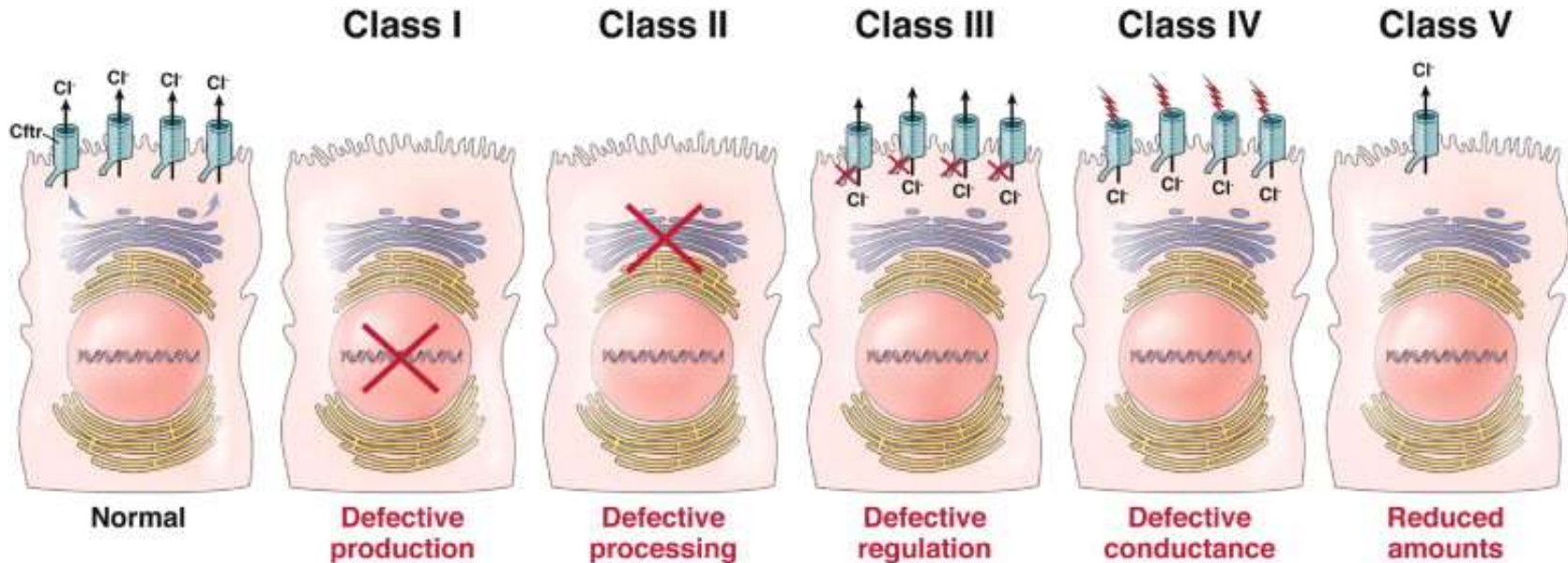


Antisense Oligonucleotide Splicing Modulation: A Novel Therapeutic Approach for CF

October 20, 2017
Israeli CF Society Conference

Classes of CFTR mutations



No CFTR production

Premature termination codons
or splicing mutations that
completely abolish protein
synthesis

**The abnormally
processed CFTR is
degraded**

**Dysregulation of
CFTR (the mutated
CFTR is not
stimulated by ATP)**

**Defective chloride
conductance or
channel gating**

**Reduced normal CFTR
mRNA transcripts and
protein synthesis**

e.g. Stop mutations W1282X, G542X

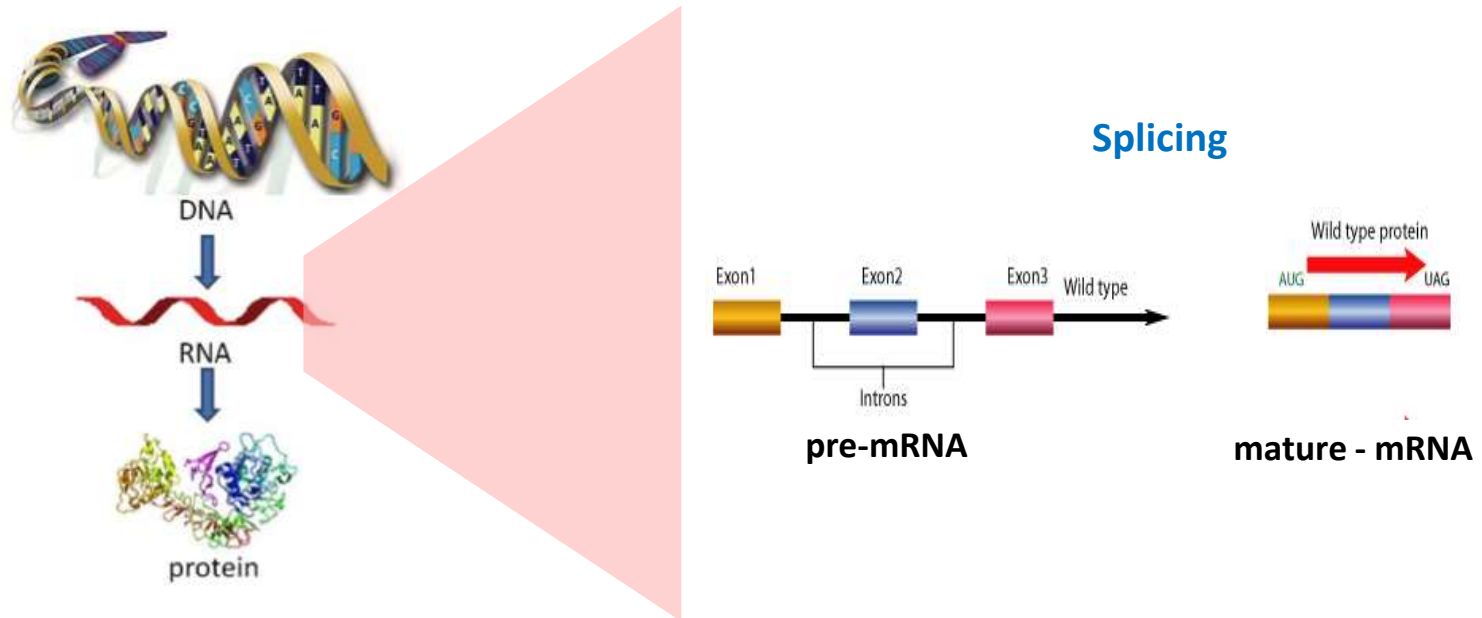
Amino acid
deletion F508del

Missense G551D

Missense R117H

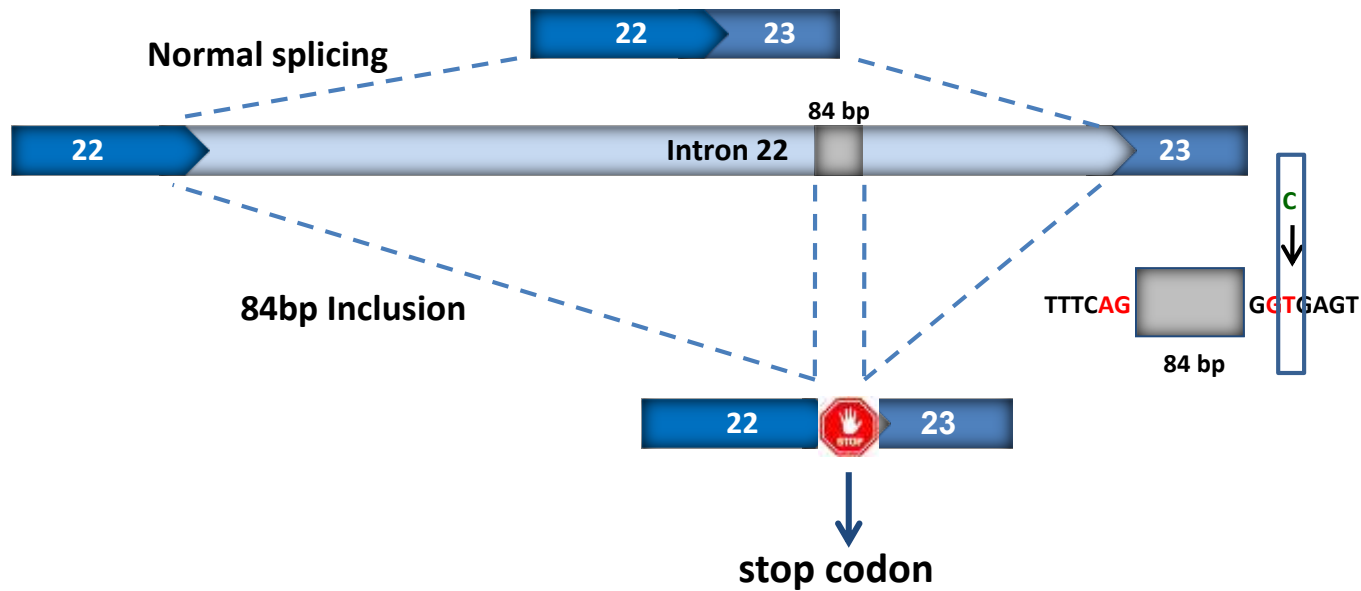
Splicing 3849+10kbC>T,
exon 10 splicing

The splicing mechanism



- **Splicing** is a cellular machinery responsible for removal of introns from pre-mRNA so that the exons are joined together to form mature RNA required for production of functional protein.
- The splicing machinery uses specific intronic and exonic sequence motives to regulate splicing efficiency.
- >150 proteins are involved in splicing.
- A significant fraction (10-15%) of disease causing mutations affects pre-mRNA splicing

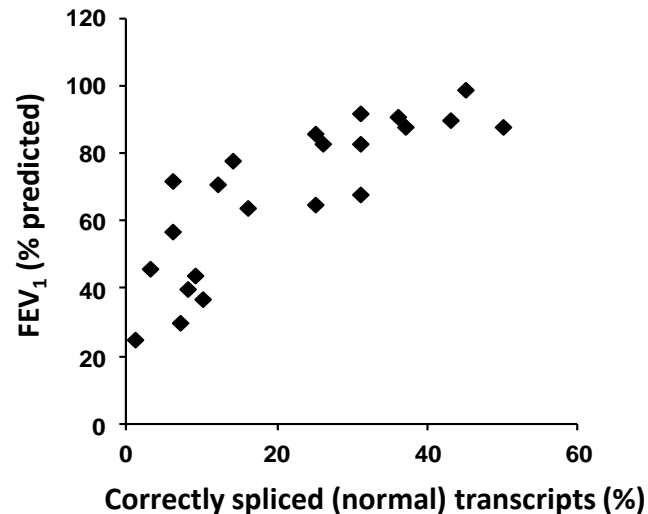
Aberrant splicing of 3849+10kb C->T mutation



Leading to degradation by the nonsense mediated mRNA decay (NMD) mechanism

Correlation between lung function and levels of normal RNA

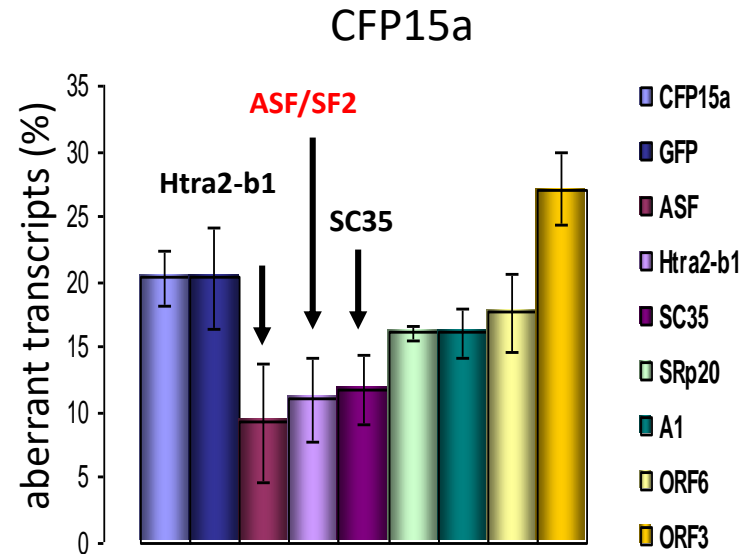
50% CFTR function is enough to be healthy (carriers)



- We previously analyzed in RNA samples from CF patients carrying splicing mutations the percentage of correctly spliced transcripts
- A correlation was found between the percentage of correctly spliced transcripts and lung function

→ **Modulation of splicing pattern towards higher levels of correctly spliced transcripts may have a clinical benefit to patients**

Modulation of 3849+10kb C->T splicing pattern in nasal epithelial cells carrying the 3849 mutation



As a proof of concept we have overexpressed several splicing factors

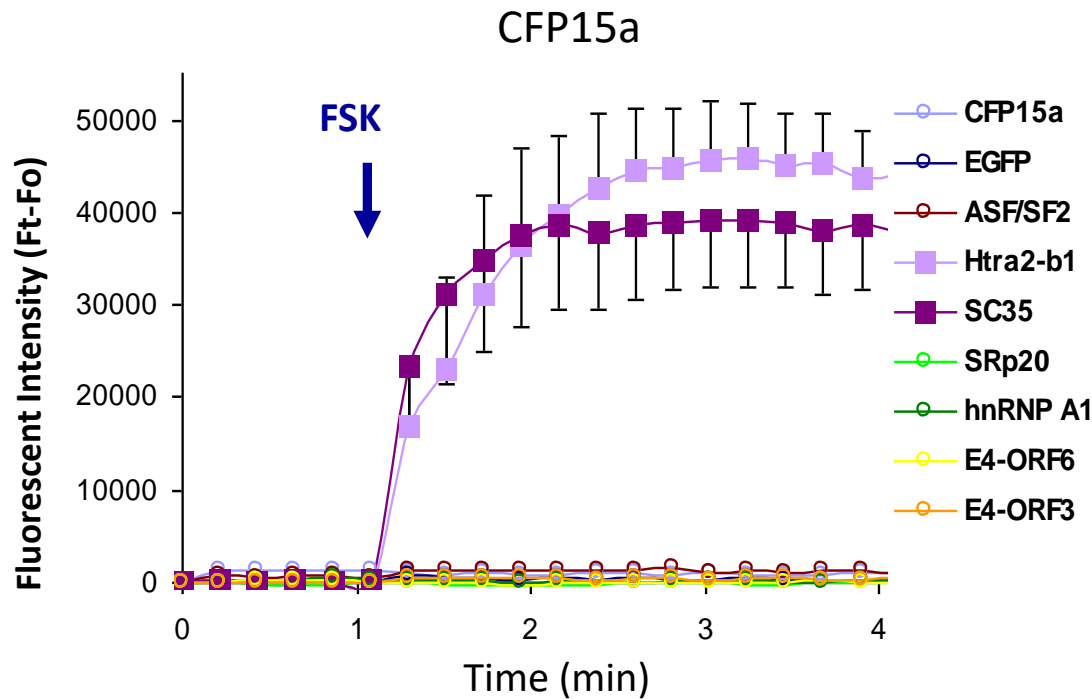
Several splicing factors led to a decrease in the levels of aberrant transcripts (less inclusion of the 84 bp cryptic exon).

Nissim-Rafinia...Kerem, EMBO Rep 2004

Nissim-Rafinia and Kerem B, *Trends in genetics* 2001

Restoration of CFTR function

in nasal epithelial cells carrying the 3849 mutation



Over expression of splicing factors, which reduced aberrant transcripts, restored the function of the CFTR channel.

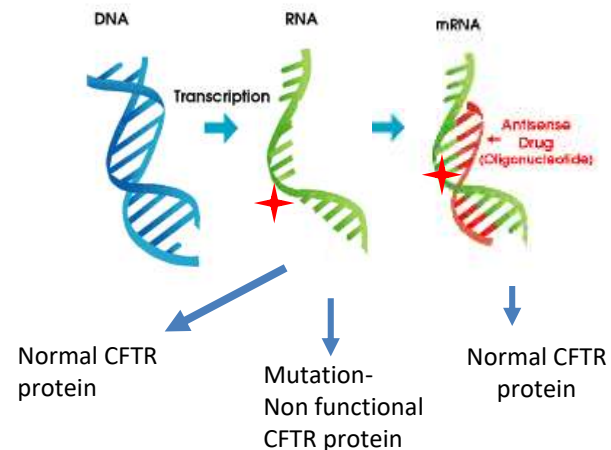
→ Reducing the level of the aberrantly CFTR spliced transcripts, containing the 84bp transcripts, restores CFTR function

Antisense oligonucleotides (ASOs)

ASOs are small synthetic nucleic acid molecules able to bind RNA

- The ASOs are chemically modified to enable:
 - Resistance to nucleases
 - Improved affinity for RNA
 - Prevention of RNase H from inducing cleavage of RNA:RNA hybrids

- ASOs can be used for:
 - mRNA down regulation
 - RNA editing
 - Splicing modulation



Antisense oligonucleotides technology for correcting splicing defects

- ASOs are designed to anneal to selected splice motifs, avoiding their recognition by the splicing machinery.
- This enables to enhance or inhibit the inclusion of a specific region in the mRNA.

IONIS and Biogen success with ASO-based drug for SMA

FDA News Release

Dec, 23, 2016

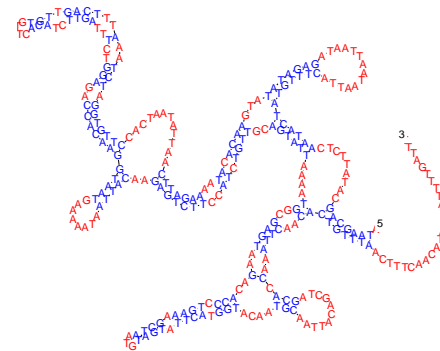
FDA approves first drug for spinal muscular atrophy

New therapy addresses unmet medical need for rare disease

- Spinraza (nusinersen) enhances exon 7 inclusion in the SMN2 gene.
- Spinraza met the primary endpoint in phase III clinical trial in infants with a severe SMA type.
- The treatment leads to a remarkable improvement in motor function and developmental milestones.

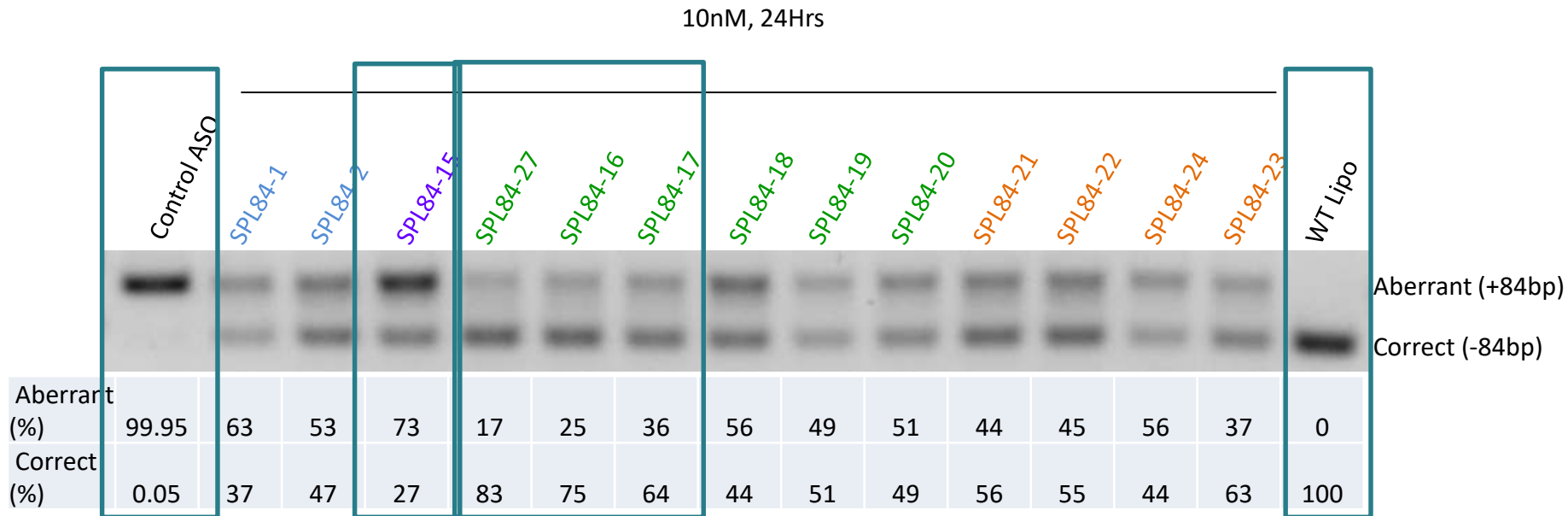
Establishment of systems for ASO design and screen

- ASO design is based on optimization of variable parameters. *SpliSense* has build an in house computational tool that supports the design of a large number of ASO sequences at the target regions.



- We established a cellular screening system stably **expressing the 3849 mutant CFTR** in FRT cells, that enables rapid and reliable RNA and functional analyses.
 - FRT cells with the **normal CFTR** sequence were also generated
- in collaboration with the lab of Prof. Steve Rowe

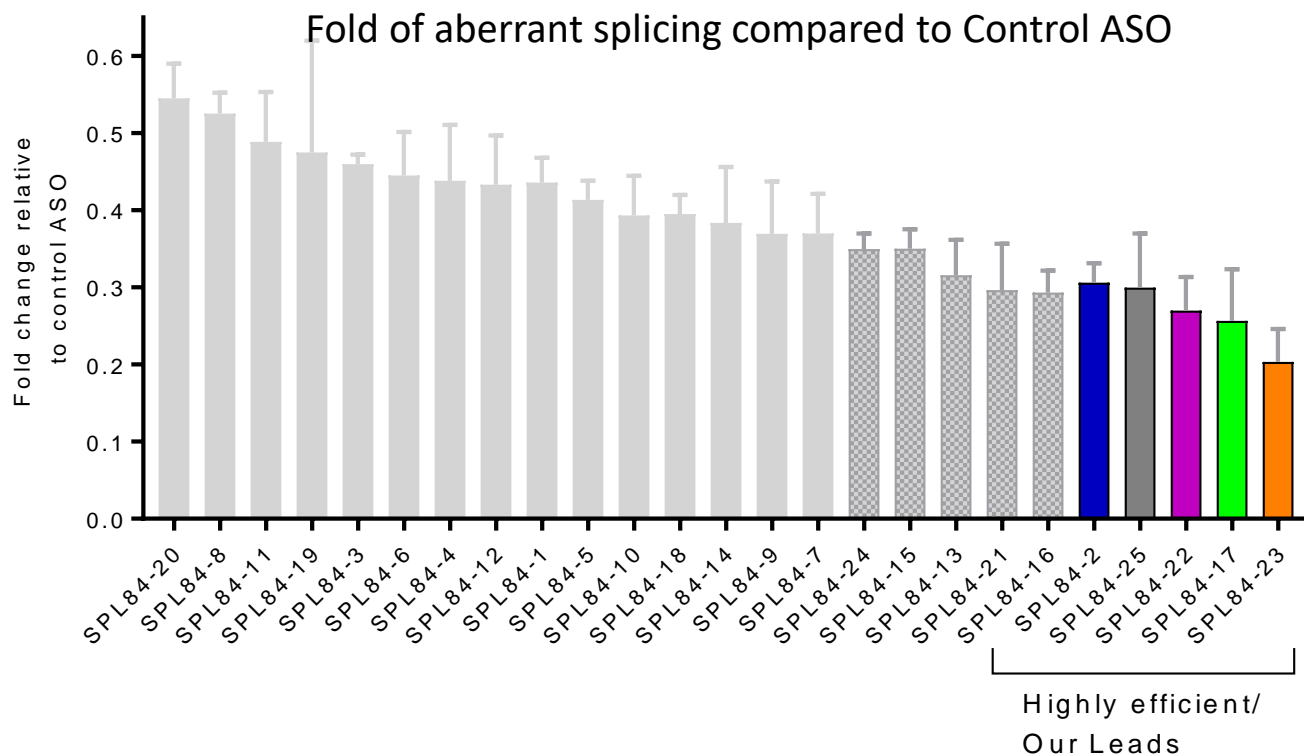
Screen for ASOs correcting 3849+10kb C->T splicing pattern



- >30 ASOs were screened for their effect of CFTR splicing in a cellular system cells stably expressing CFTR cDNA carrying the 3849+10kb C-to-T mutation
- An example of a PCR gel showing the different effects of the ASOs in inducing correct CFTR splicing compared to control ASO

Correction of
splicing pattern

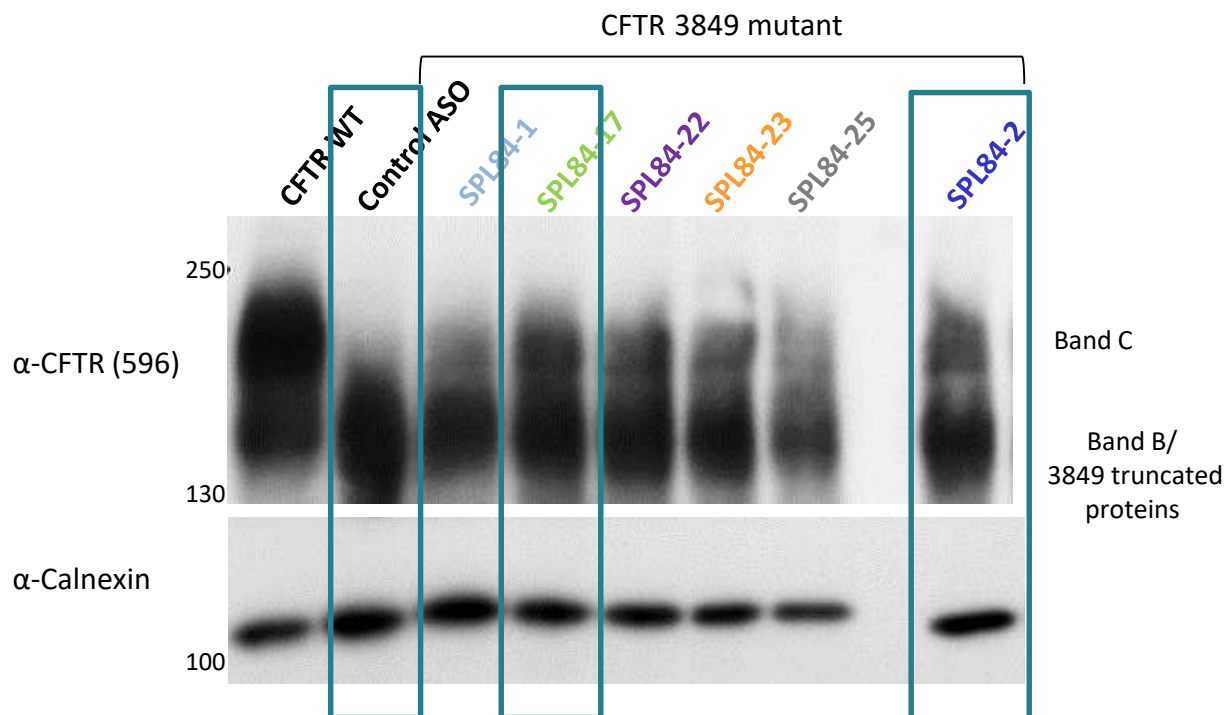
Screen for ASOs correcting 3849+10kb C->T splicing pattern



- qRT-PCR results of the ASOs screen.
- The values shown are the average fold decrease in the levels of the aberrant transcripts relative to the levels in cells treated with control ASO.
- The 5 best ASOs were chosen for further analysis

Correction of
splicing pattern

The effect of SpliSense lead ASOs on CFTR protein levels

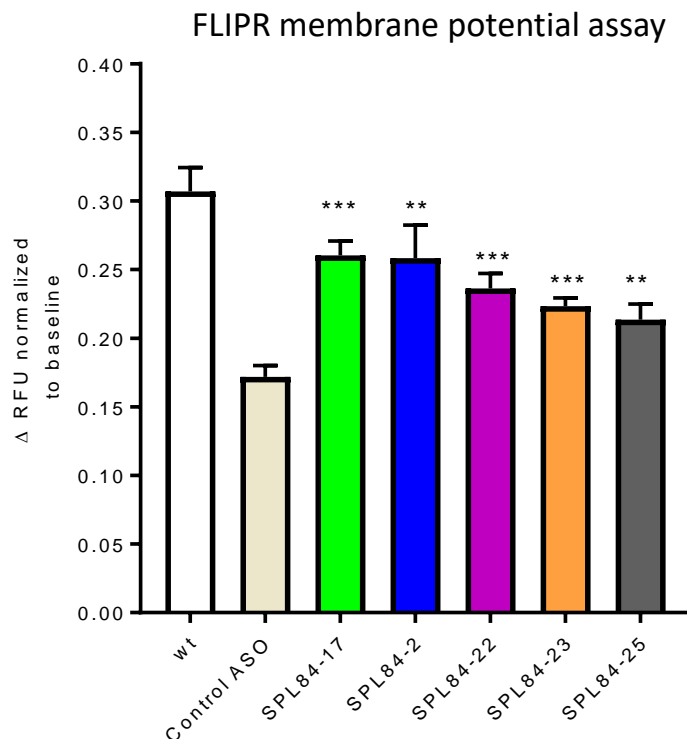


- Lead ASOs induce the generation of **normal full length mature CFTR protein (WT sequence)**.
- SPL84-17 and SPL84-2 lead to the formation of the highest levels of normal CFTR protein.

Correction of
splicing pattern

Normal protein
production

The effect of *Splisense* lead ASOs on the channel function



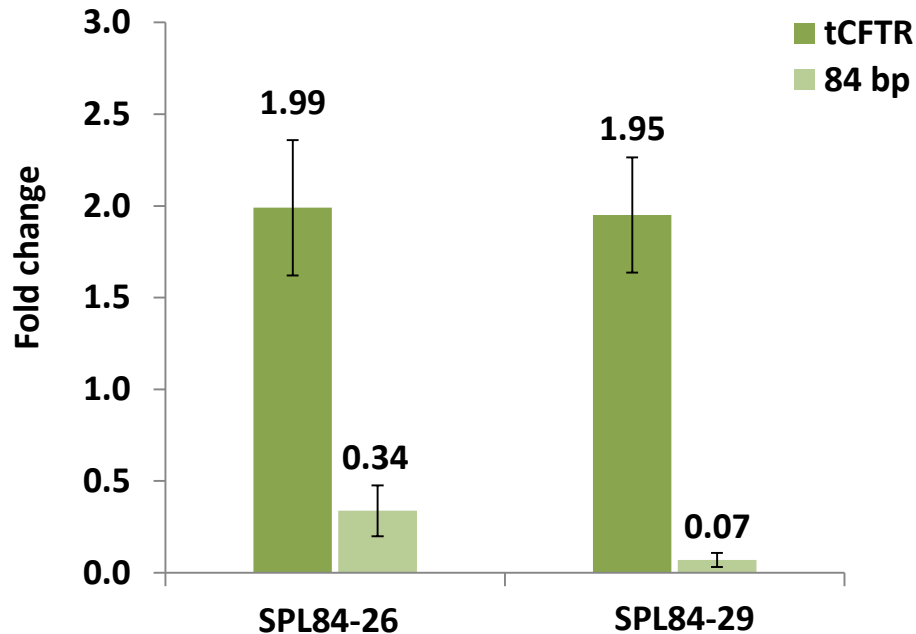
- SPL84-17 and SPL84-2 also have the strongest effect on CFTR function.
- Transfection of SPL84-17 to FRT 3849 mutant cells recovers CFTR activity to >80% of wild type.

Correction of
splicing pattern

Normal protein
production

Restoration of Channel
function

The effect of ASOs on the CFTR splicing pattern in primary Human Bronchial Epithelial (HBEs) cells



Following ASO transfection the levels of aberrantly spliced CFTR transcripts are reduced in primary HBEs carrying the 3849+10kb C>T mutation.

Summary

- ASOs were screened to find the top ASOs leading to the stronger effect on 3849 +10Kb C-to-T mutation.
- The chosen lead ASOs have a dosage effect with very high affinity, thus having an effect already at very low concentrations.
- The lead ASOs result in the formation of normal full length CFTR protein (WT sequence) and thus restore the CFTR function.
- Preliminary results in HBE, derived from a 3849/F508del patient, show a significant reduced levels of aberrantly spliced transcripts.
- Functional assays in HNEs using Ussing Chamber measurements are under way.

Thanks

Kerem's CF group

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