).



[1]CF registry data. [2]Heijerman *et al*. Lancet. 2019;394:1940–1948. [3]Middleton *et al*. N Engl J Med 2019;381:1809-1819.

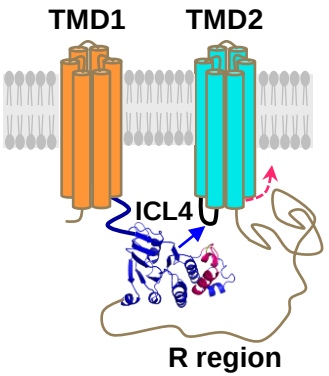
NBD1 INSTABILITY AND DEFECTIVE CFTR DOMAIN-DOMAIN ASSEMBLY ARE CENTRAL DRIVERS OF ΔF508-CFTR DYSFUNCTION

ΔF508-CFTR's errors in domain assembly and its critical NBD1 instability must be addressed.



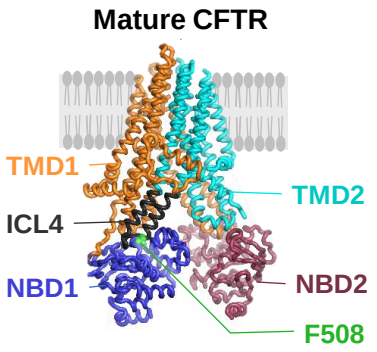
NBD1 domain folding/stability

ΔF508 = Irreversible NBD1 unfolding at physiological temperatures

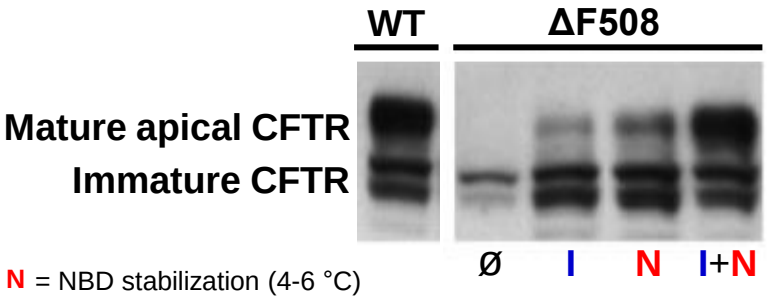


Domain-domain assembly

ΔF508 = Failed assembly of the domain-domain interface between NBD1 and ICL4



Proof of Hypothesis: Stabilizing NBD1 with Second-Site Mutations



N = NBD stabilization (4-6 °C)
I = ICL4 interface restoration

From Thibodeau 2010

CFTR suppressor mutations that stabilize NBD1 and the NBD1-ICL4 interface fully restore ΔF508-CFTR maturation and function to WT levels, providing a roadmap to more effective therapies.

Thibodeau *et al*. J Biol Chem. 2010 Nov 12;285(46):35825-35.

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IMPORTANCE OF NBD1 STABILIZATION TO FULL CORRECTION OF ΔF508-CFTR

Alterations to the processes of domain folding and assembly may both contribute to the misfolding and rescue of ΔF508 CFTR. These data provide evidence for the direct biochemical and biophysical alterations of NBD1 with the ΔF508 and -3M suppressor mutations, demonstrating a critical role of NBD1 folding in CFTR maturation.

"The CF-causing Mutation F508 Affects Multiple Steps in CF Transmembrane Conductance Regulator Biogenesis" *(Thibodeau 2010)*

Correction of both NBD1 energetics and domain interface is required to restore ΔF508 CFTR folding and function

Wael M. Rabeh[1,2,3], Florian Bossard[1], Haijin Xu[1], Tsukasa Okiyoneda[1], Miklos Bagdany[1], Cory M. Mulvihill[1], Kai Du[1], Salvatore di Bernardo[1], Yuhong Liu[4], Lars Konermann[4], Ariel Roldan[1], and Gergely L. Lukacs[1,2]

"Correction of both NBD1 Energetics and Domain Interface is Required to Restore F508 CFTR Folding and Function" *(Rabeh 2011)*

Combinations of different NBD1 changes had additive corrective effects on ΔF508 maturation that correlated with their ability to increase NBD1 thermostability. These effects were much larger than those caused by interface modification alone and accounted for most of the correction achieved by modifying both the domain and the interface. Thus, NBD1 stabilization plays a dominant role in overcoming the ΔF508 defect.

"Restoration of NBD1 Thermal Stability is Necessary and Sufficient to Correct ΔF508 CFTR Folding and Assembly" *(He 2014)*

The results indicate that both NBD1 folding and interaction with ICL4 are altered by the ΔF508 mutation and that correction of either individual process is only partially effective. By contrast, combination of mutations that counteract both defects restores ΔF508 maturation and function to wild-type levels.

"Requirements for Efficient Correction of F508 CFTR Revealed by Analyses of Evolved Sequences" *(Mendoza 2012)*

Recent studies revealed that ΔF508-NBD1 is thermodynamically and kinetically destabilized at physiological temperature and suggested that NBD1 stabilization would effectively counteract ΔF508-CFTR misprocessing[25,26]

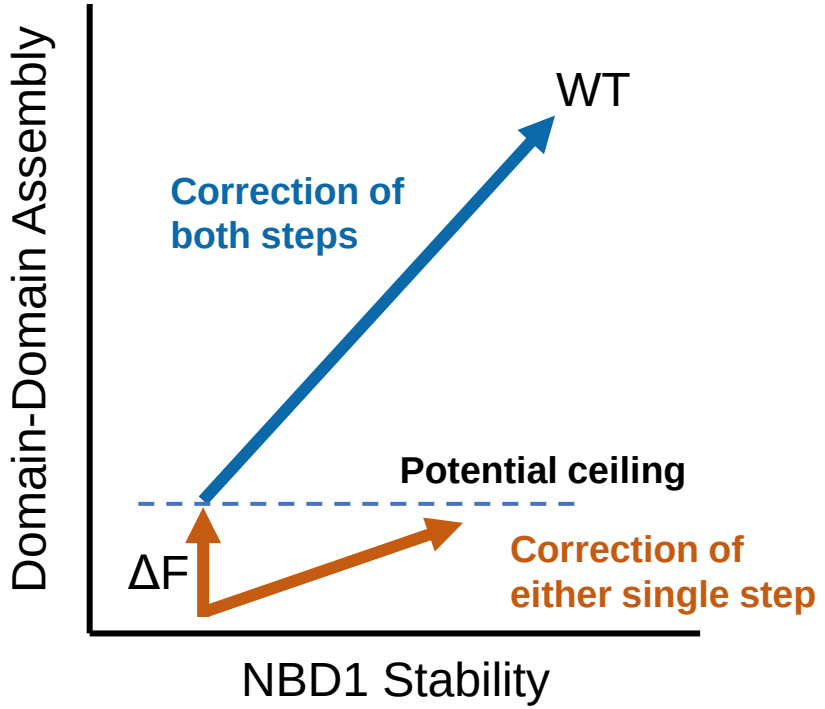
"Mechanism-based Corrector Combination Restores F508-CFTR Folding and Function" *(Okiyoneda 2013)*

Restoration of NBD1 Thermal Stability Is Necessary and Sufficient to Correct ΔF508 CFTR Folding and Assembly

"Restoration of NBD1 Thermal Stability is Necessary and Sufficient to Correct F508 CFTR Folding and Assembly" *(He 2014)*

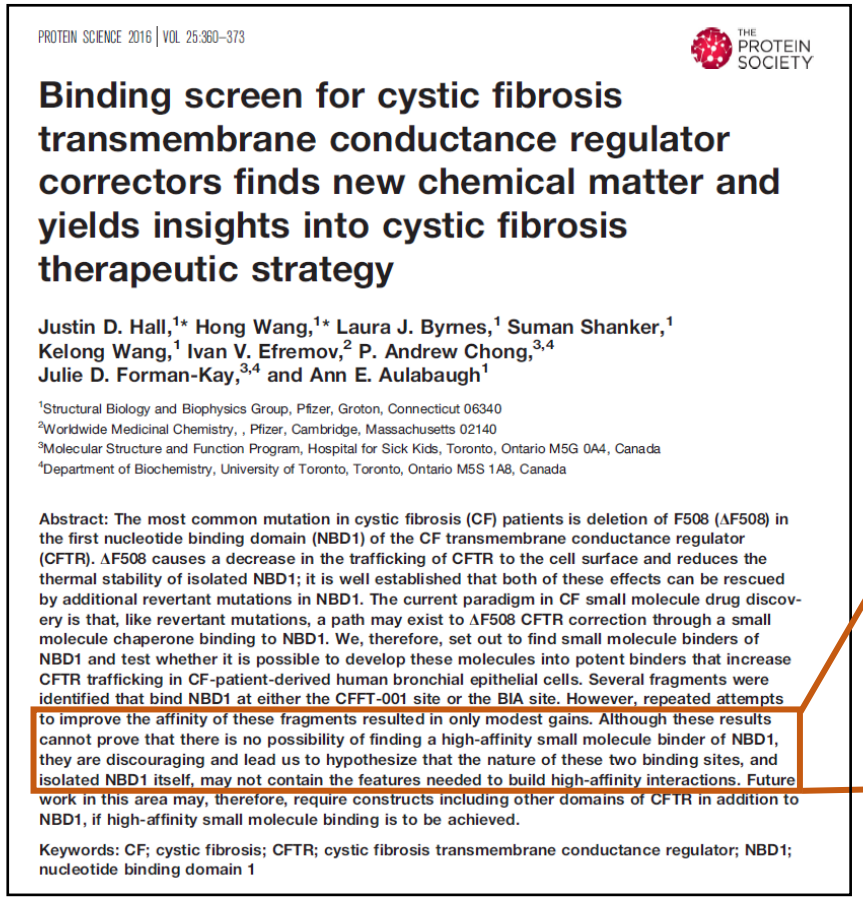
The co-translational rescue of ΔF508 NBD1 misfolding in CFTR by I539T advocates this domain as the most important drug target for cystic fibrosis.

"The Primary Folding Defect and Rescue of F508 CFTR Emerge during Translation of the Mutant Domain" *(Hoelen 2010)*



Adapted from Mendoza 2012, Rabeh 2011 and others

LACK OF HIT-FINDING SUCCESS AND INHERENT DIFFICULTY AS A TARGET LED NBD1 TO BE CONSIDERED "UNDRUGGABLE"



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THE PROTEIN SOCIETY

Binding screen for cystic fibrosis transmembrane conductance regulator correctors finds new chemical matter and yields insights into cystic fibrosis therapeutic strategy

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Abstract: The most common mutation in cystic fibrosis (CF) patients is deletion of F508 (ΔF508) in the first nucleotide binding domain (NBD1) of the CF transmembrane conductance regulator (CFTR). ΔF508 causes a decrease in the trafficking of CFTR to the cell surface and reduces the thermal stability of isolated NBD1; it is well established that both of these effects can be rescued by additional revertant mutations in NBD1. The current paradigm in CF small molecule drug discovery is that, like revertant mutations, a path may exist to ΔF508 CFTR correction through a small molecule chaperone binding to NBD1. We, therefore, set out to find small molecule binders of NBD1 and test whether it is possible to develop these molecules into potent binders that increase CFTR trafficking in CF-patient-derived human bronchial epithelial cells. Several fragments were identified that bind NBD1 at either the CFFT-001 site or the BIA site. However, repeated attempts to improve the affinity of these fragments resulted in only modest gains. Although these results cannot prove that there is no possibility of finding a high-affinity small molecule binder of NBD1, they are discouraging and lead us to hypothesize that the nature of these two binding sites, and isolated NBD1 itself, may not contain the features needed to build high-affinity interactions. Future work in this area may, therefore, require constructs including other domains of CFTR in addition to NBD1, if high-affinity small molecule binding is to be achieved.

Keywords: CF; cystic fibrosis; CFTR; cystic fibrosis transmembrane conductance regulator; NBD1; nucleotide binding domain 1

Hall 2016 (Pfizer),
"Although these results cannot prove that there is no possibility of finding a high-affinity small molecule binder of NBD1, they are discouraging and lead us to hypothesize that the nature of these two binding sites, and isolated NBD1 itself, may not contain the features needed to build high-affinity interactions."

TO FULLY NORMALIZE ΔF508-CFTR, SIONNA IS DEVELOPING NOVEL MODULATORS THAT ADDRESS THE KEY DRIVERS OF ITS DYSFUNCTION

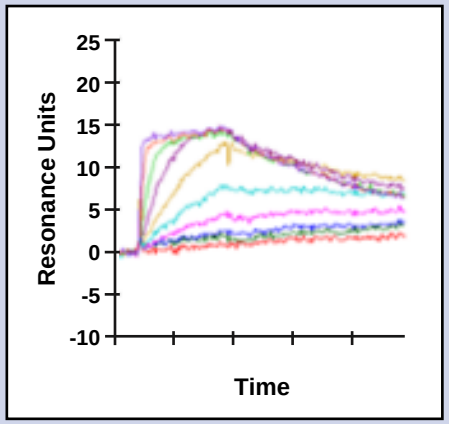
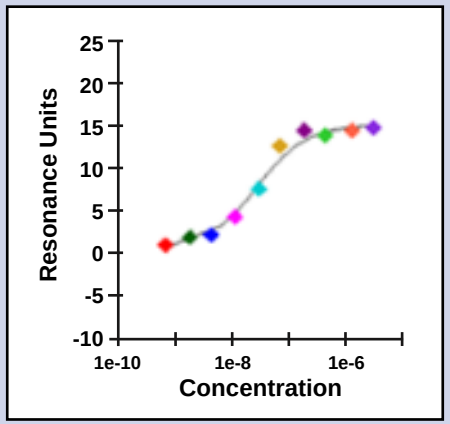
- **We leverage 12+ years of investment by Sionna, CFF, Genzyme, and Sanofi.**
  - >600 FTE years, >$120MM spent
  - Discovery: millions of compounds assessed in over a dozen screens
  - Optimization: >10,000 novel compounds across multiple series synthesized/characterized
  - >200 *in vivo* PK studies, >15 multiple-dose tox studies conducted
- **First-in-class NBD1 stabilizer SION-638 is IND enabled.**
  - Additional NBD1 stabilizers are being advanced.
- **Sionna is advancing modulators with mechanisms of action complementary to NBD1.**
  - ICL4 and TMD1-directed correctors and back-ups are advancing to IND.
- **Complementary assets enable proprietary, potentially clinically-superior combo therapy.**

Hypothesis: Stabilize NBD1 + Improve Domain Assembly = Fully Restorative Therapy

WE IDENTIFIED HIGH AFFINITY DRUG-LIKE SMALL MOLECULE NBD1 LIGANDS

Surface Plasmon Resonance (SPR)

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

SPR Sensorgram WT NBD1	Dose Response WT NBD1
	

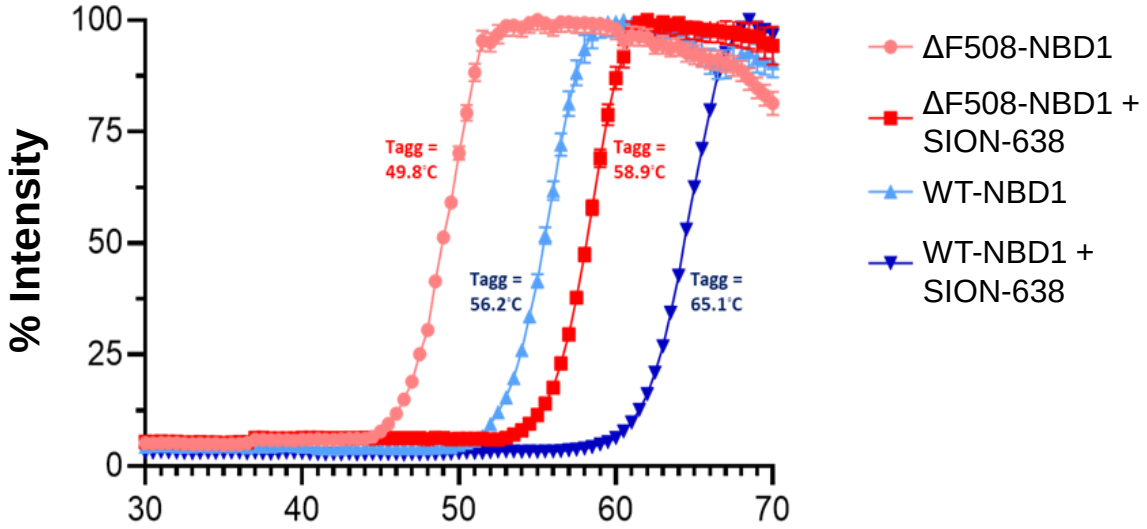
SION-638 binds to ΔF508 and WT NBD1 isoforms with a KD < 50 nM.

Thousands of NBD1 ligands were synthesized.

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

NBD1 LIGANDS LIKE SION-638 INCREASE ΔF508-NBD1 STABILITY

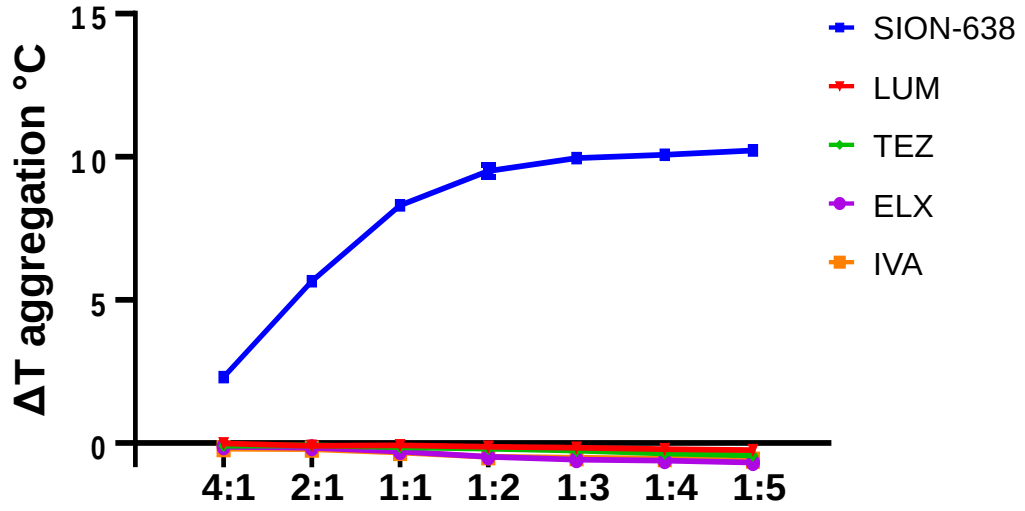
Differential Static Light Scattering (DSLS)



Temperature °C

N=7, error bars indicate standard error

SION-638 increases ΔF508-NBD1 stability above WT-NBD1 levels



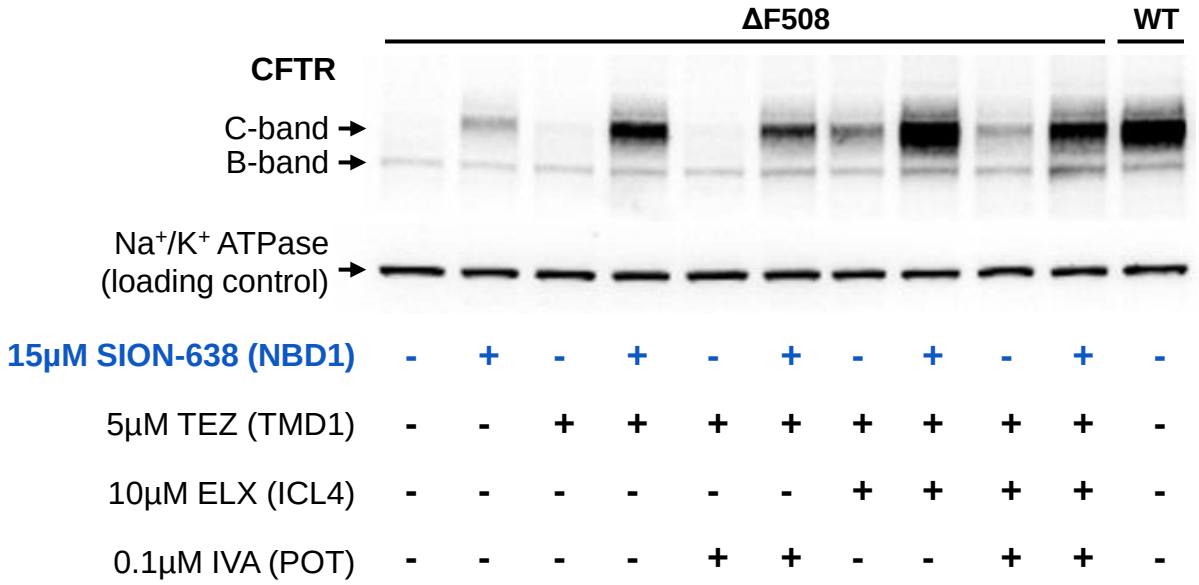
Ratio of ΔF508-NBD1 Protein to Compound

N=4; error bars indicate standard deviation

Approved CFTR modulators do not meaningfully impact NBD1 stability

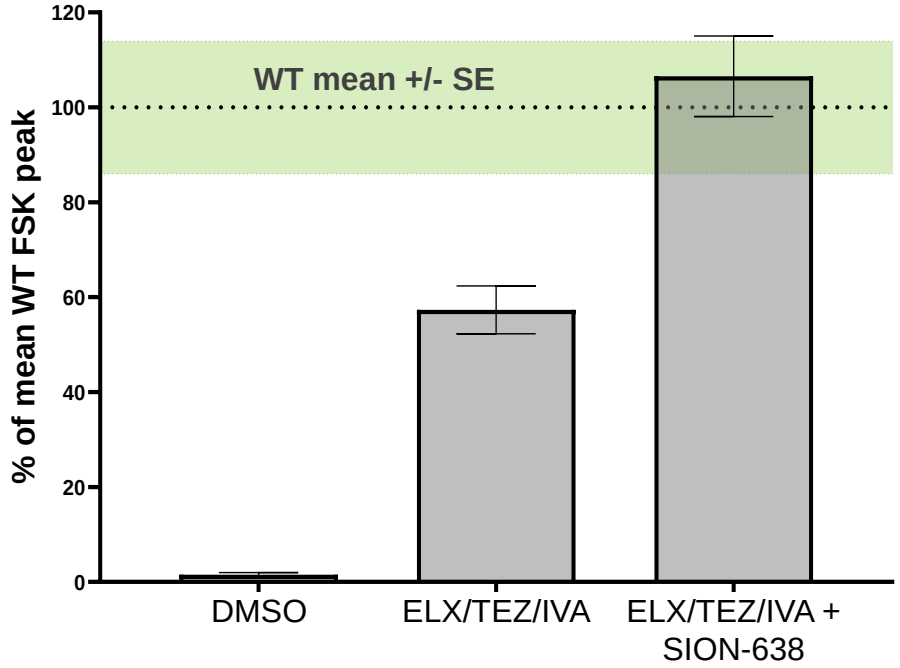
SION-638 CORRECTS ΔF508-CFTR MATURATION AND FUNCTION TO WT LEVELS WHEN COMBINED WITH APPROVED CFTR MODULATORS

CFSMEo- CFTR Western



SION-638 increases ΔF508-CFTR C-band levels to WT when combined with SoC modulators.

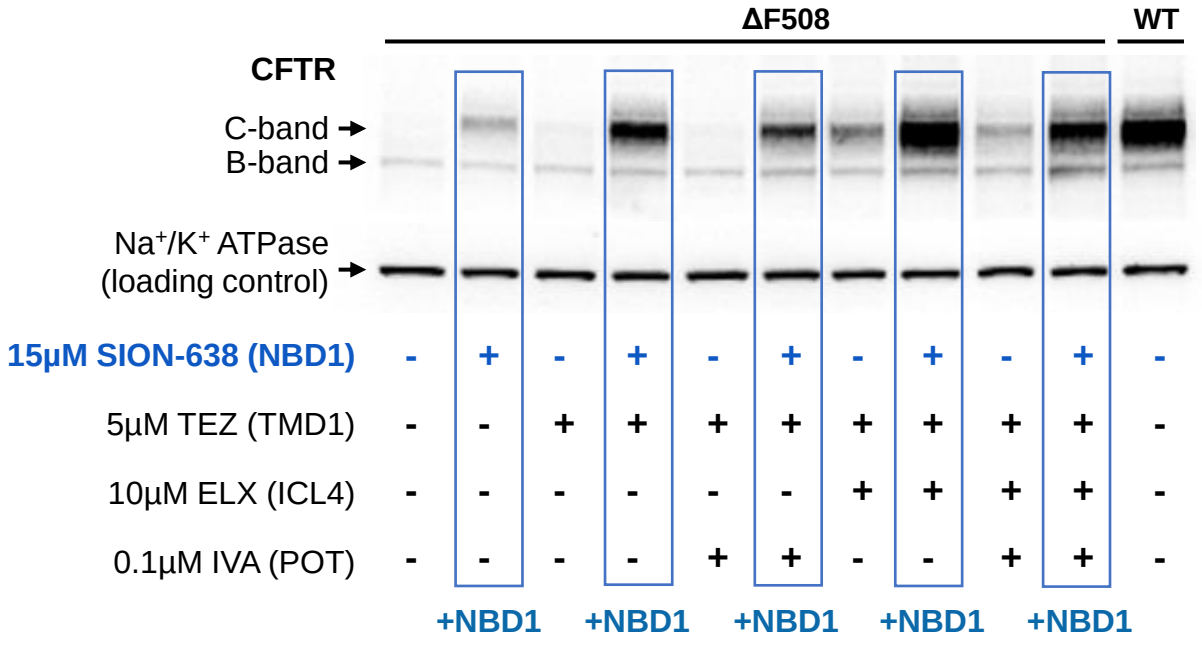
CFHBE Ephys - 9 ΔF508/ΔF508, 8 WT HBEs



CFTR activity in ΔF508/ΔF508 CFHBEs achieves mean WT levels in combo with complementary modulators.

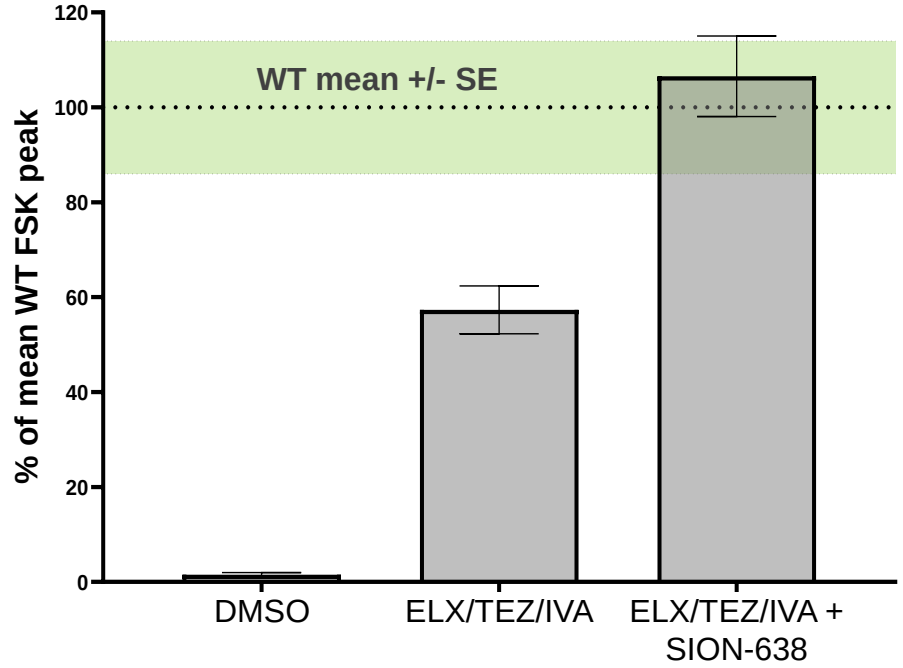
SION-638 CORRECTS ΔF508-CFTR MATURATION AND FUNCTION TO WT LEVELS WHEN COMBINED WITH APPROVED CFTR MODULATORS

CFSMEo- CFTR Western



SION-638 increases ΔF508-CFTR C-band levels to WT when combined with SoC modulators.

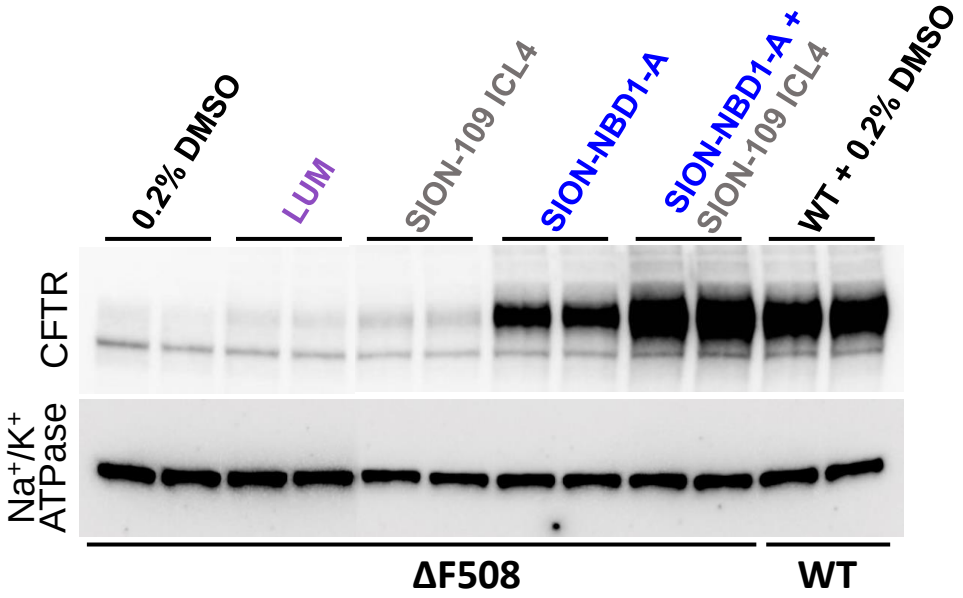
CFHBE Ephys - 9 ΔF508/ΔF508, 8 WT HBEs



CFTR activity in ΔF508/ΔF508 CFHBEs achieves mean WT levels in combo with complementary modulators.

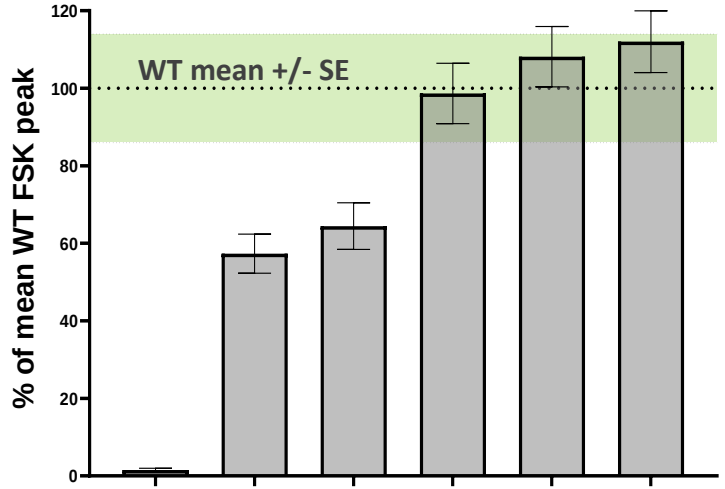
WE ARE ADVANCING ADDITIONAL NBD1 AND COMPLEMENTARY CFTR MODULATORS

CFSMEo- CFTR Western



3μM LUM – TMD1 corrector
3μM SION-109 – Sionna ICL4 corrector
3μM SION-NBD1-A – Sionna NBD1 stabilizer

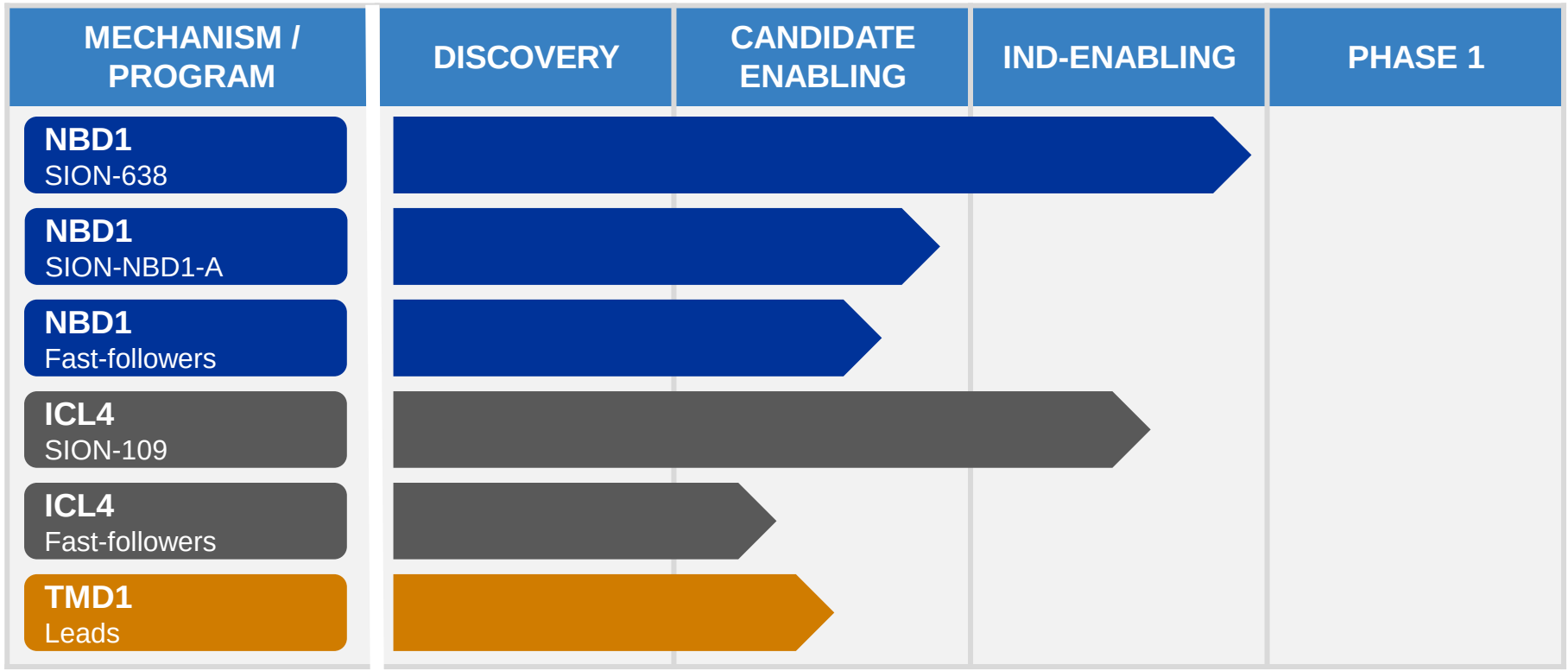
CFHBE Ephys - 9 ΔF508/ΔF508, 8 WT HBEs



1μM SION-NBD1-A	-	-	+	+	+	+
1μM SION-109 (ICL4)	-	-	-	+	-	+
0.1μM SION-TMD1	-	-	-	-	+	+
3μM TEZ	-	+	-	-	-	-
3μM ELX	-	+	-	-	-	-
0.3μM IVA	-	+	-	-	-	-

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

THE SIONNA CFTR MODULATOR PIPELINE



Anchored by NBD1, our goal is to advance complementary modulators to deliver new therapies of potentially higher efficacy to patients with ΔF508 and other responsive mutations.

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Sionna Science Team

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QUESTIONS & ANSWERS

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