XXVII Congresso Italiano della Fibrosi Cistica
XVII Congresso Nazionale della Società Italiana per lo studio della Fibrosi Cistica
Napoli, 22.10.2021

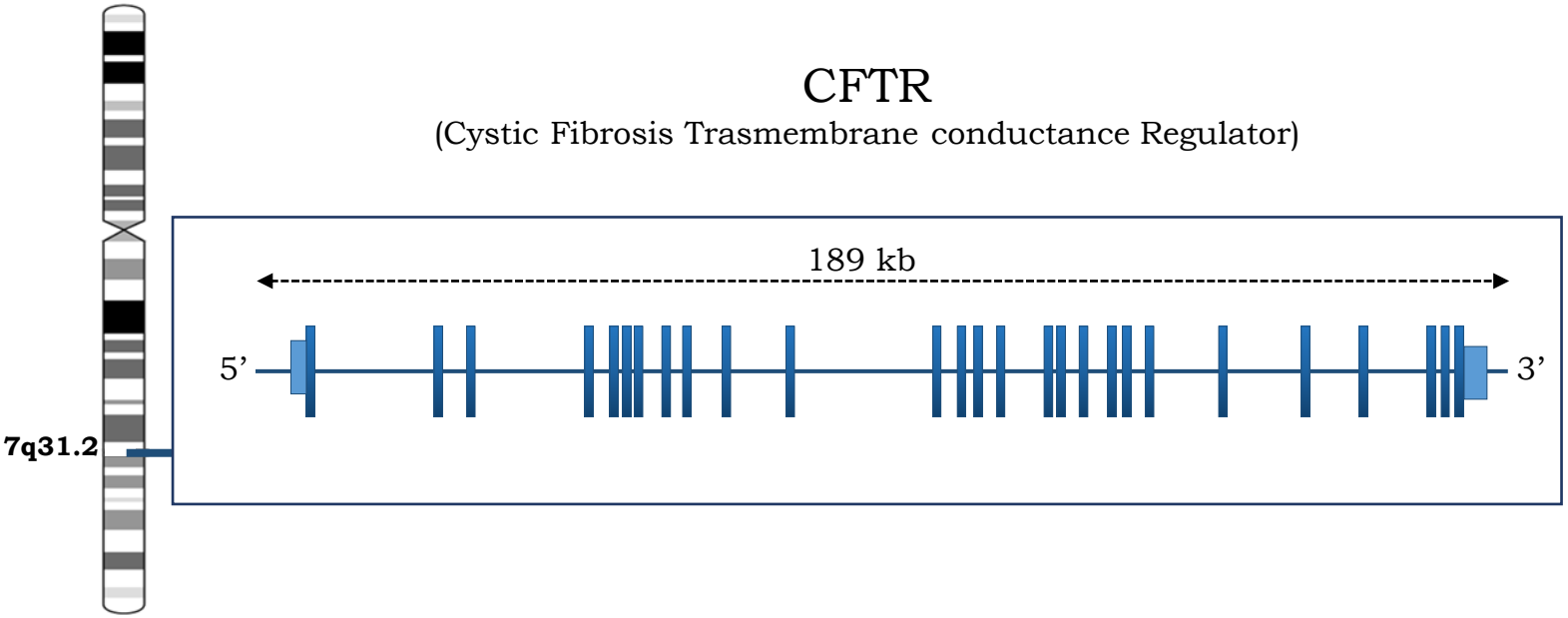
Correzione farmacologica del difetto di splicing

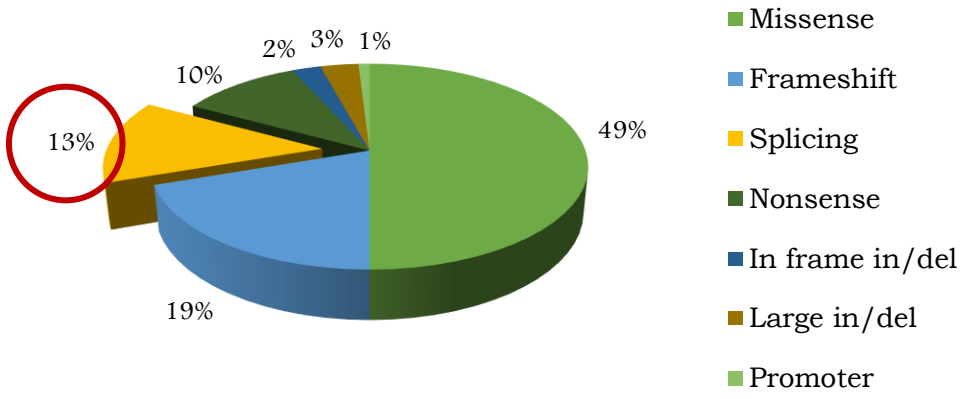
Letizia Straniero PhD

Lab of Medical Genetics & RNA Biology – Humanitas University

HUMANITAS UNIVERSITY

CFTR


CFTR
(Cystic Fibrosis Trasmembrane conductance Regulator)
189 kb
5'
3'
7q31.2


49%
19%
13%
10%
2%
3%
1%
Missense
Frameshift
Splicing
Nonsense
In frame in/del
Large in/del
Promoter

CF & medicina personalizzata

- CFTR potenziatori
- CFTR correttori

⬇

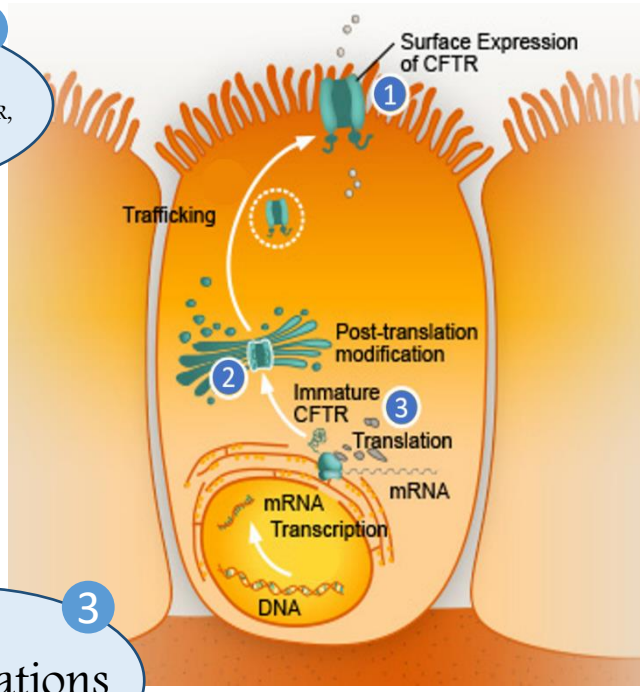
Combinazioni

- Molecole per il read-through

1 gating mutations
G551D, G178R, S549N, S549R, G551S, G970R, G1244E, S1251N, S1255P, and G1349D.

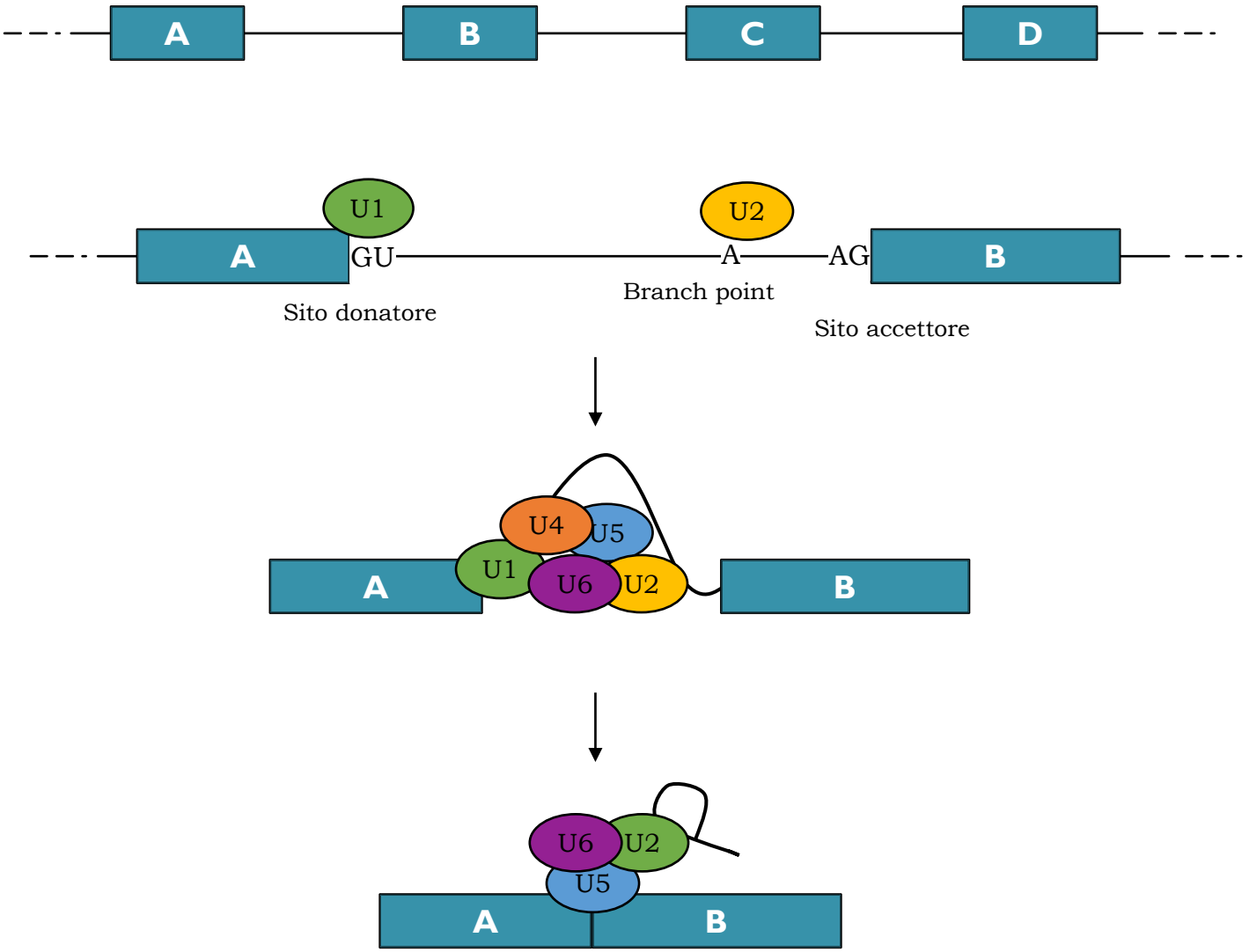
2 F508del

3 stop mutations

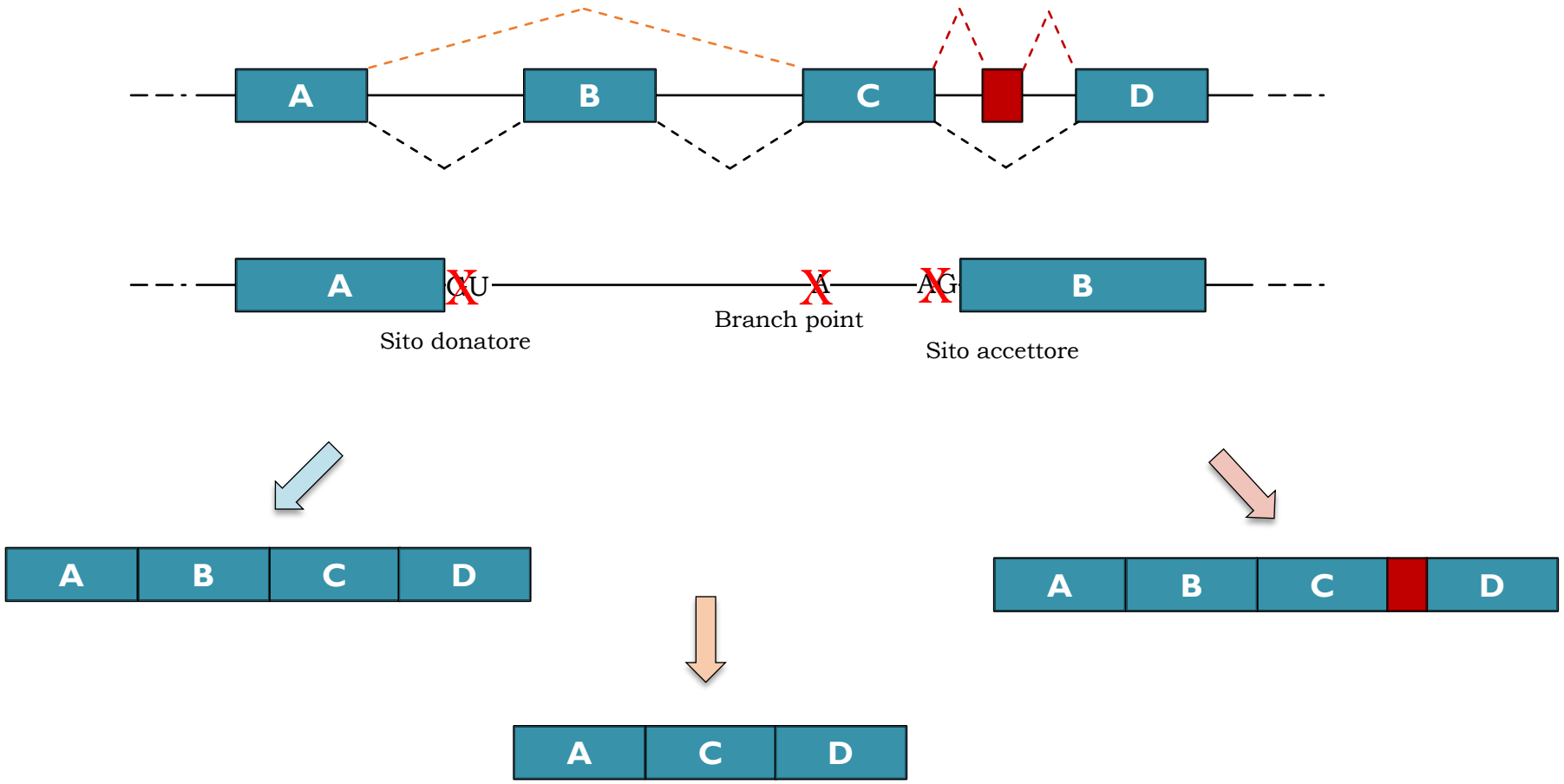


- Mutazioni di splicing??

Processo di splicing



Difetti di splicing



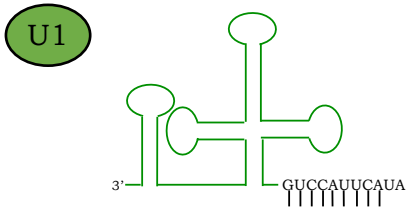
Strategie per la correzione dello splicing

1. Oligonucleotidi antisenso (ASO)

Diverse terapie basate su ASO sono state approvate dall'FDA e alcune anche dall'EMA (AIFA).
Esempio: SPINRAZA per il trattamento dell'atrofia muscolare spinale (SMA)

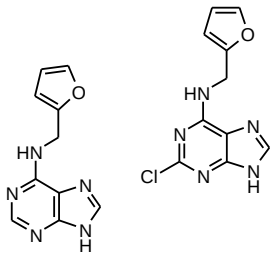
→ molte altre sono in fase preclinica

2. U1 modificati



Vari U1 modificati sono stati testati per il trattamento di diverse patologie (come ad esempio: SMA, emofilia A e B, talassemia, …), ma solo in modelli preclinici

3. Piccole molecole

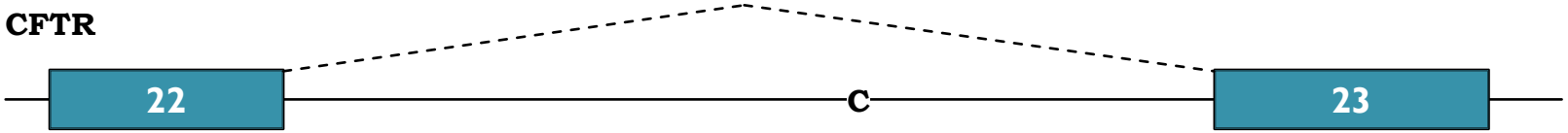


Branaplam e Risdiplam sono attualmente in trial clinici (rispettivamente di fase 2 e 3) in pazienti affetti da SMA.

Varie molecole (già approvate per altre indicazioni terapeutiche) sono state testate in modelli preclinici di diverse patologie.

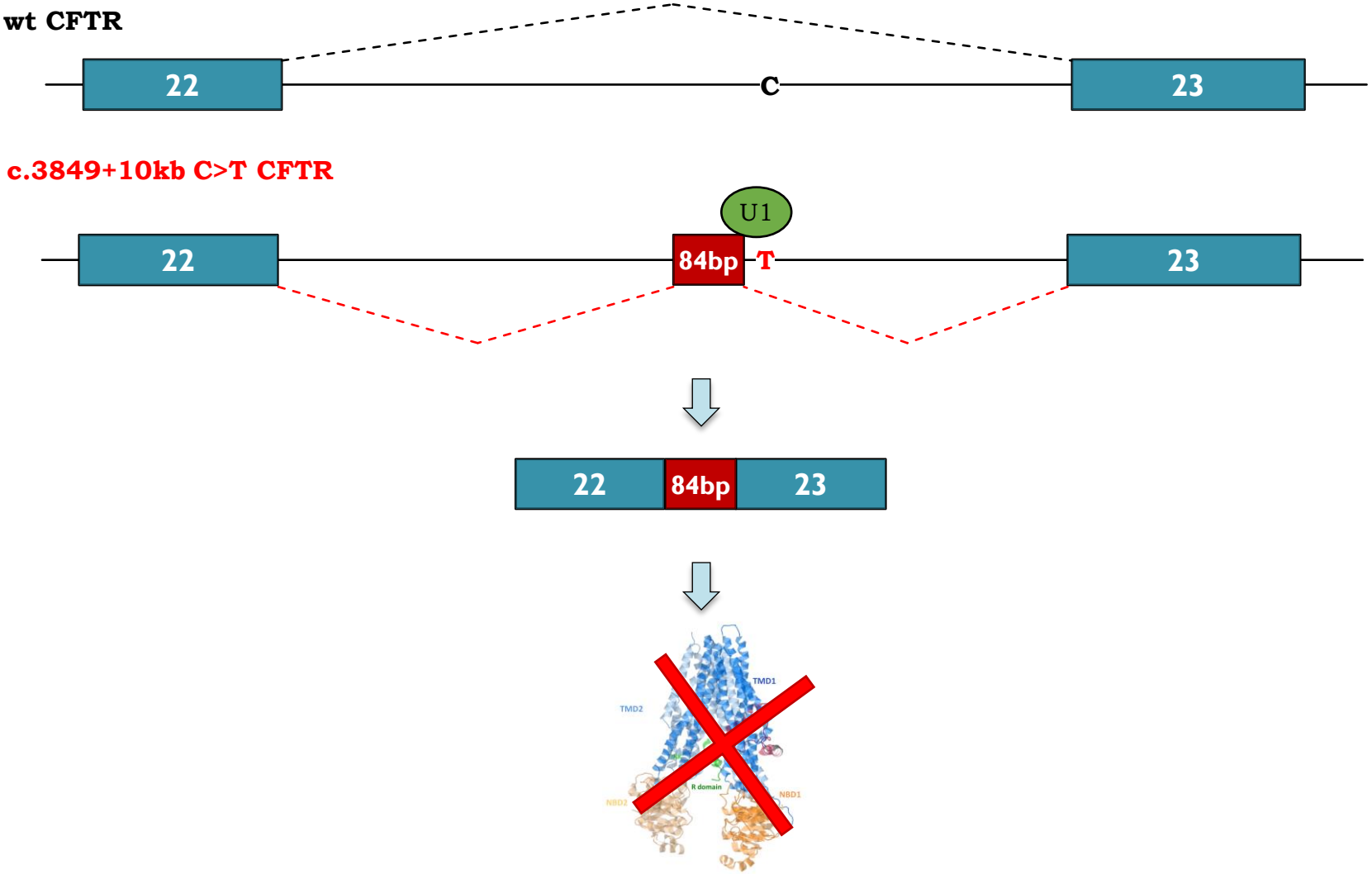
Oligonucleotidi Antisenso

wt CFTR



Oren et al., Journal of Cystic Fibrosis, 2021

Oligonucleotidi Antisenso



Oren et al., Journal of Cystic Fibrosis, 2021

Oligonucleotidi Antisenso

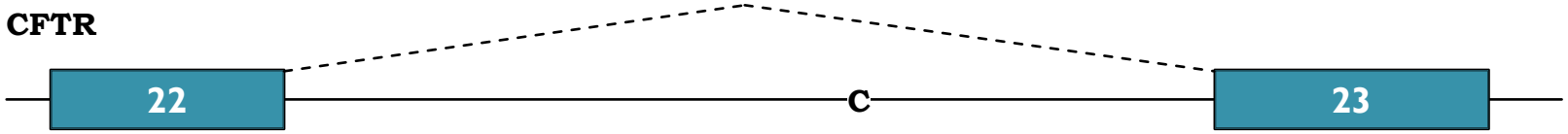

Journal of Cystic Fibrosis

Original article

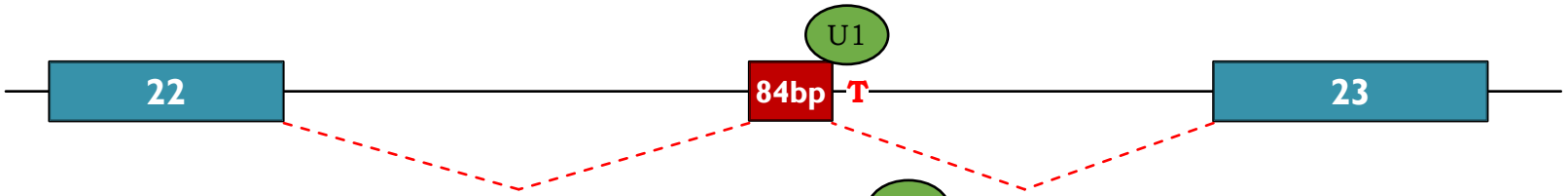
Antisense oligonucleotide-based drug development for Cystic Fibrosis patients carrying the 3849+10 kb C-to-T splicing mutation

Yifat S. Oren[a,b,1], Michal Irony-Tur Sinai[a,1], Anita Golec[c,1], Ofra Barchad-Avitzur[a], Venkateshwar Mutyam[d], Yao Li[d], Jeong Hong[c], Efrat Ozeri-Galai[a], Aurélie Hatton[c], Chen Leibson[a], Liran Carmel[e], Joel Reiter[f], Eric J. Sorscher[d], Steve D. Wilton[g,h], Eitan Kerem[a], Steven M. Rowe[d], Isabelle Sermet-Gaudelus[c,i,*], Batsheva Kerem[a,*]

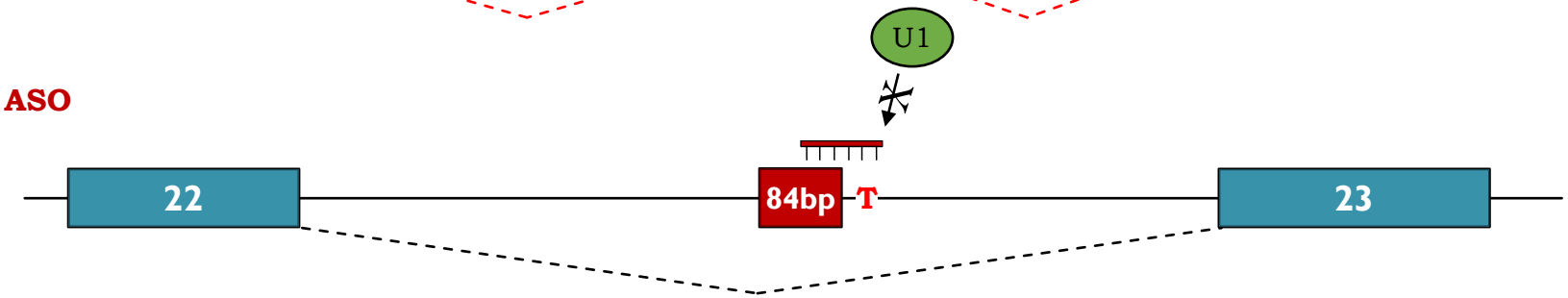
wt CFTR



c.3849+10kb C>T CFTR



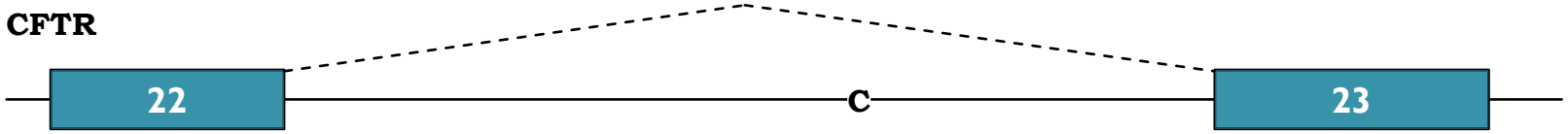
+ ASO



Oren et al., Journal of Cystic Fibrosis, 2021

Oligonucleotidi Antisenso

wt CFTR



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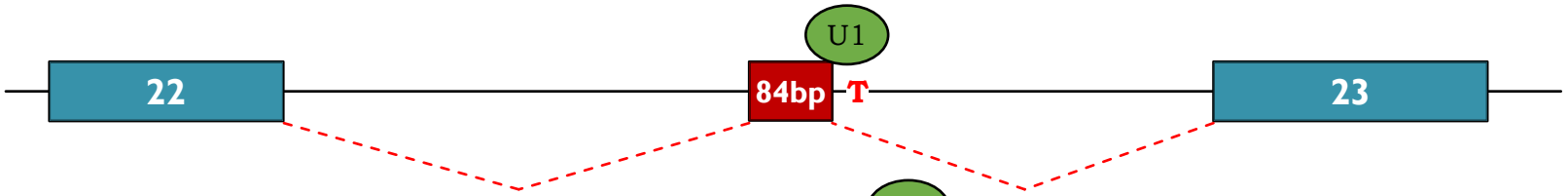
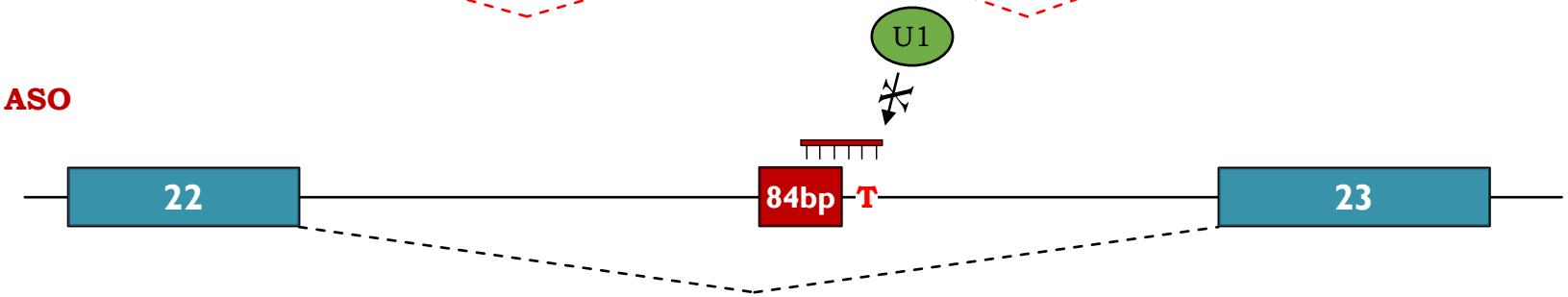
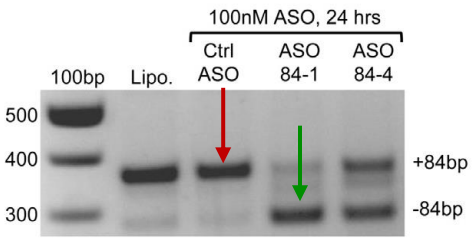


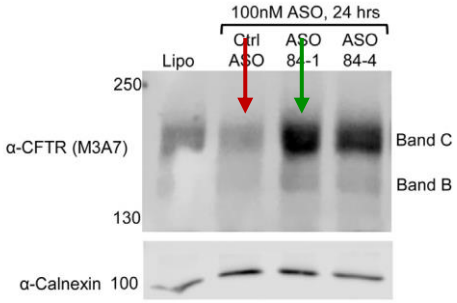
+ ASO



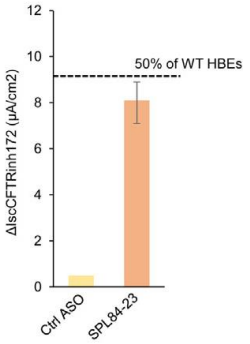
RNA



Proteina



Attività del canale



Oren et al., Journal of Cystic Fibrosis, 2021

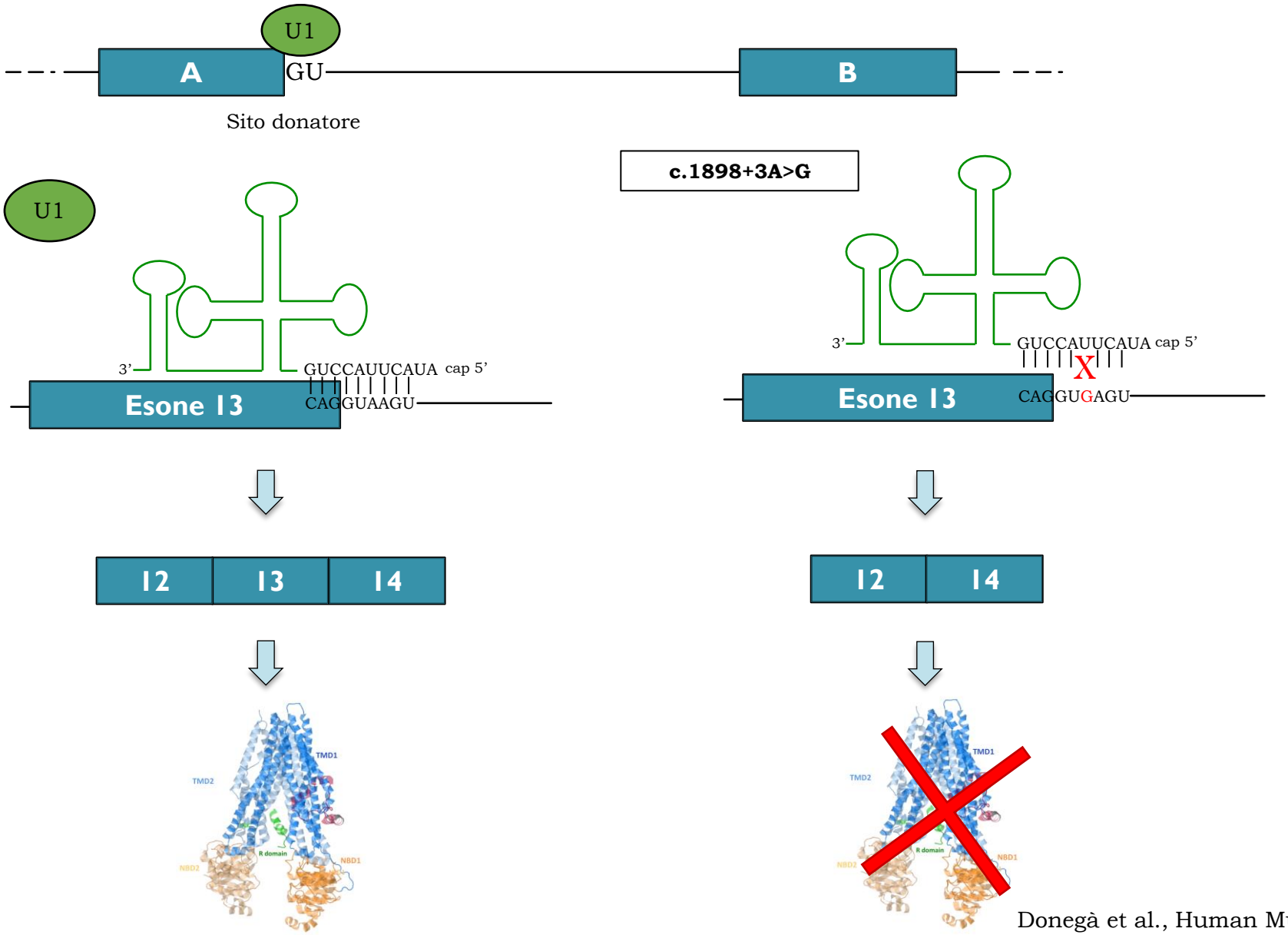
U1-modificati

RESEARCH ARTICLE

Human Mutation HGVS WILEY

Rescue of common exon-skipping mutations in cystic fibrosis with modified U1 snRNAs

Stefano Donegà[1] | Malgorzata Ewa Rogalska[1] | Giulia Pianigiani[1] | Susana Igreja[2] | Margarida Duarte Amaral[2] | Franco Pagani[1]



Donegà et al., Human Mutation, 2020

U1-modificati

RESEARCH ARTICLE

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Rescue of common exon-skipping mutations in cystic fibrosis with modified U1 snRNAs

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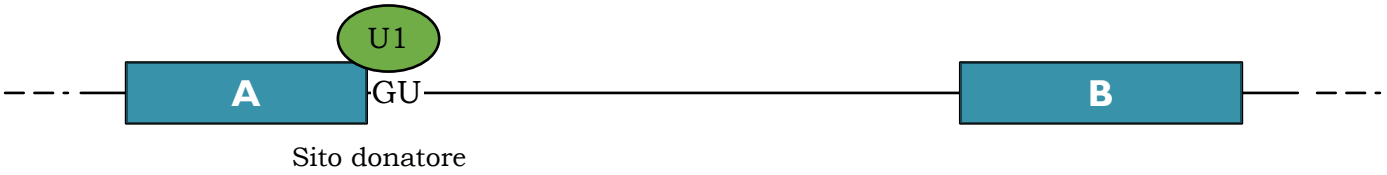
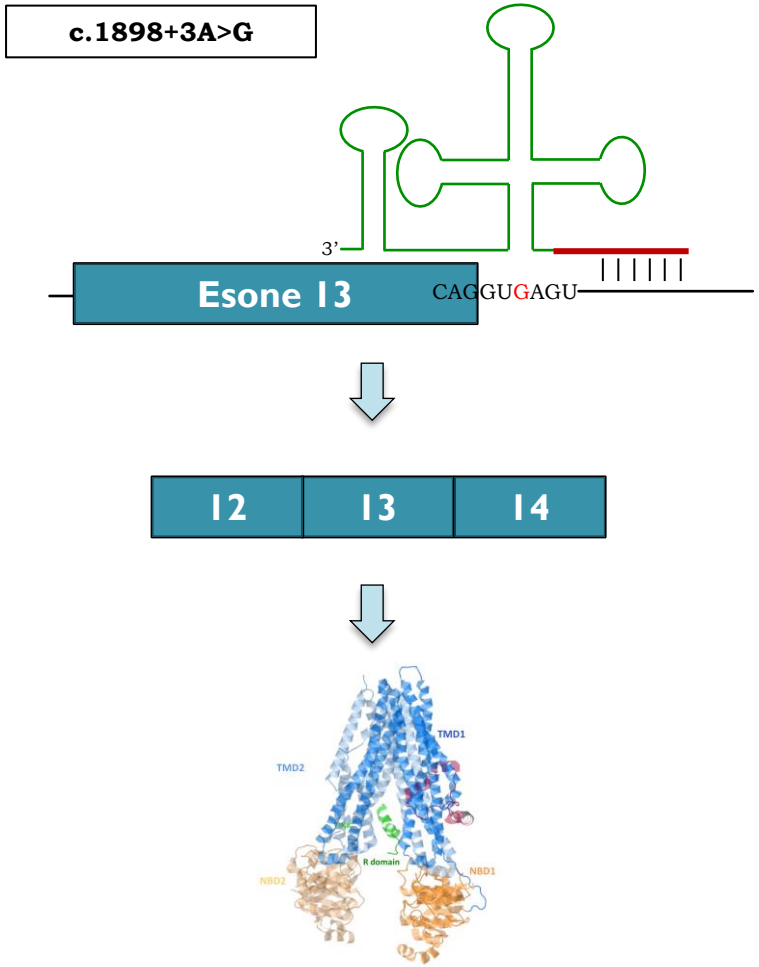
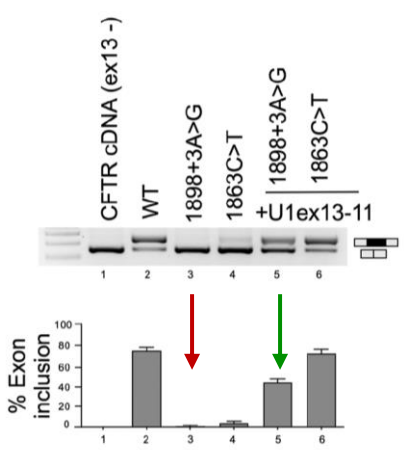


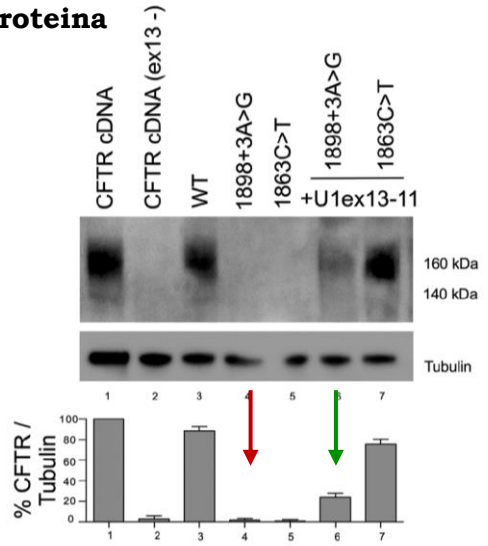
Sito donatore



RNA



Proteina



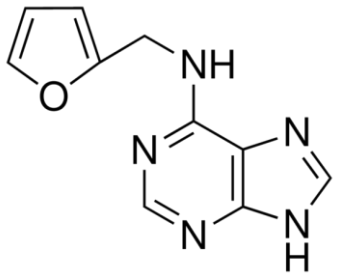
Altre mutazioni: c.711+3A>C, c.711+3A>G, c.711+5G>A, c.1863C>T, c.2789+5G>A, c.3120G>A

Donegà et al., Human Mutation, 2020

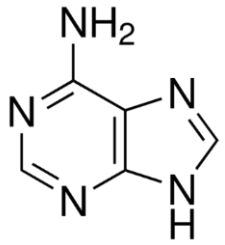
Piccole molecole: Kinetina

- N6-furfuriladenina
- ormone vegetale
- effetti anti-aging

Kinetina



Adenina



Human Molecular Genetics, 2004, Vol. 13, No. 4 **429–436**
DOI: 10.1093/hmg/ddh046
Advance Access published on January 6, 2004

Rescue of a human mRNA splicing defect by the plant cytokinin kinetin

Susan A. Slaugenhaupt[1,2,*], James Mull[1,2], Maire Leyne[1,2], Math P. Cuajungco[1,2], Sandra P. Gill[1,2], Matthew M. Hims[1,2], Fabiola Quintero[1,2], Felicia B. Axelrod[3] and James F. Gusella[1,2]

European Journal of Human Genetics (2010) 18, 614–617

www.nature.com/ejhg

SHORT REPORT

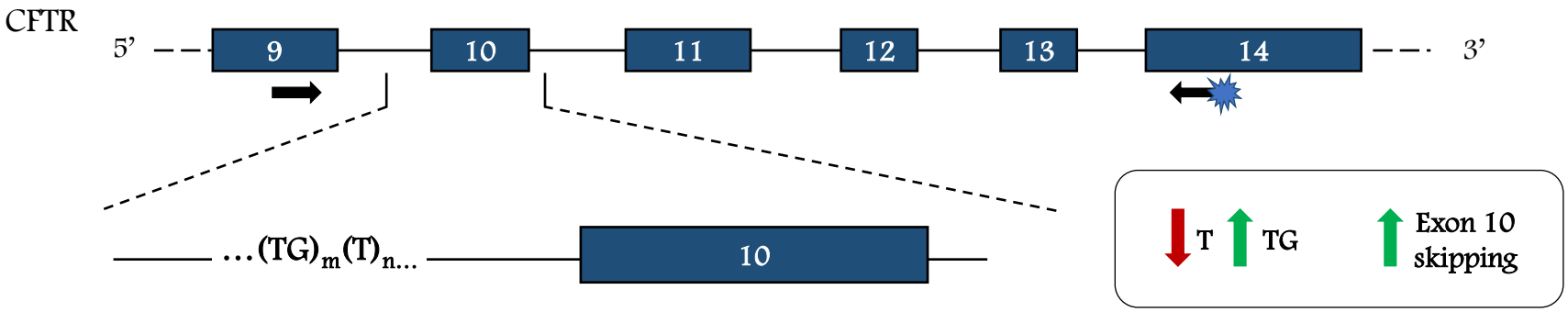
Modulation of aberrant *NF1* pre-mRNA splicing by kinetin treatment

Eva Pros[1], Juana Fernández-Rodríguez[1], Llúcia Benito[2], Anna Ravella[3], Gabriel Capellá[1], Ignacio Blanco[2], Eduard Serra[4] and Conxi Lázaro*[,1]

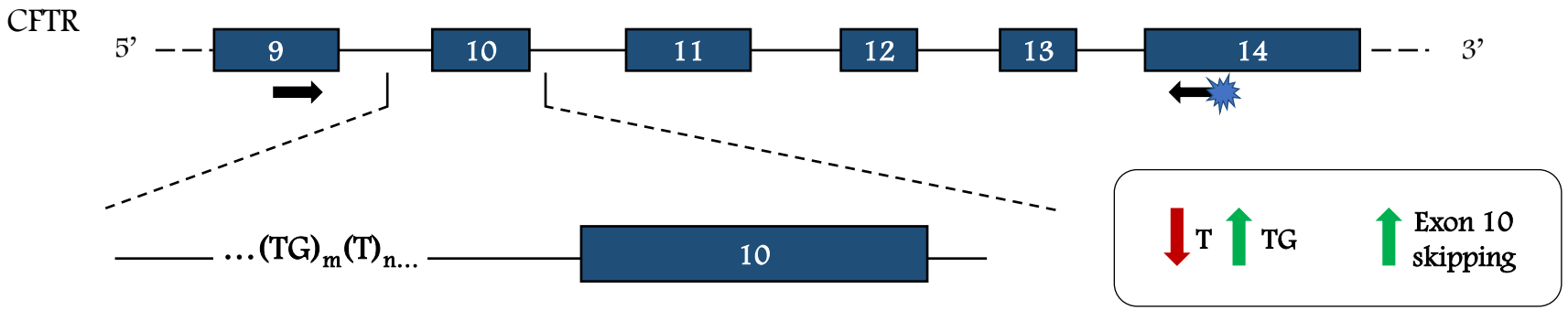
Kinetin improves *IKBKAP* mRNA splicing in patients with familial dysautonomia

Felicia B. Axelrod, Leonard Liebes, Gabrielle Gold-von Simson, Sandra Mendoza, James Mull, Maire Leyne, Lucy Norcliffe-Kaufmann, Horacio Kaufmann, and Susan A. Slaugenhaupt

Polimorfismo poly-T

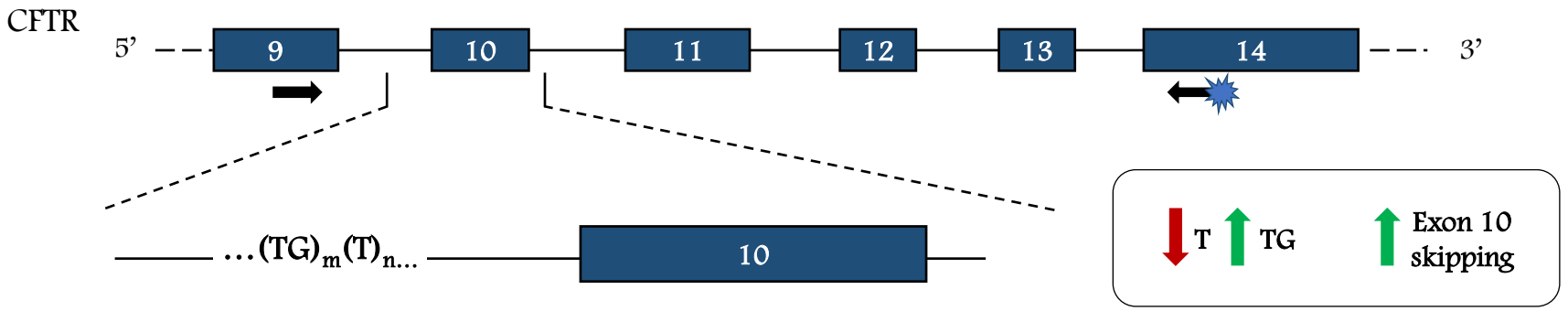


Polimorfismo poly-T



3.9%	84.7%	11.4%
allele 5T	allele 7T	allele 9T

Polimorfismo poly-T



3.9%

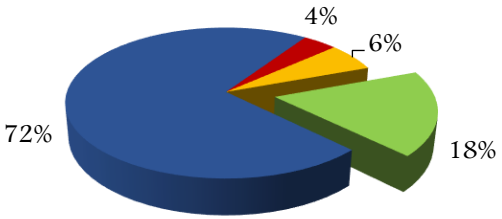
allele 5T

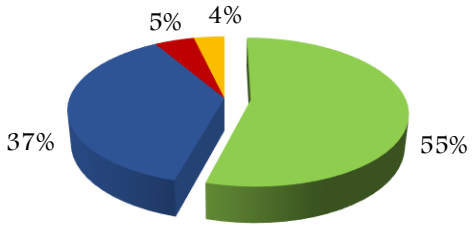
84.7%

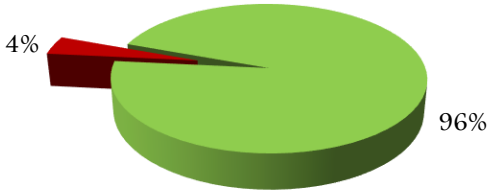
allele 7T

11.4%

allele 9T







wild type

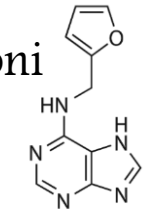
ex10 skipping

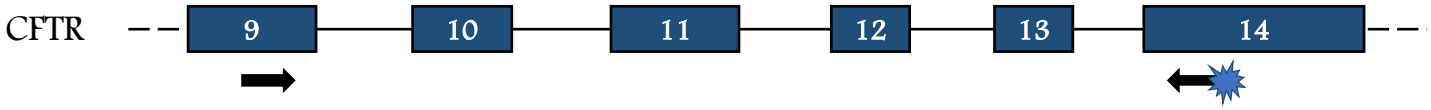
ex13 skipping

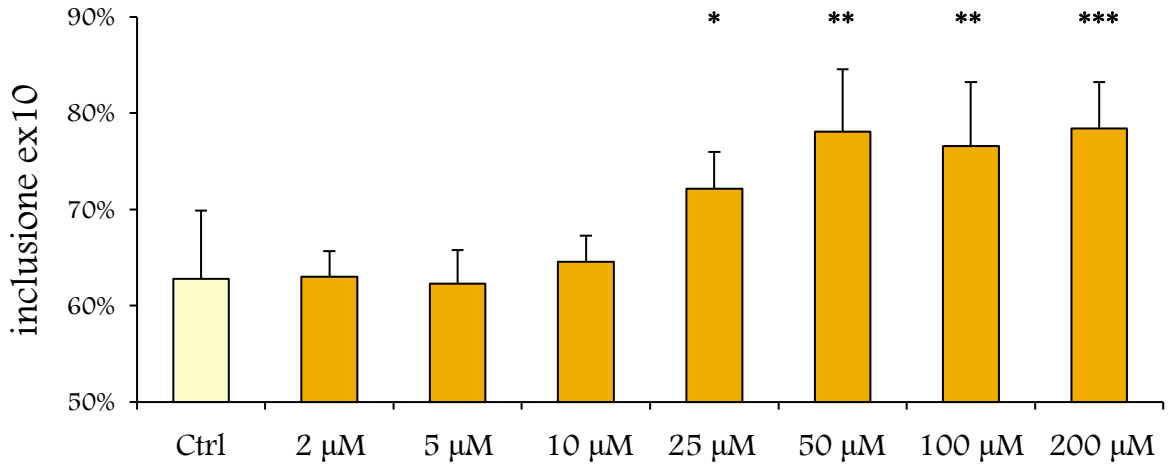
ex10 and ex13 skipping

Kinetina

Abbiamo trattato le cellule **Caco-2** (omozigoti 7T) per **24h** con diverse concentrazioni della molecola



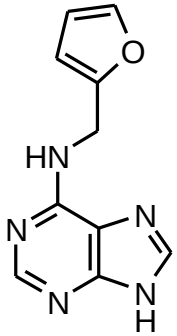




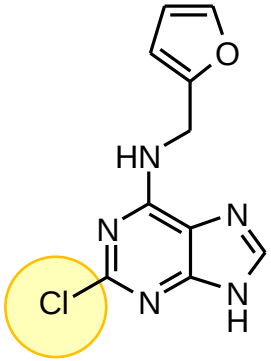
➡ Kinetina ha un considerevole effetto sullo splicing dell'esone 10

RECTAS (RECTifier of Aberrant Splicing)

kinetina



2Cl-kinetina



PNAS

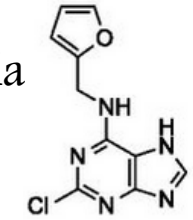
CrossMark click for updates

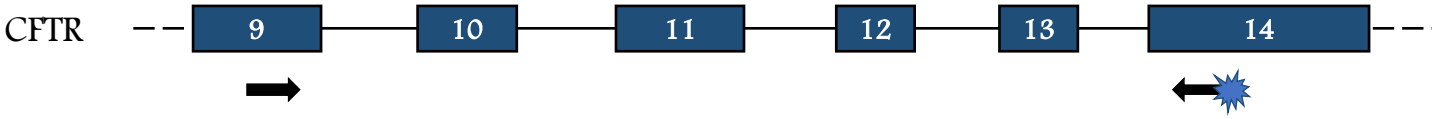
Rectifier of aberrant mRNA splicing recovers tRNA modification in familial dysautonomia

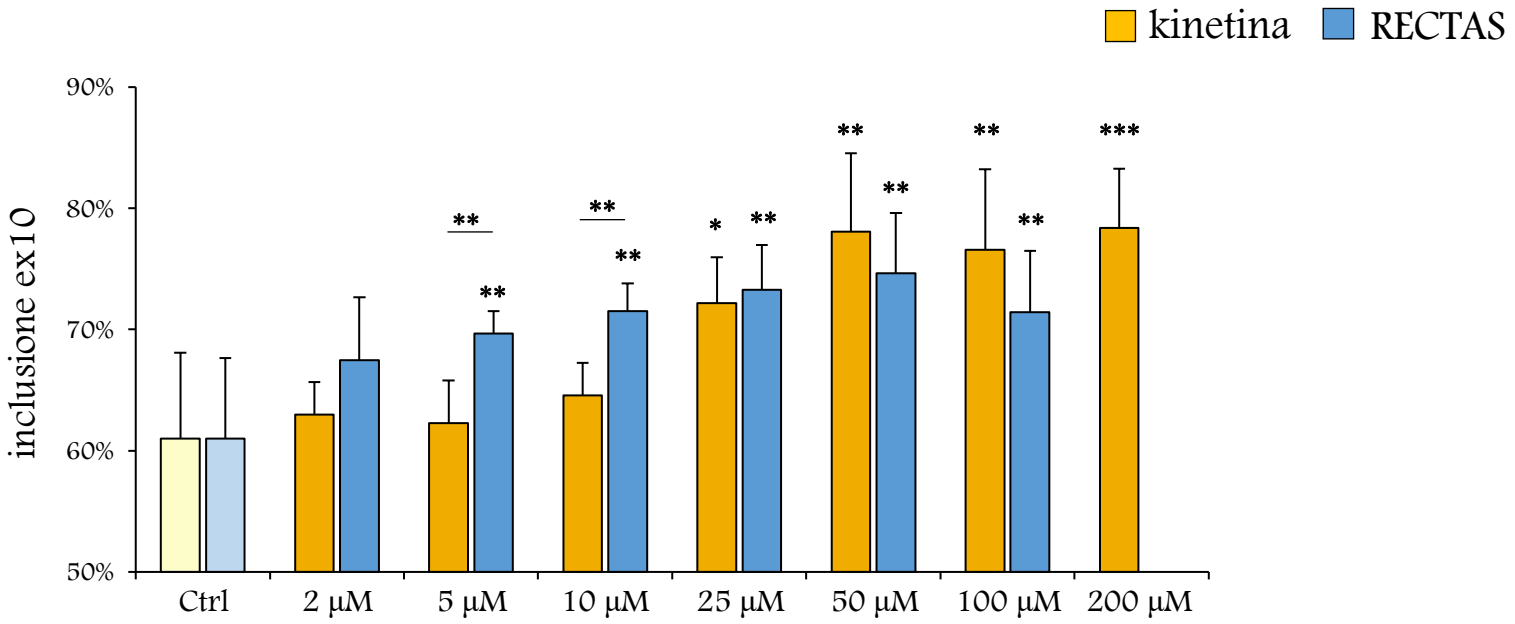
Mayumi Yoshida[a], Naoyuki Kataoka[b,1], Kenjyo Miyauchi[c], Kenji Ohe[a,d], Kei Iida[a,e], Suguru Yoshida[f], Takayuki Nojima[g], Yukiko Okuno[a,e], Hiroshi Onogi[a,h], Tomomi Usui[i], Akihide Takeuchi[a], Takamitsu Hosoya[f], Tsutomu Suzuki[c], and Masatoshi Hagiwara[a,1]

RECTAS

Abbiamo testato in cellule **Caco-2** per **24h** differenti concentrazioni della molecola



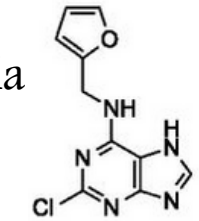


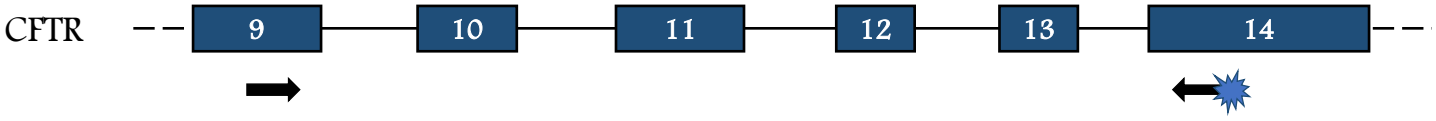


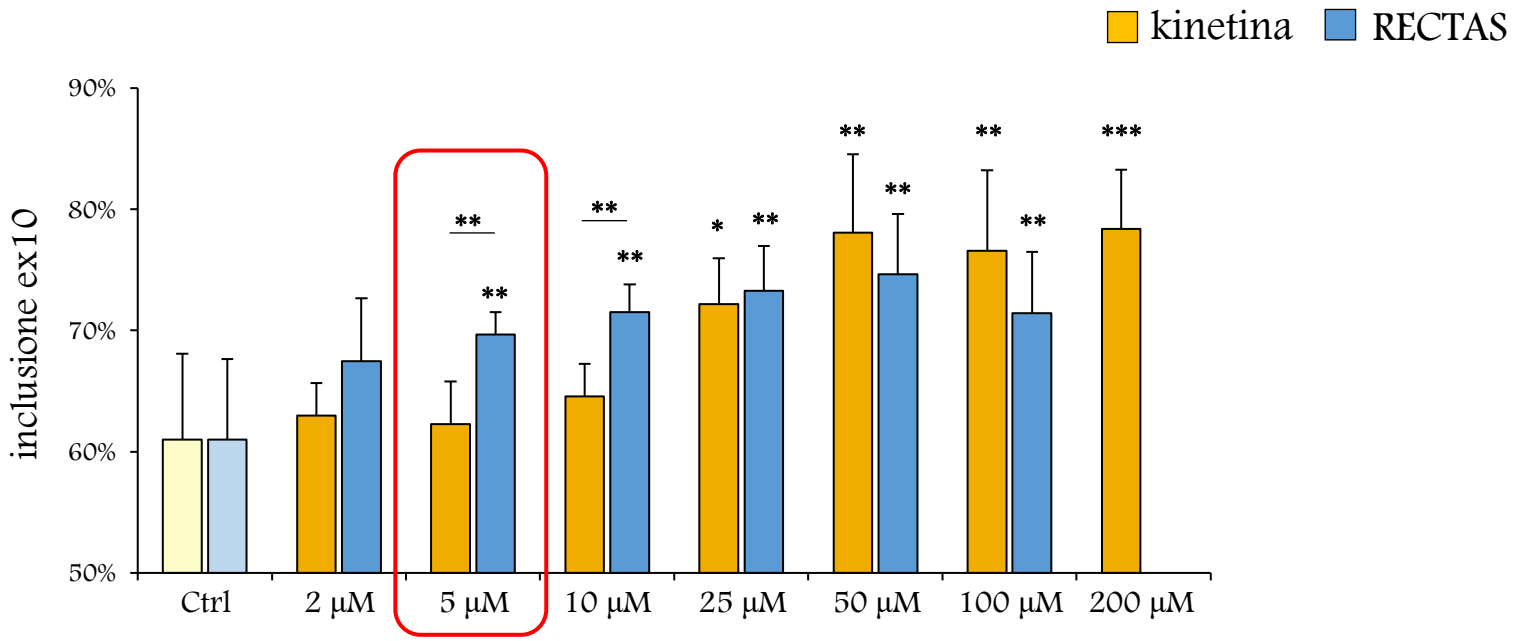
➡ RECTAS corregge lo splicing dell'esone 10 come kinetina, ma a basse dosi

RECTAS

Abbiamo testato in cellule **Caco-2** per **24h** differenti concentrazioni della molecola



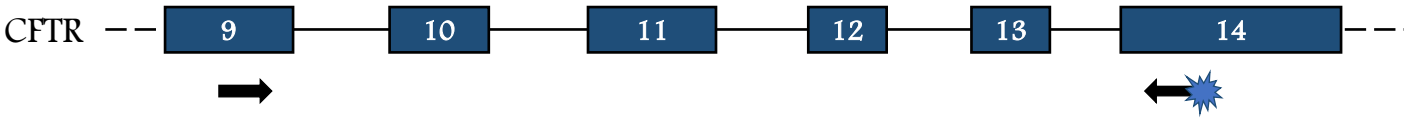


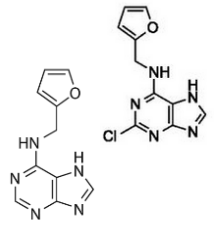


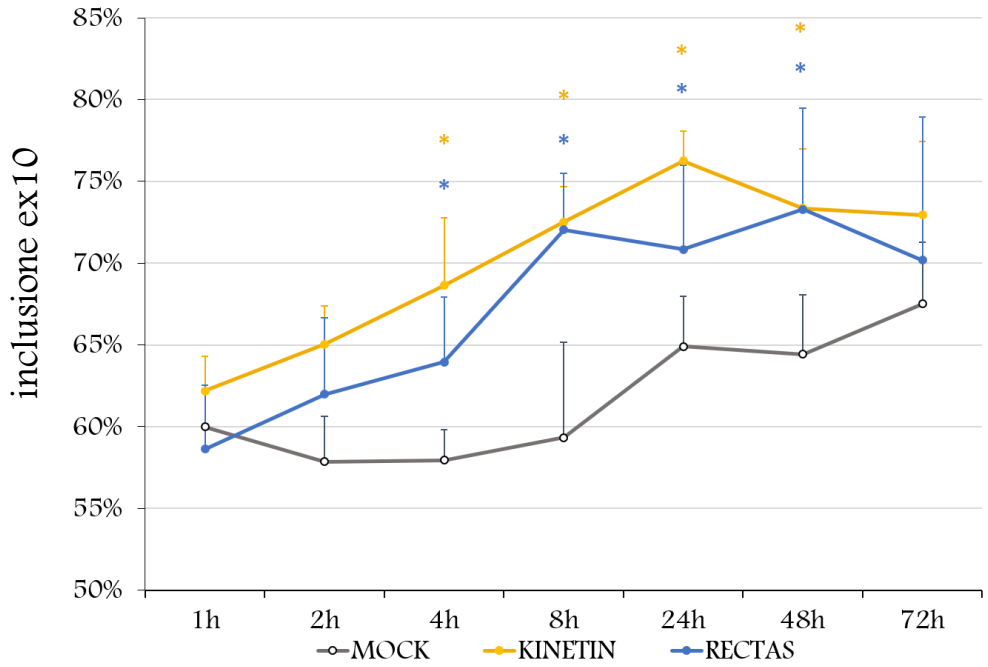
➡ RECTAS corregge lo splicing dell'esone 10 come kinetina, ma a basse dosi

Kinetina & RECTAS: time-course

Gli esperimenti sono stati condotti con la dose massima testata

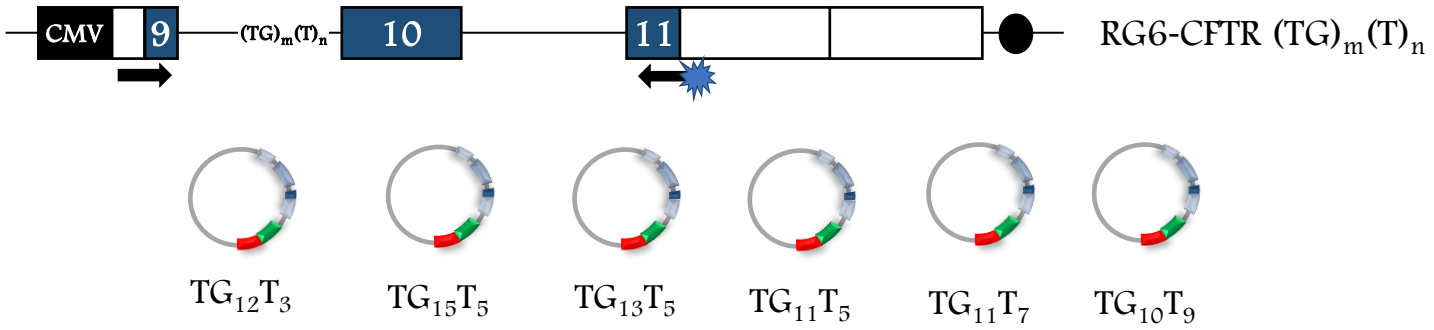


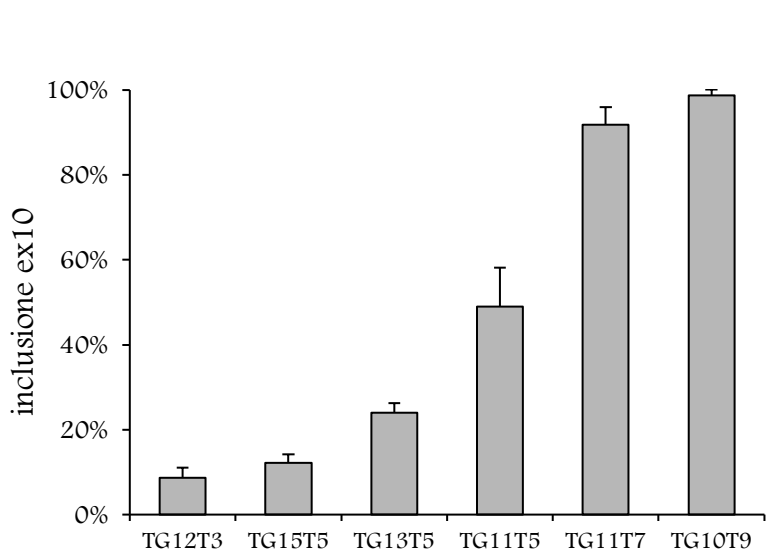


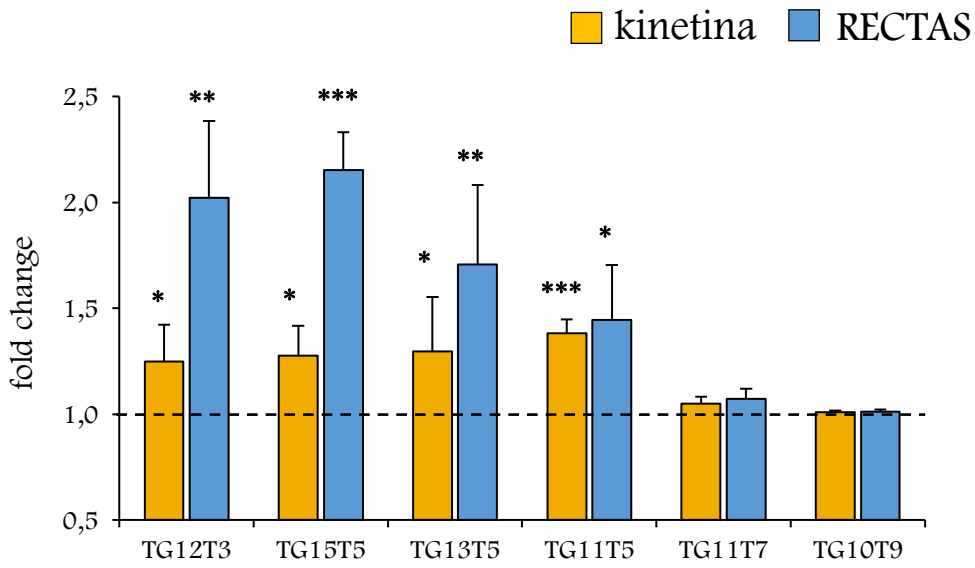


➡ L'inclusone dell'esone 10 aumenta significativamente dopo 4h di trattamento

Kinetina & RECTAS: diversi genotipi







Analoghi della Kinetina

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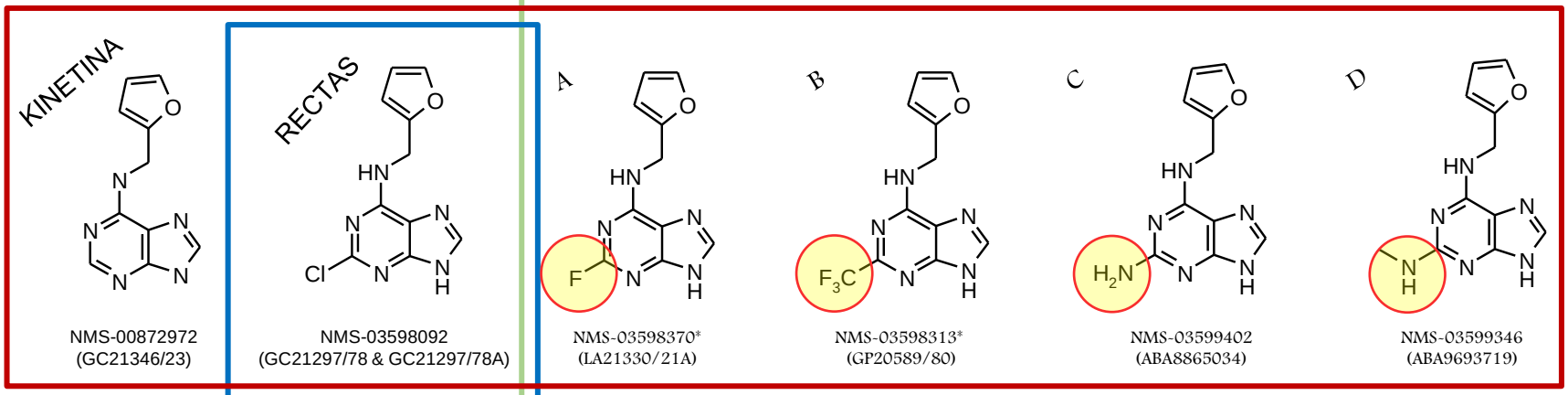
"2-position exploration"



NMS-00872972 (GC21346/23)

NMS-03598092 (GC21297/78 & GC21297/78A)

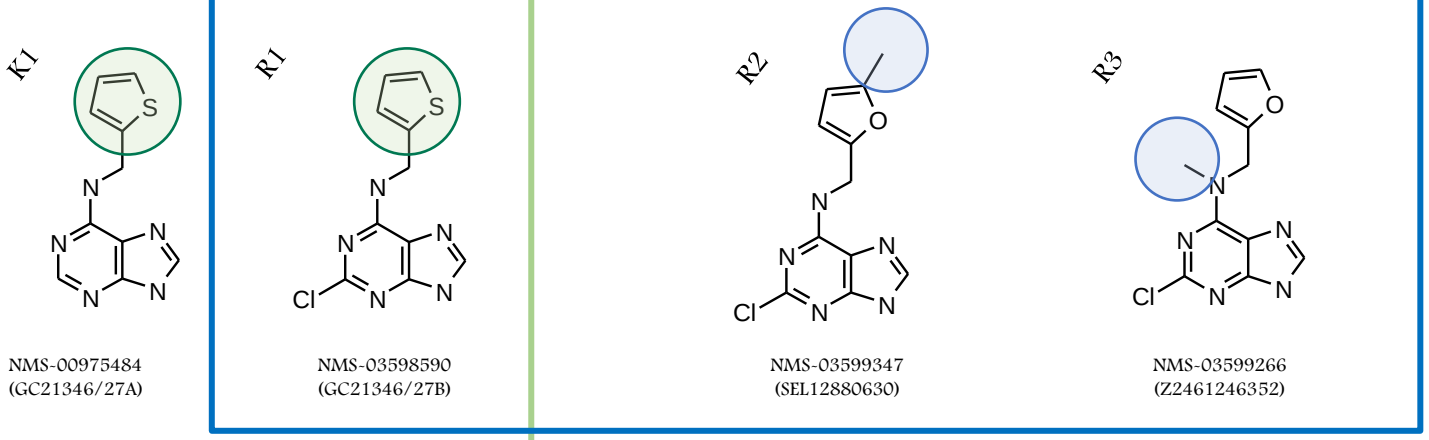
NMS-03598370* (LA21330/21A)

NMS-03598313* (GP20589/80)

NMS-03599402 (ABA8865034)

NMS-03599346 (ABA9693719)

" analoghi di RECTAS "



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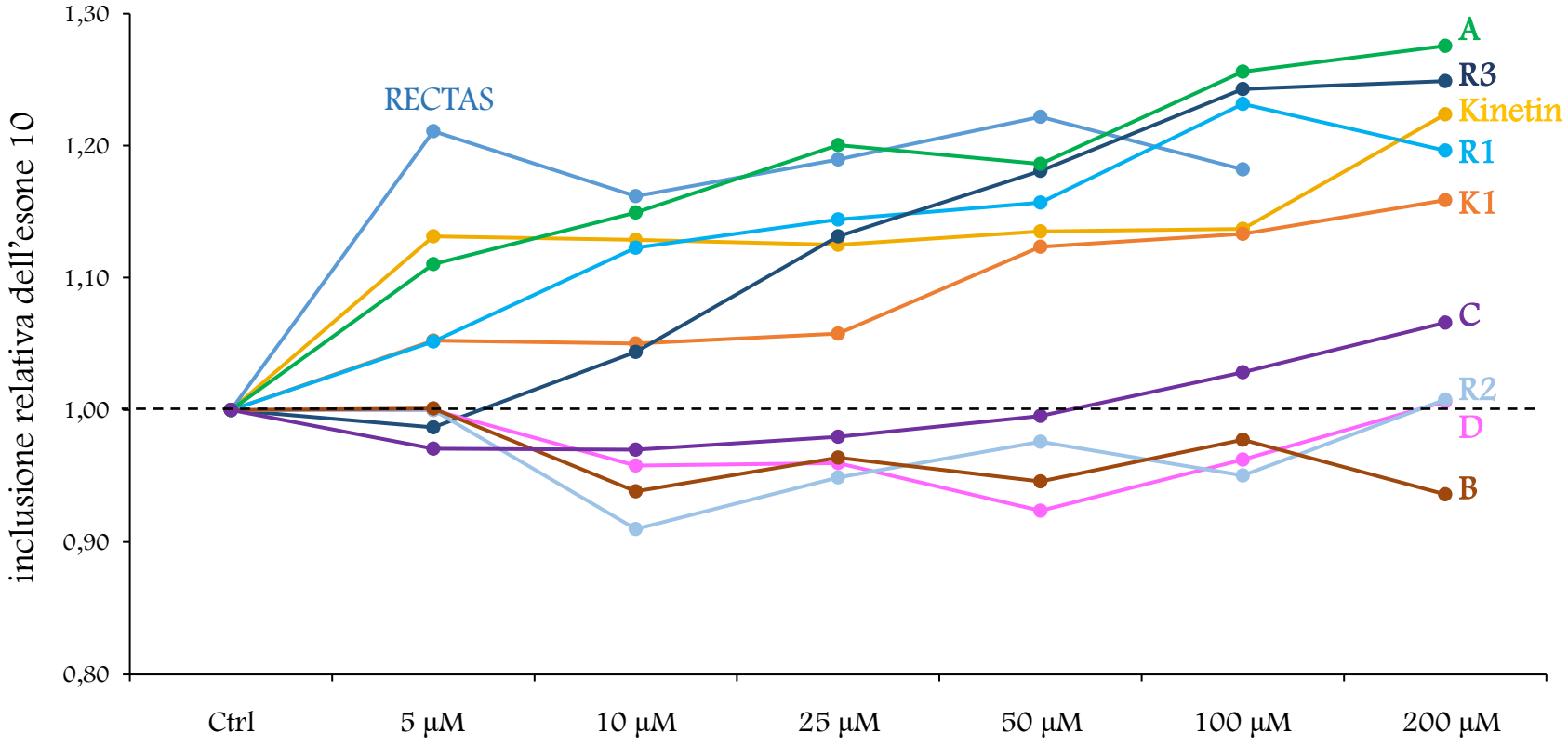
NMS-03598590 (GC21346/27B)

NMS-03599347 (SEL12880630)

NMS-03599266 (Z2461246352)

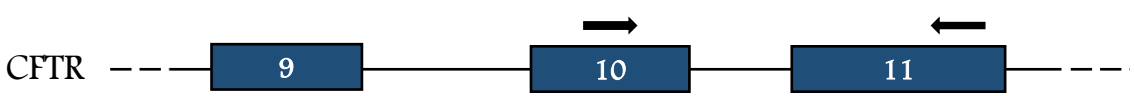
Analoghi della Kinetina

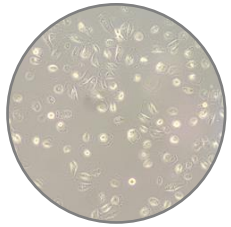
Abbiamo testato gli analoghi della kinetina selezionati trattando le cellule **Caco-2** per **24h**



➡ RECTAS è la molecola più attiva a basse dosi

Cellule da brushing nasale



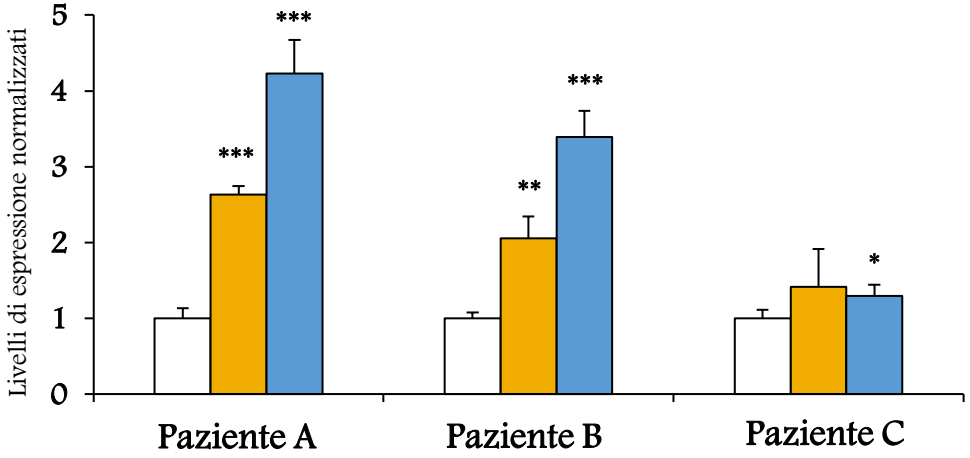


Ctrl kinetina RECTAS

Paziente A $(TG)_{10} - (T)_7$ / c.1717-1 G>A
$(TG)_{12} - (T)_5$ / wt

Paziente B $(TG)_{10} - (T)_7$ / G542X
$(TG)_{12} - (T)_5$ / wt

Paziente C $(TG)_{10} - (T)_7$ / R553X
$(TG)_{12} - (T)_5$ / wt



➡ Il trattamento con RECTAS aumenta l'inclusione dell'esone 10 nelle cellule di pazienti CF

Conclusioni

- Kinetina ha un considerevole effetto sullo splicing dell'esone 10
- RECTAS è in grado di correggere lo splicing dell'esone 10 come kinetina, ma a dosi inferiori
- Il trattamento con RECTAS aumenta l'inclusione dell'esone 10 in cellule di pazienti CF

Prospettive future

➡ confermare l'effetto a livello della proteina

➡ valutare la possibile correzione di altre mutazioni di splicing in CFTR

Ringraziamenti

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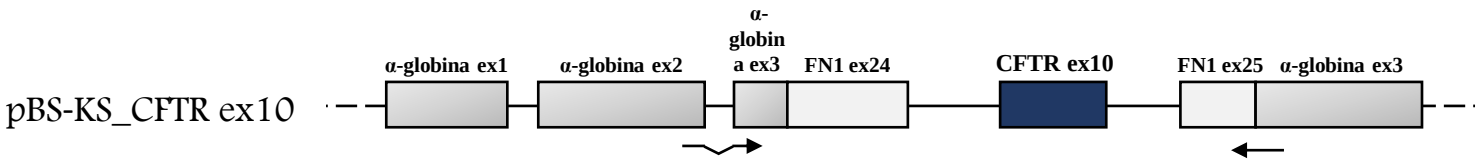
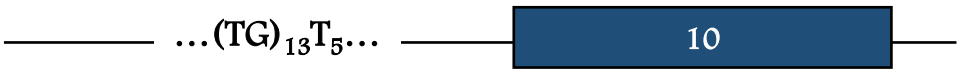
Nerviano Medical Sciences

Christian Orrenius

Fondazione Ricerca Fibrosi Cistica - Onlus
www.fibrosicisticaricerca.it

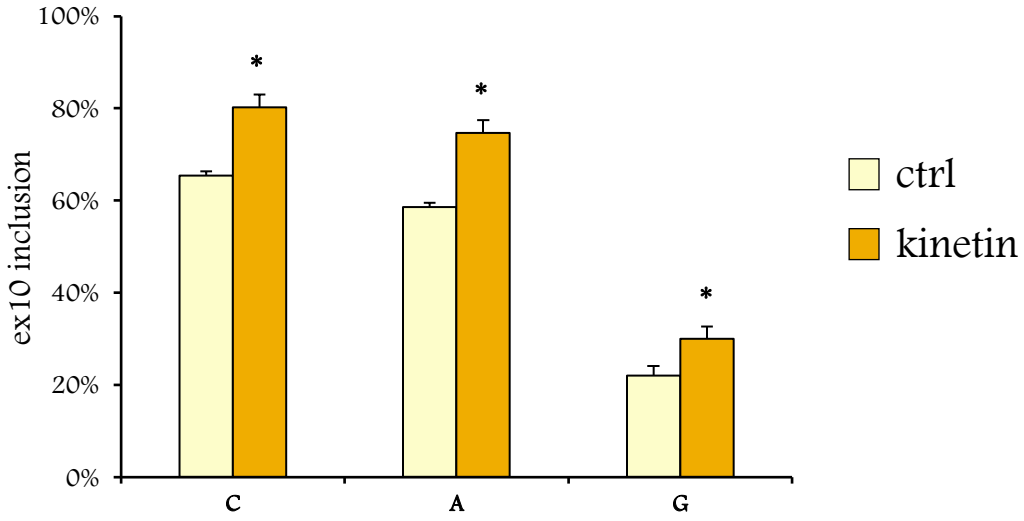
Molecular mechanism: in-cis element





…CACTGGAGCAGG**C**AAGgtagttcttttgttcttcact…

…CACTGGAGCAGG**T**AAGgtagttcttttgttcttcact…



Molecular mechanism: in-trans factor

FEBS Letters 580 (2006) 1339–1344

TDP43 depletion rescues aberrant CFTR exon 9 skipping

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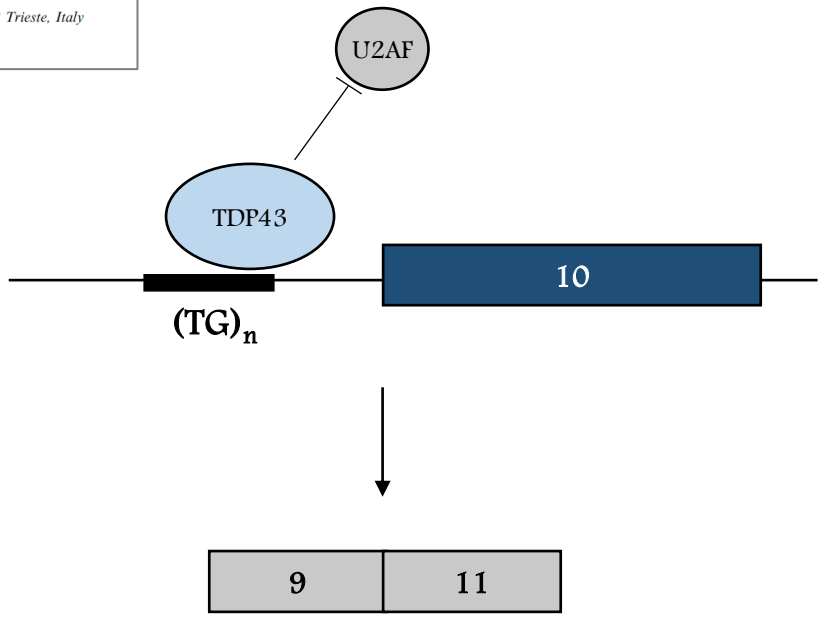
Received 3 January 2006; revised 18 January 2006; accepted 19 January 2006



exon 10 skipping

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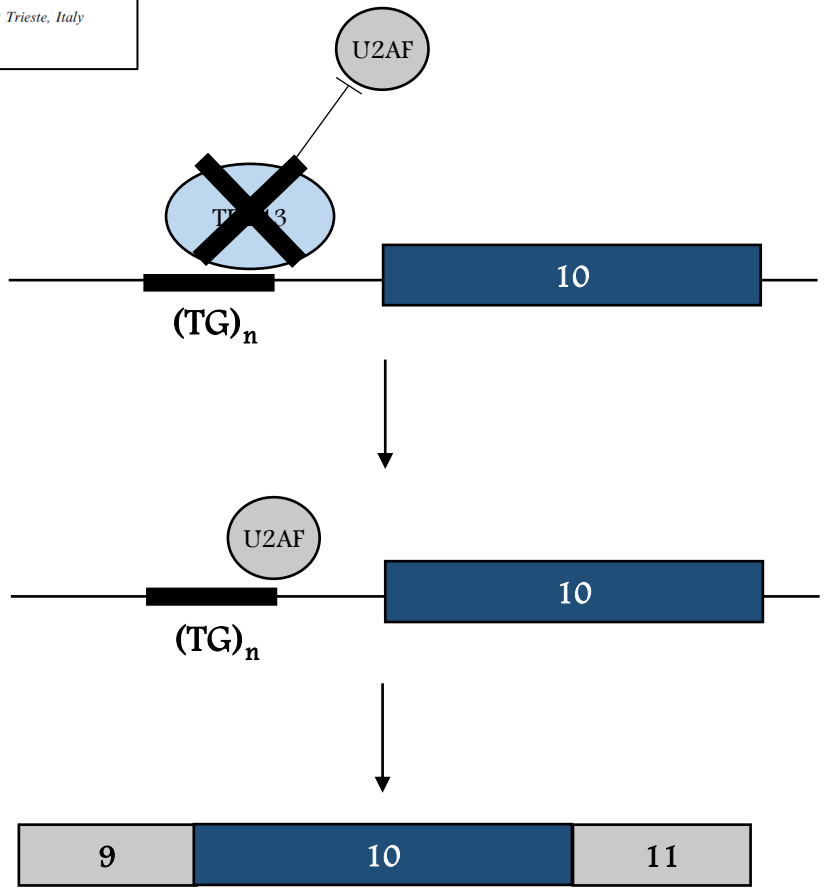

exon 10 inclusion

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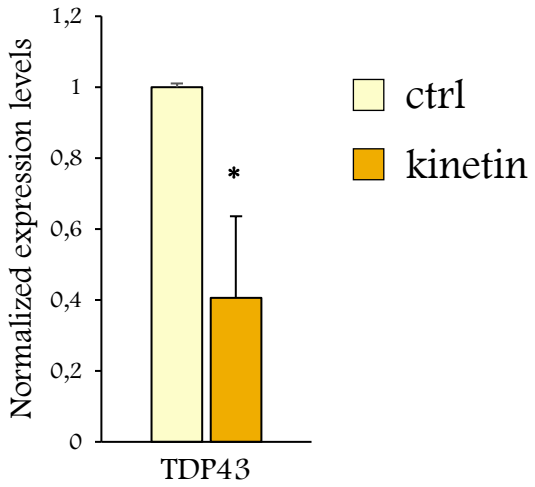
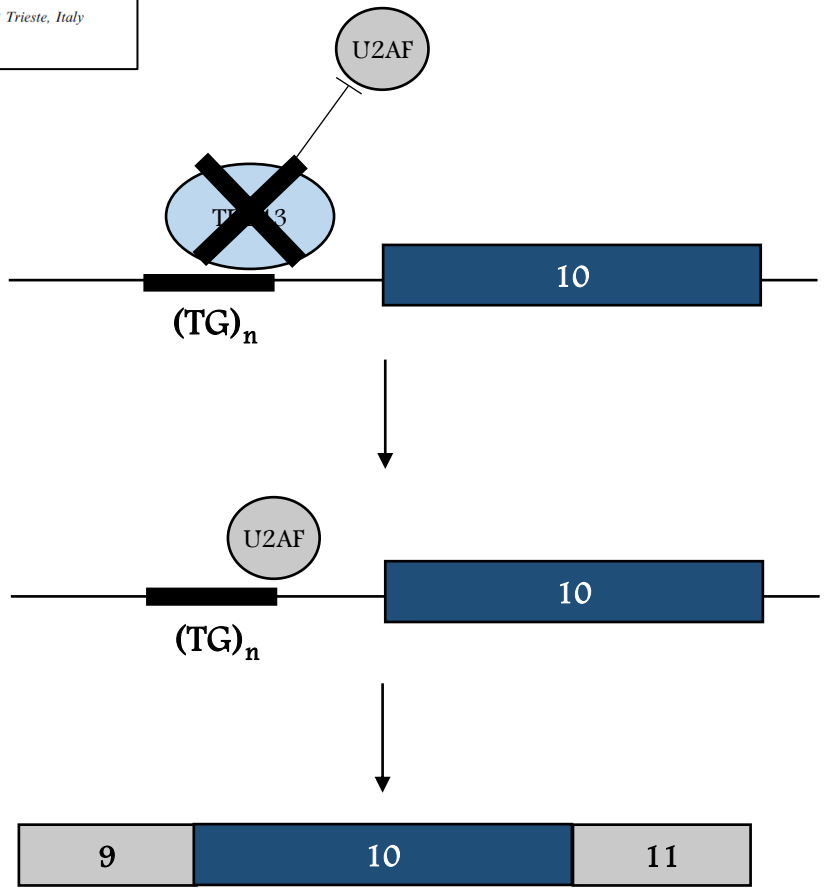
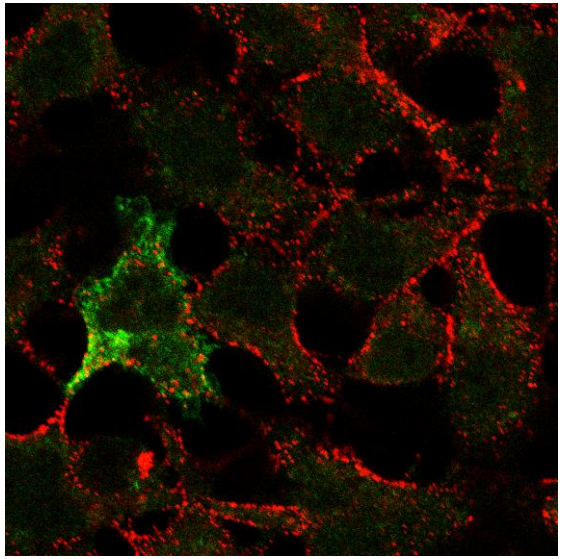




exon 10 inclusion

CFTR

caveolin 1



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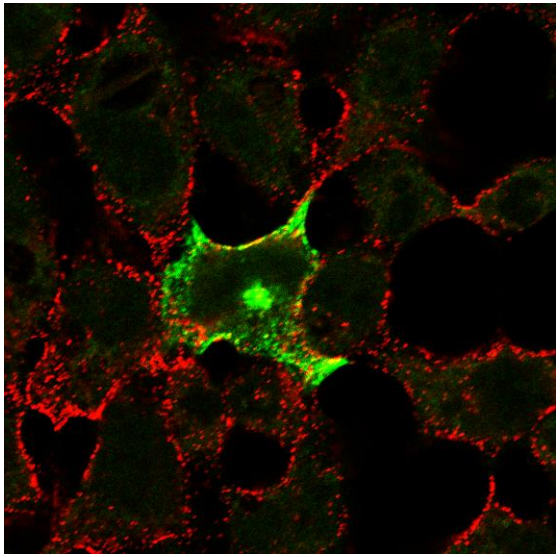

RECTAS