New therapies - the potential of splicing modulation as mono and combined therapy

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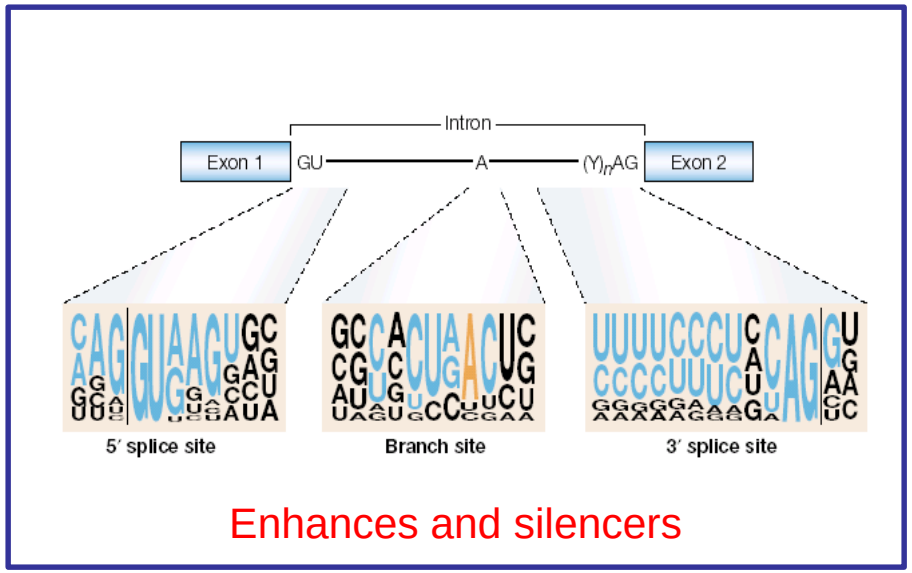
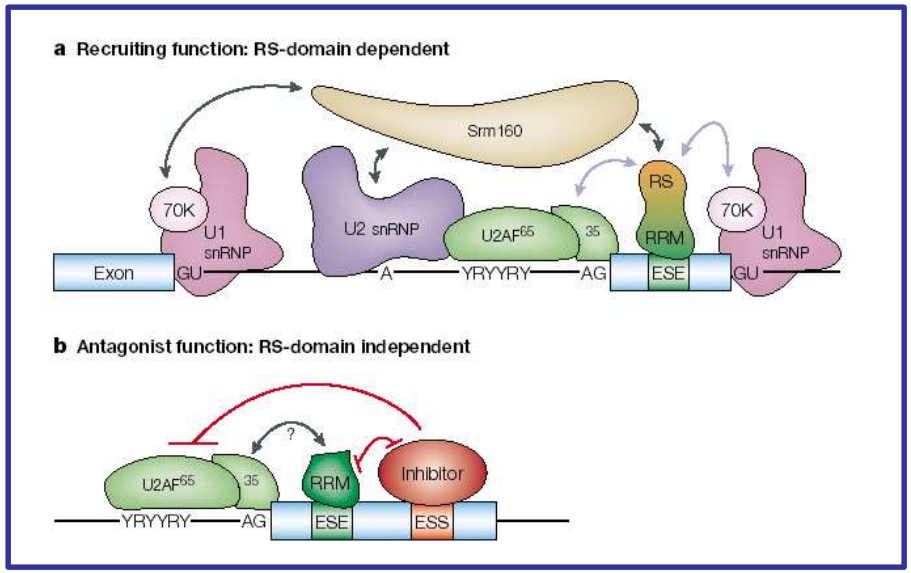
The Annual Israeli CF Society Conference,
November 6, 2015

Interaction of cis and trans splicing elements

DNA -> RNA -> Protein



Enhances and silencers



Cis motifs (DNA sequence) and their interactions with trans elements (splicing factors) affect splicing efficiency.

Cartegni et al., Nat Rev 2002

CF MUTATIONS

- The CFTR gene has 27 exons, that are all required for the normal function of the gene.

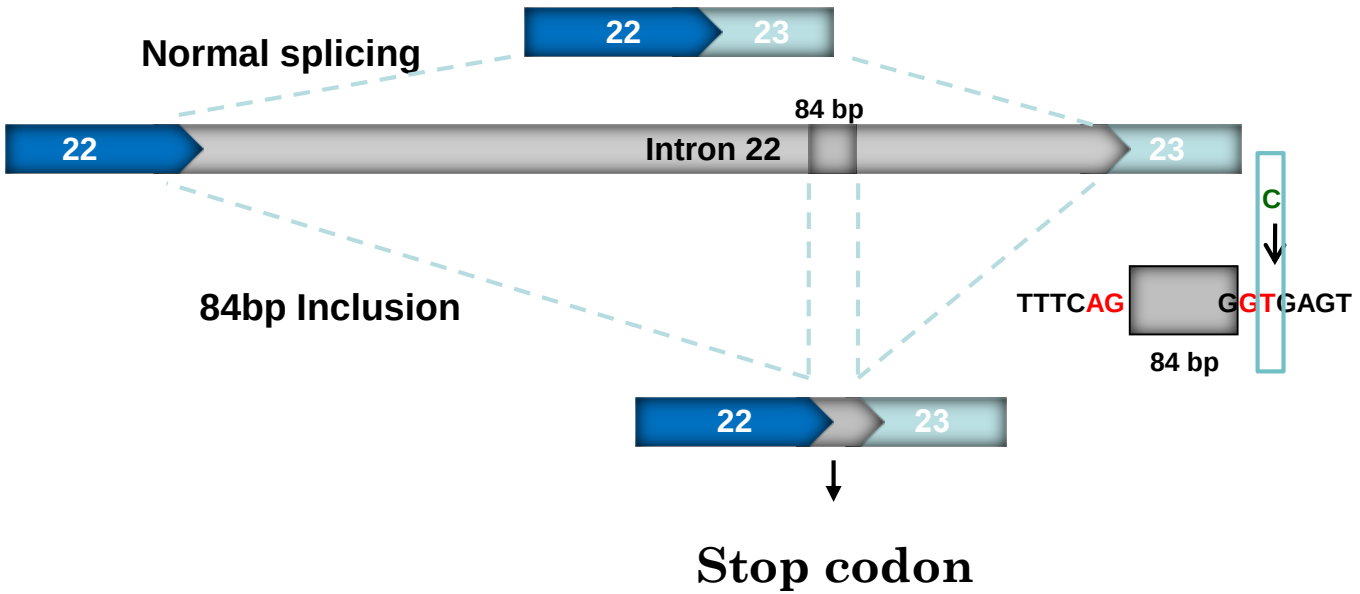
- No functional alternative spliced isoforms were found.

- There are ~2000 different disease-causing mutations in the CFTR gene.

- 10-15% of the mutations are splicing mutations.

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

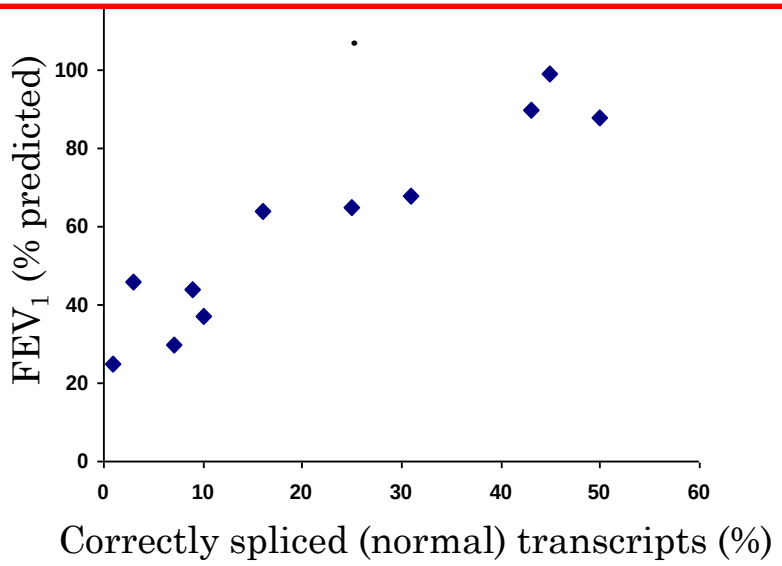
The mutation affects >850 patients in Europe and US



- Leading to degradation by the nonsense mediated mRNA decay (NMD) mechanism.
- The mutation leads to the generating of both correctly and aberrantly spliced transcripts.

Correlation between splicing pattern and disease severity

This led to the conclusion that the splicing machinery is a modulator of disease severity in patients carrying the 3849 +10kb C-to T mutation.



- The disease severity is correlated with the level of correctly spliced transcripts (not including the 84 bp).

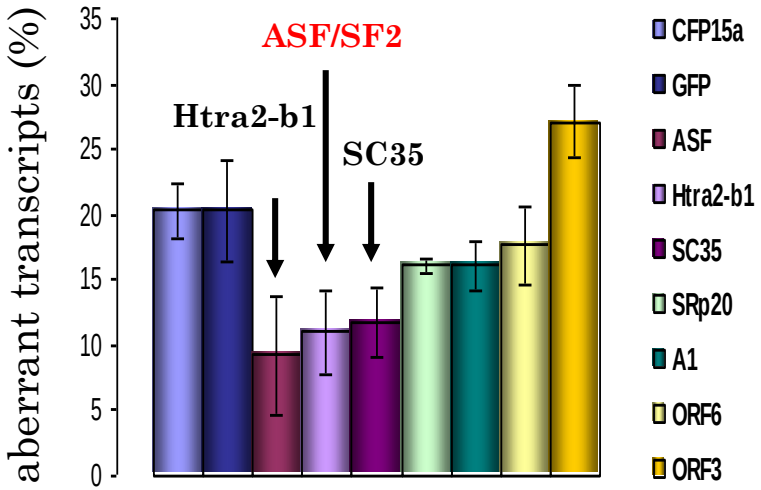
Splicing Modulation – Proof of Concept

The effect of overexpression of splicing factors on:

- The CFTR splicing pattern
- The CFTR protein function

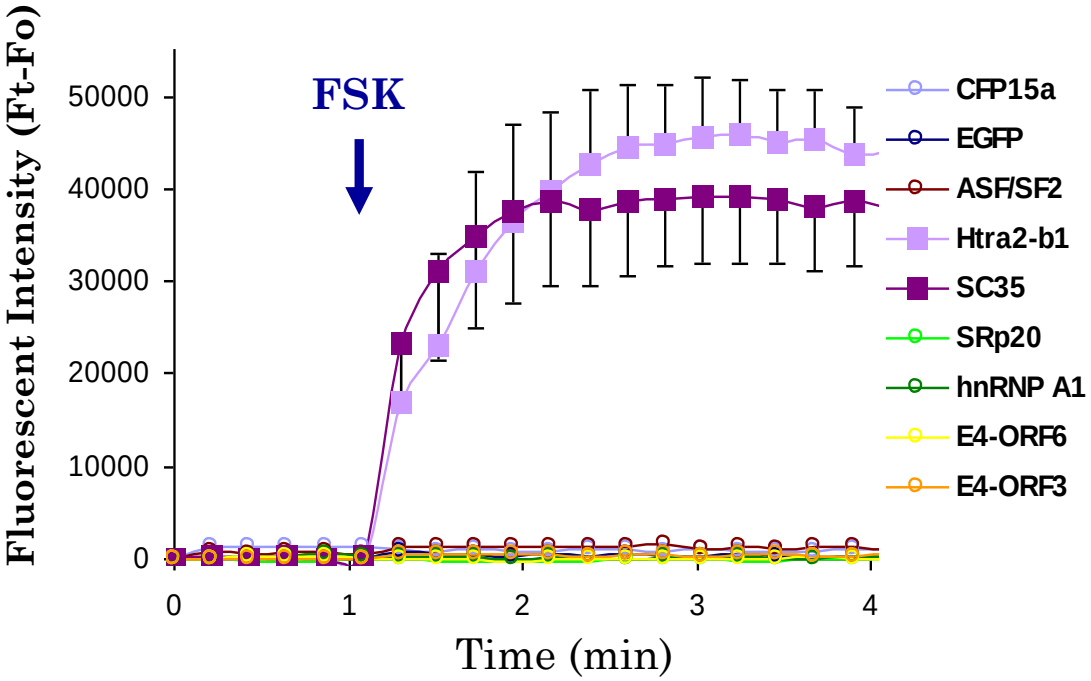
- Nasal epithelial cell line (CFP15a) from a patient carrying the 3849 mutation.

Modulation of 84bp splicing pattern in nasal epithelial cell line (*CFP15a*)



Three of the splicing factors led to a decrease in the levels of aberrant transcripts.

Restoration of CFTR function in CFP15a



- Expression of two splicing factors, which reduced the aberrantly splicied transcripts, restored the function of the CFTR channel.

- The splicing factor ASF/SF2, which also led to reduced level of aberrant transcripts, did not restore CFTR function.

Conclusion

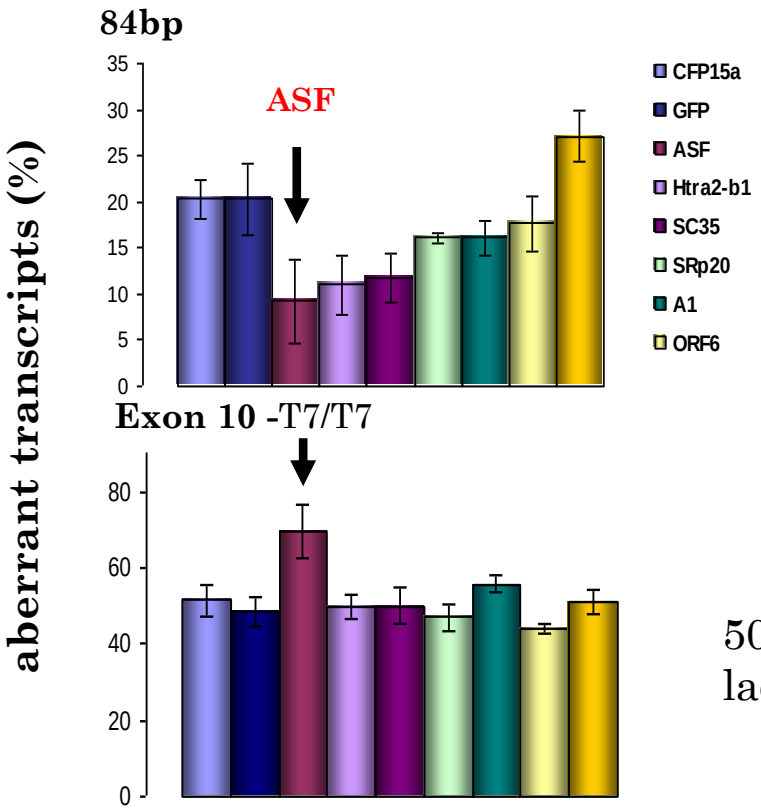
Reducing the level of aberrantly spliced transcripts was sufficient for restoration of the CFTR function.

Question

Why ASF/SF2 over-expression did not restore the CFTR function?

For this we investigated the effect of ASF/SF2 on the splicing pattern of all CFTR exons.

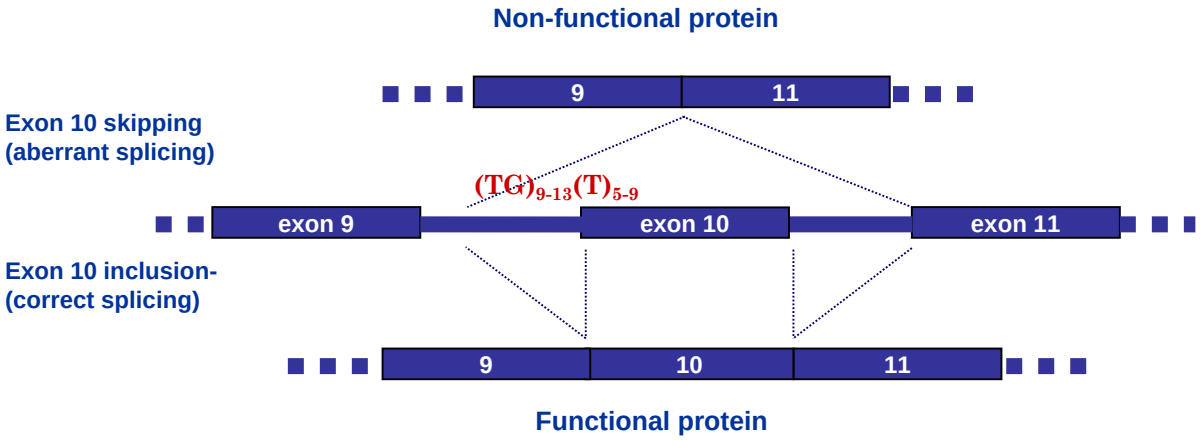
Modulation of 84bp & Exon 10 splicing pattern in CFP15a



50% of the CFTR transcripts lack Exon 10

- Non of the CFTR exons were affected by ASF/SF2 except for exon 10, in which the level of exon 10 skipping (aberrant splicing) was increased.
- Hence, although the level of aberrantly spliced transcripts carrying the 84bp cryptic decreased, the level of aberrantly spliced exon 10 transcripts increased.
- CFTR protein lacking exon 10 is non-functional.
- Overall, there was no correction of the CFTR function.

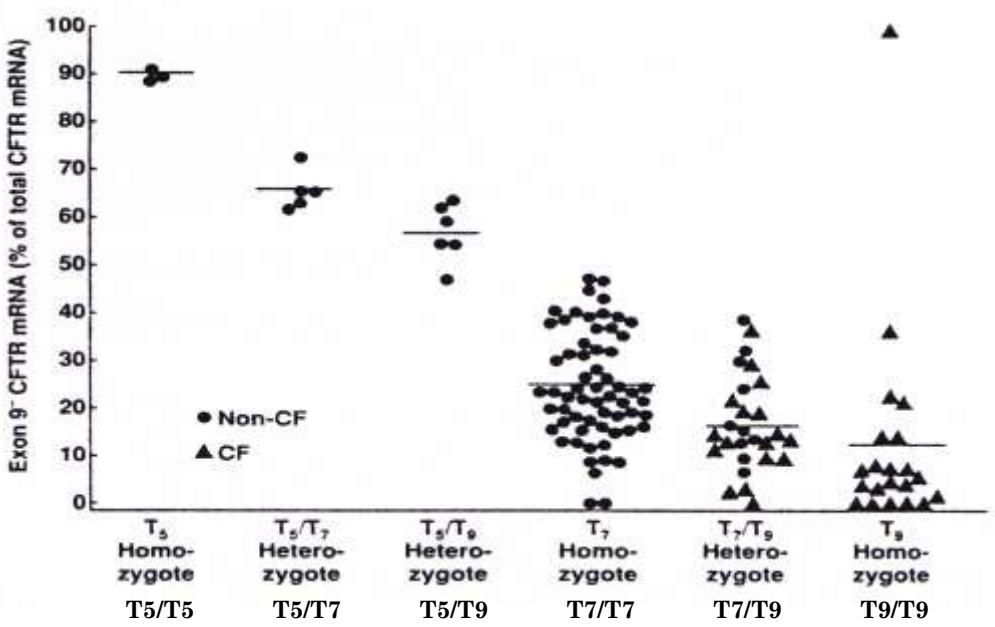
Exon 10 skipping in normal individuals and CF patients



CFTR exon 10 skipping is found in normal individuals as well as in CF patients.

Exon 10 skipping in normal individuals and CF patients

Average skipping:	90	66	57	25	17	13

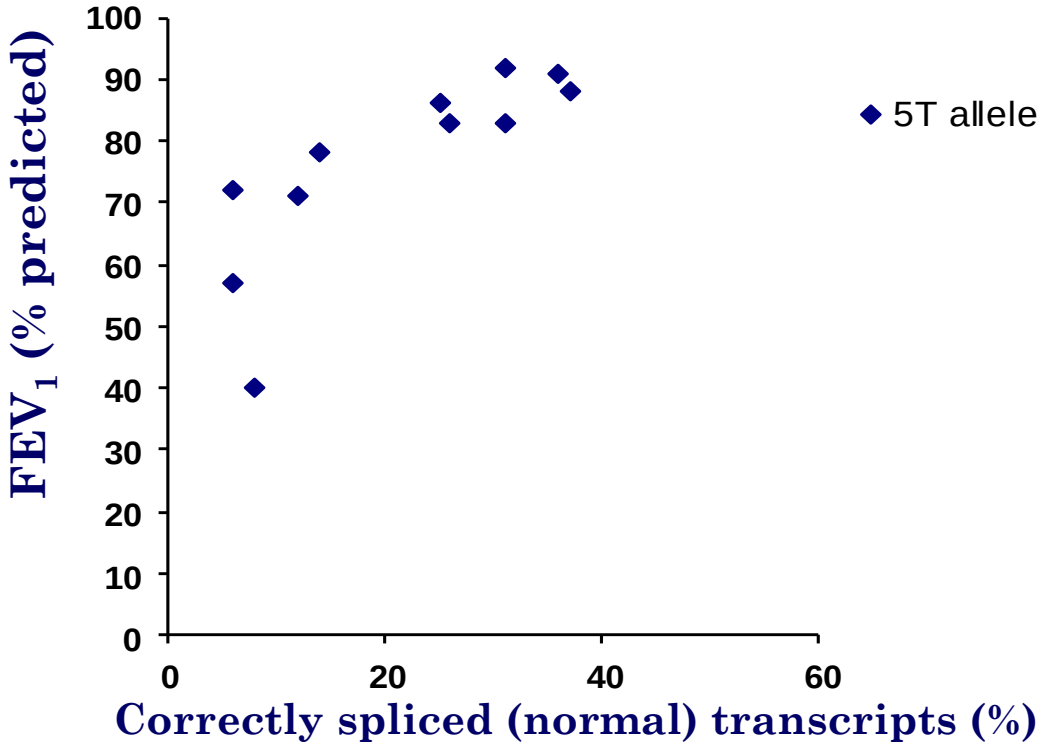


There is a correlation between the length of the poly T tract and levels of aberrantly spliced exon 10 transcripts.

However, for each given genotype there is a large variability in the level of the aberrantly spliced transcripts.

Chu et al., Nat Genet 1993

Association between lung function and the level of exon 10 correct splicing



The level of correct splicing of exon 10 correlates with lung disease severity in patients carrying the 5T allele (the shortest pyrimidine tract allele)

Chiba-Falek …Kerem, Genomics 1998

The effect of the polyT tract on disease severity R117H

- R117H is among the most common class IV mutation (defective chloride conductance), occurs at a worldwide frequency approaching 0.5%.
- The mutation is found on the background of with 7T or 5T.
- Longer PolyT produces more correctly spliced transcripts and more functional CFTR proteins, in these patients.
- A more severe disease is found in patients carrying the R117H mutation with 5T than in 7T.

(Rogan M.P. et al., 2011)

Conclusions

- Reducing the level of aberrantly spliced transcripts, containing the 84bp cryptic exon, can restore the CFTR function in patients carrying the 3849 mutation.

- The level of correct exon 10 splicing affects the response to splicing modulation of the 84 bp cryptic exon.

- Any therapeutic approach aimed at correcting the CFTR function could benefit from increasing exon 10 inclusion.

Antisense Oligonucleotides (AOs) approach for splicing modulation

- Small synthetic nucleic acid molecules able to bind specific intronic or exonic sites of pre-mRNA.

- The AOs are designed to anneal to selected splice motifs, avoiding their recognition by the splicing machinery.

- Masking splice junctions or splicing enhancers is expected to reduce exon recognition, promoting exon skipping (3849 mutation).

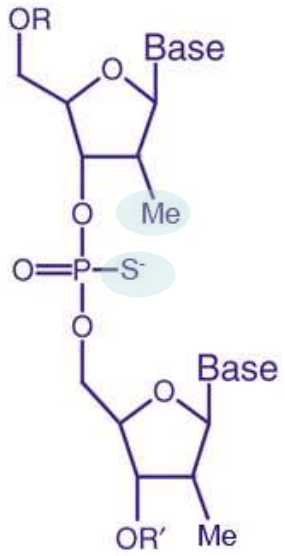
- While masking splicing silencers is expected to promote exon inclusion (exon 10).

Splicing Modulation by ASOs

The effect of ASOs on:

- The splicing pattern of the 84bp cryptic exon.
- The CFTR protein function.
- In respiratory epithelial cells from CF patients carrying the endogenous 3849 +10kb C-to-T CFTR allele.
- To identify ASOs that will affect cells from different patients (variable response).

Antisense Oligo Nucleotide Approach



2'-O-methyl-phosphorothioates

Dr. Michal Irony

Dr. Yifat Oren

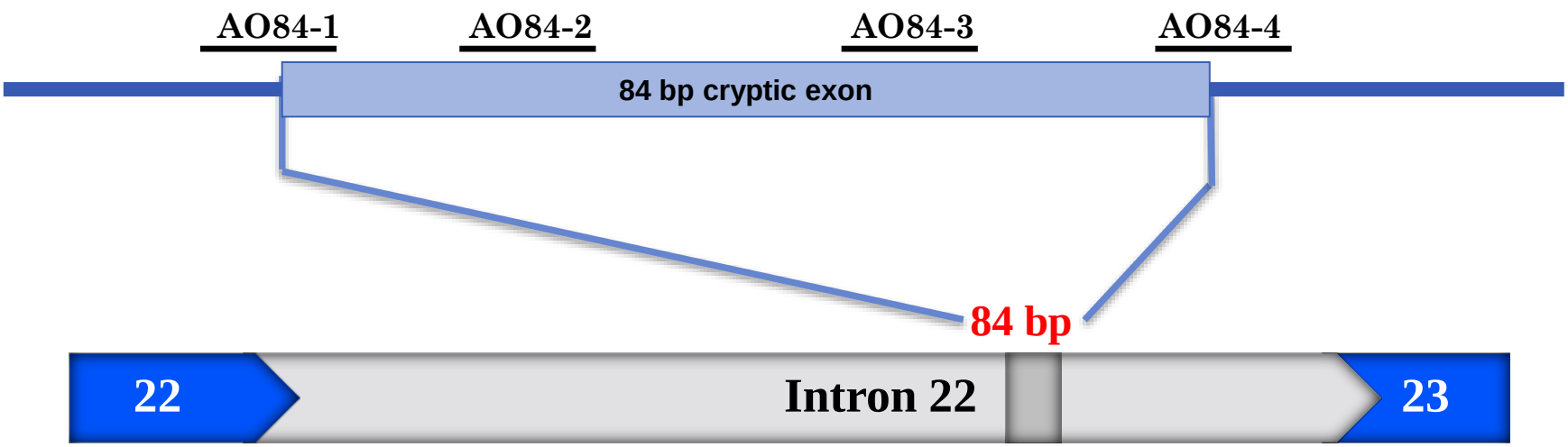
Bar Gindi

Dr. Efrat Ozeri-Galai

- Contains around ~20 nucleotides.
- Replacement of the negatively charged oxygen by sulfur.
- Methylation of the hydroxyl group at the 2nd position of the ribose.

Suppress abnormal splicing arising from the CFTR 3849+10kb C->T mutation

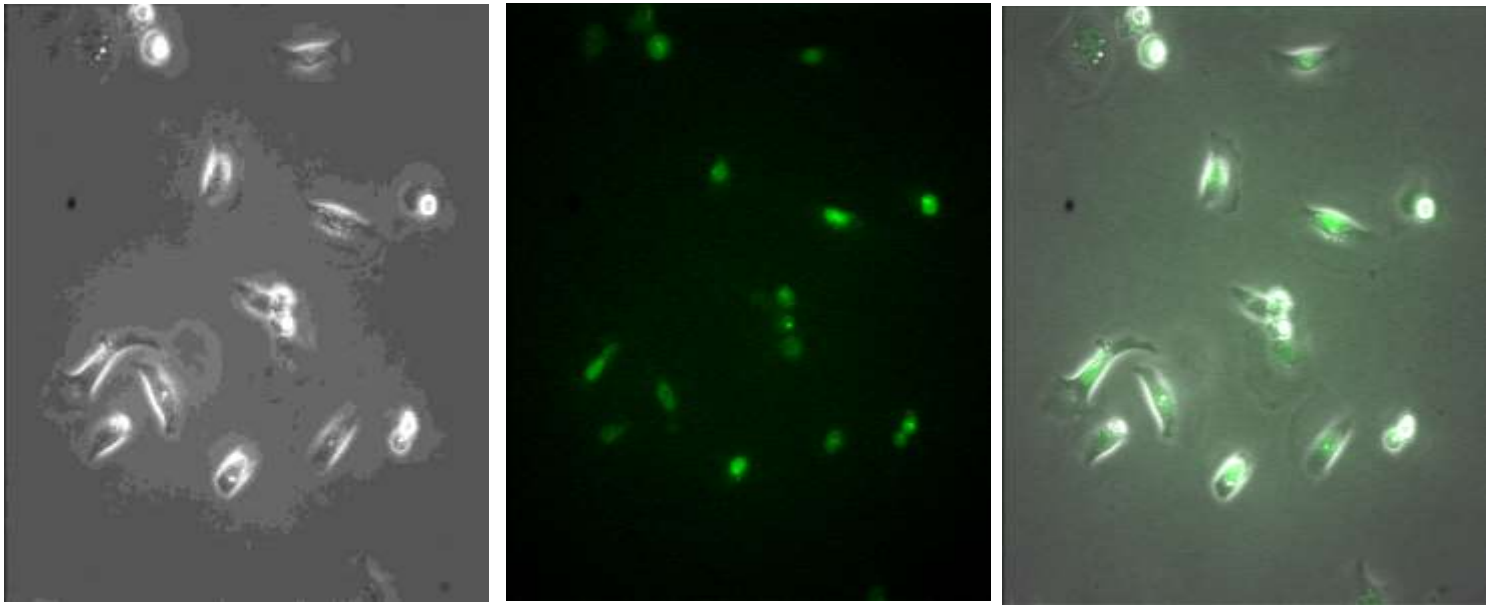
Positions of ASOs binding sites (OMPs)



ASOs were designed to mask splicing elements promoting recognition of the 84 bp cryptic exon in order to reduce aberrant splicing.

Human Splice Finder
(http://www.umd.be/HSF/)

AO delivery by transfection into CFP15a cells



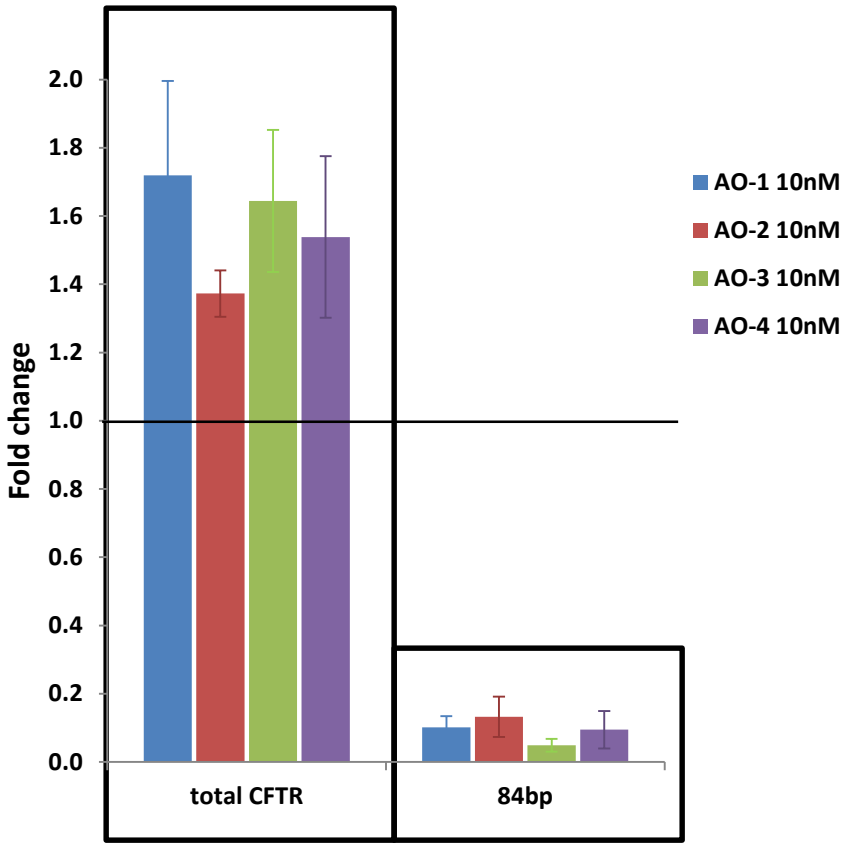
Bright field FITC Merge

FITC-labeled AO targeted to correct **the 3849+10kb mutation**

Lipofectamine 2000 Transfection reagent

High delivery efficiency into the nucleus

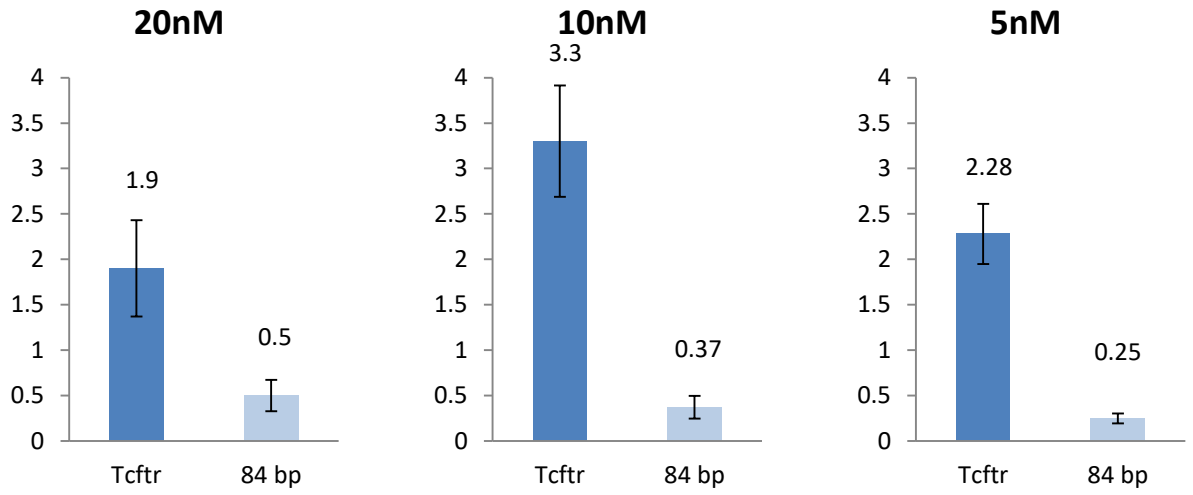
The effect of transfected ASOs on the CFTR splicing pattern in the nasal epithelial cell line (CFP15a)



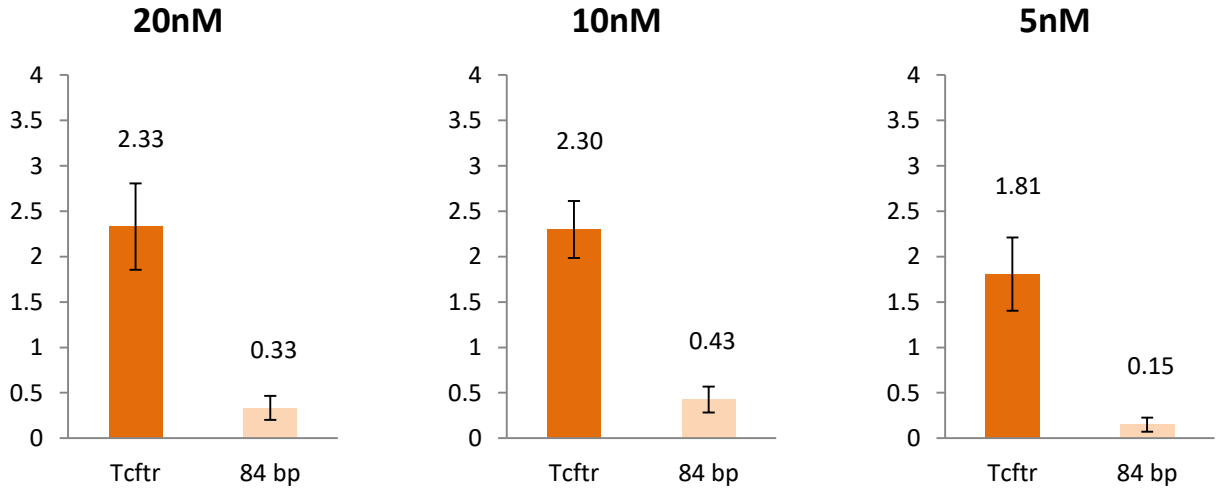
- Significant reduction in the level of aberrantly spliced transcripts.
- A marked elevation of correctly spliced transcripts, reflecting the effect of NMD on the aberrantly spliced transcripts.

The effect of AO84-1 and AO84-4 on the CFTR splicing pattern in nasal epithelial cell line (CFP15a)

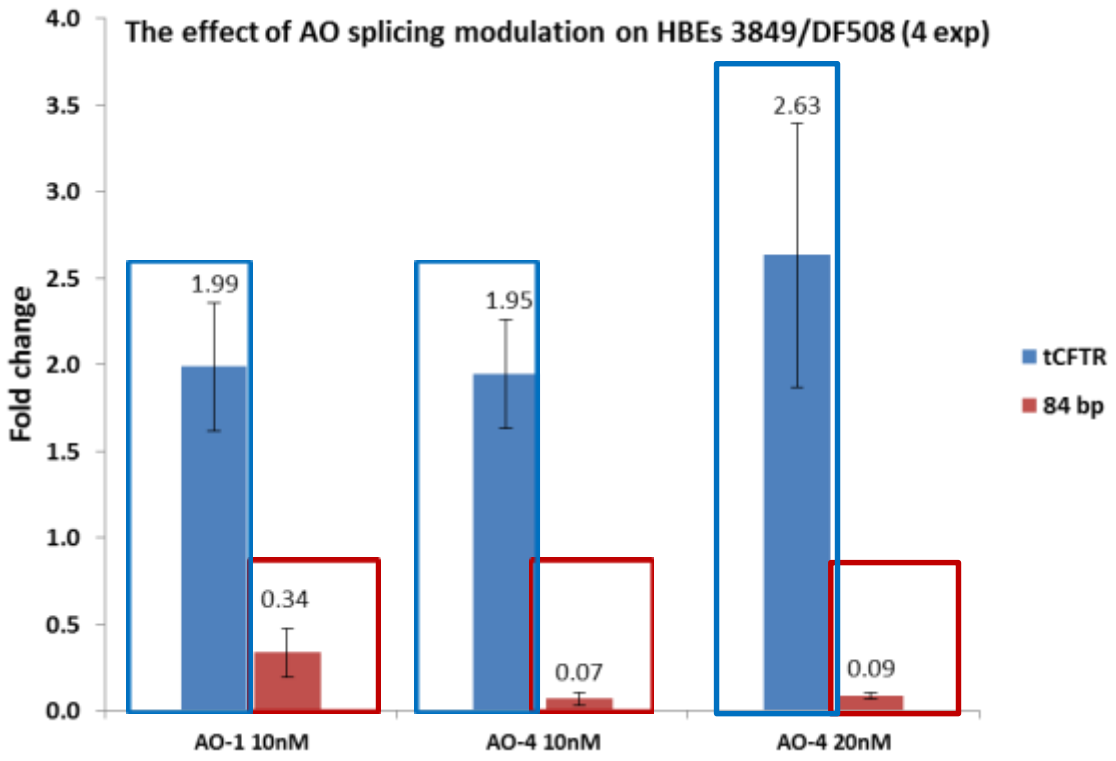
AO84-1
24h treatment with the indicated concentration



AO84-4
24h treatment with the indicated concentration

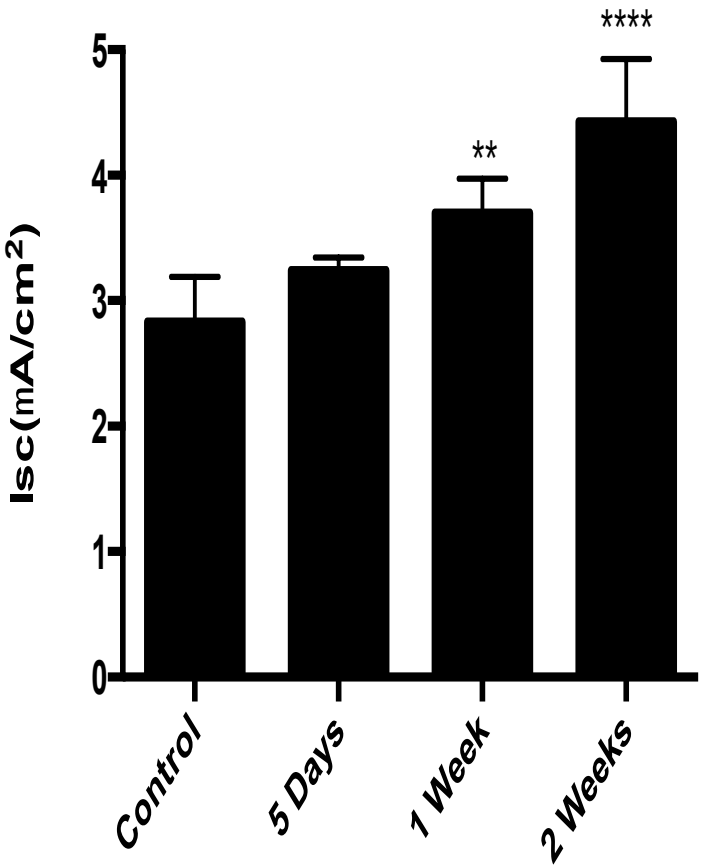


The effect of ASOs on the CFTR splicing pattern in Primary Human Bronchial Epithelial Cells carrying the 3849+10kb C->T mutation



- A significant reduction in the level of aberrantly spliced transcripts.
- A marked elevation of correctly spliced transcripts, also reflecting efficient NMD in these cells.

AO-4 treatment with no transfection improves the CFTR function in Well-Differentiated Primary Human Nasal Epithelial Cells



**P<0.01, **** P<0.001

Short circuit current (Isc)

100nM AO84-4, no transfection

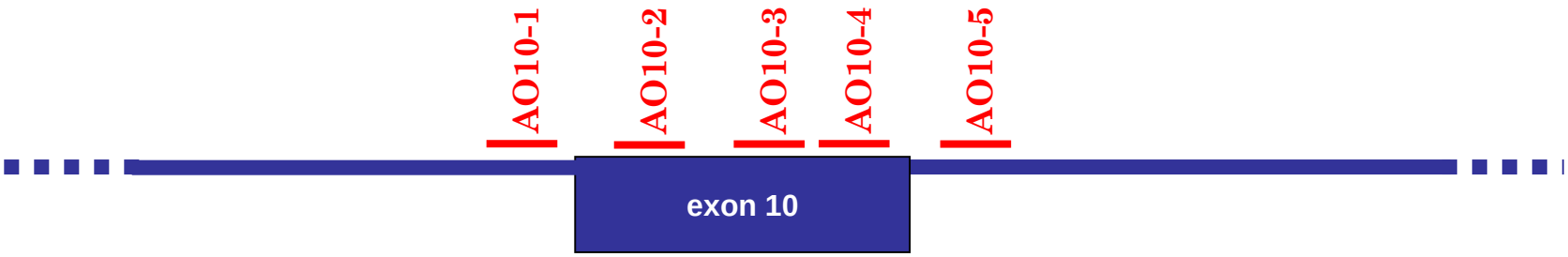
Collaboration with Steve Rowe

Conclusion

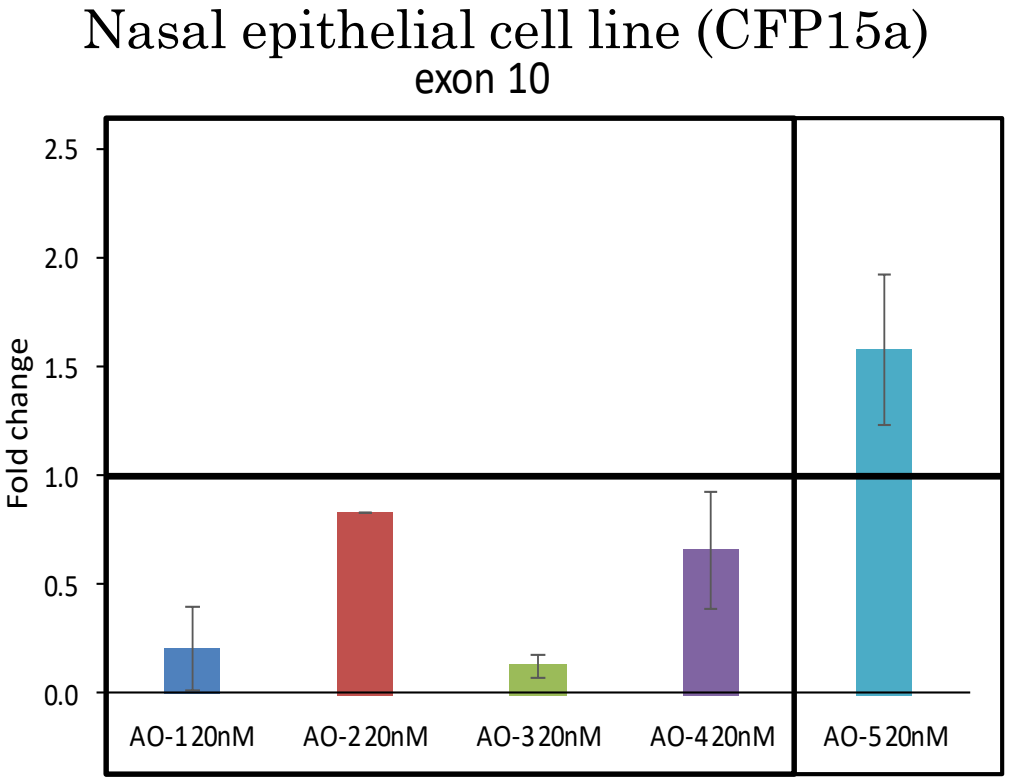
ASOs have the potential to correct the CFTR function in human epithelial cells carrying the endogenous allele with the 3849+10kb C-to-T splicing mutation.

Treatment Modality: Promote Exon 10 Retention

Mask splice-silencing elements that compromise recognition of exon 10

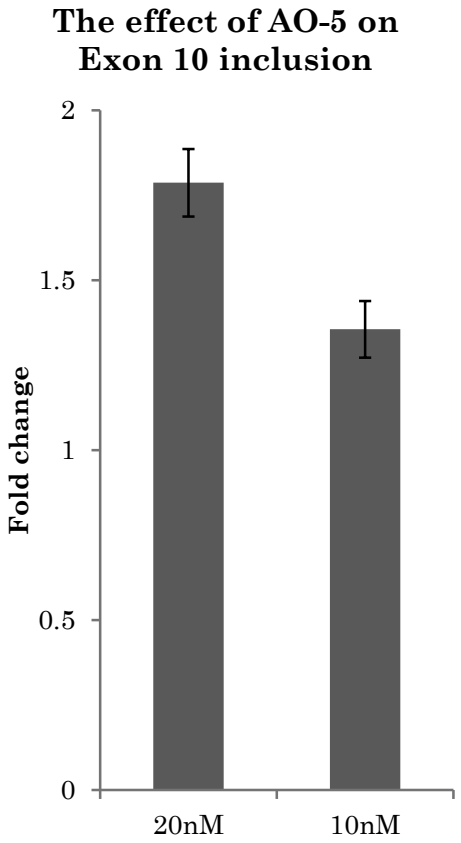


The effect of AOs on the level of CFTR transcripts including exon 10



- AO10-5 showed a significant increase in correctly spliced exon 10 transcripts.
- Preliminary new results from HBEs 5T/5T with AO10-5 showing 3-5 fold increase in exon 10 inclusion.
- Additional AOs are currently synthesized at other DNA sequences along exon 10 region.

Dose dependent effect of AO-5 on the level of CFTR transcripts including exon 10



- 10nM - 40% increase in exon 10 inclusion.
- 20nM - 80% increase in exon 10 inclusion.

Future prospects

- AOs can increase exon 10 inclusion.
- Therapeutic approach aimed at correcting the CFTR function could benefit from increasing exon 10 inclusion.

Hebrew University

Kerem's Lab

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