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SOCIETÀ ITALIANA
PER LO STUDIO DELLA FIBROSI CISTICA

QUAL È IL FUTURO PROSSIMO DELLA RICERCA IN FC?

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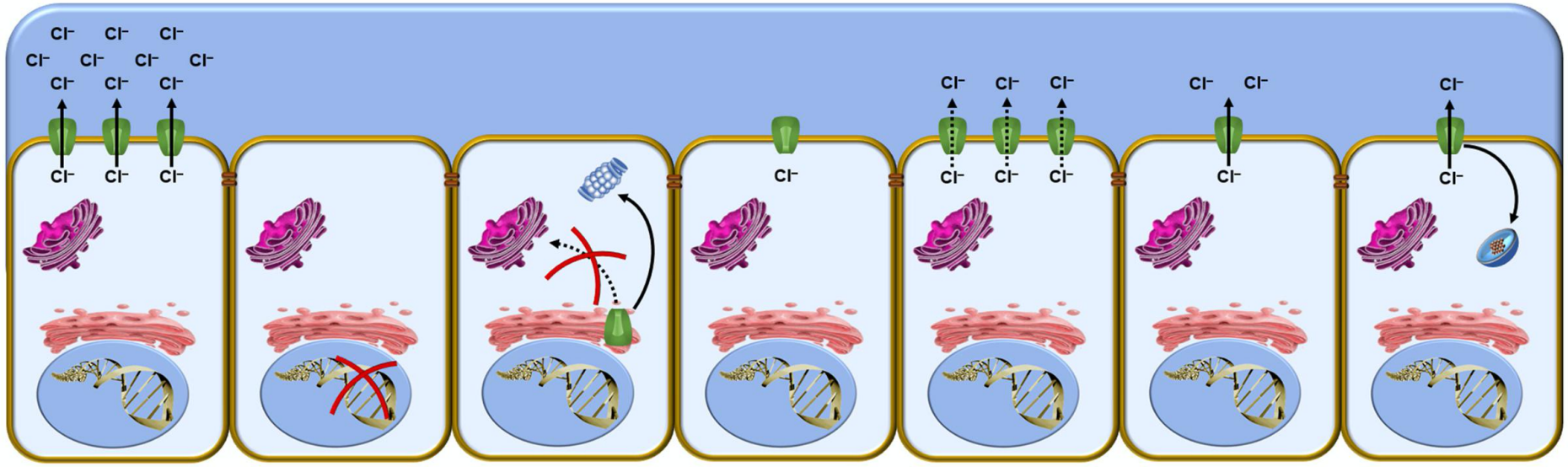
POTENZIALE CONFLITTO D'INTERESSI DA DICHIARARE

Tipo di affiliazione o supporto finanziario	*Sponsor*
Relatore a corsi di aggiornamento	Vertex Pharmaceuticals

Meccanismi con cui le mutazioni determinano la perdita di funzione della proteina CFTR



	wt-CFTR	I	II	III	IV	V	VI
Defect types		**No protein**	**No traffic**	**No function**	**Less function**	**Less protein**	**Less stable**
Mutation examples		G542X R553X W1282X	G85E ΔI507 **ΔF508** N1303K	V520F S549R G551D	R117H R334W S1235R	A455E 1680-886A>G 2657+5G>A	**rΔF508** Q1412X
Required approaches		Rescue protein synthesis	Correct protein folding	Restore channel conductance	Restore channel conductance	Maturation / Correct misplicing	Promote protein stability

La ricerca ha permesso di identificare farmaci modulatori, come potenziatori e correttori, che possono recuperare la funzione di CFTR:

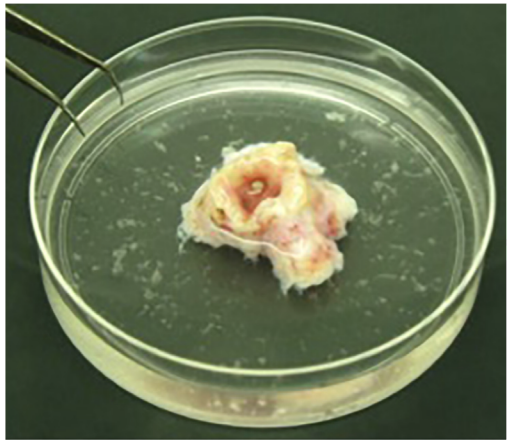
- Ivacaftor
- Lumacaftor/Ivacaftor
- Tezacaftor/Ivacaftor
- Elexacaftor/Tezacaftor/Ivacaftor

sono stati approvati per l'uso sui pazienti con determinate mutazioni.

Tuttavia, molti pazienti FC (circa il 30% in Italia) hanno mutazioni non incluse in quelle per le quali sono stati sviluppati i modulatori di CFTR, definite “mutazioni orfane”, con sensibilità sconosciuta ai modulatori CFTR.

Alcune di queste mutazioni, però, potrebbero essere responsive ai modulatori conosciuti.

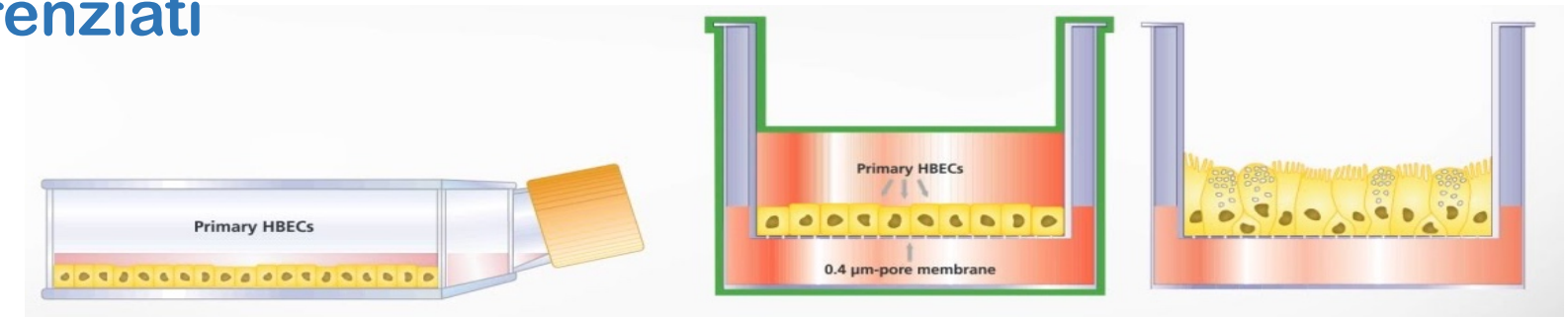
Ruolo fondamentale delle colture primarie di cellule epiteliali bronchiali da pazienti FC nella validazione dell'efficacia dei modulatori identificati



Cellule epiteliali bronchiali umane

Isolate dai bronchi primari di pazienti FC sottoposti a trapianti polmonare

Le cellule basali possono essere espanse in vitro e riprogrammate in epiteli differenziati



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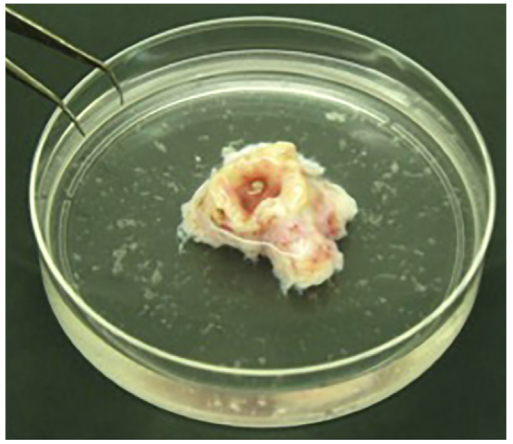
Ruolo fondamentale delle colture primarie di cellule epiteliali bronchiali da pazienti FC nella validazione dell'efficacia dei modulatori identificati



Limiti dell'uso delle cellule epiteliali bronchiali:

- Le cellule ottenute derivano in genere da pazienti con mutazioni severe
- E' difficile avere cellule da pazienti con genotipi «rari»

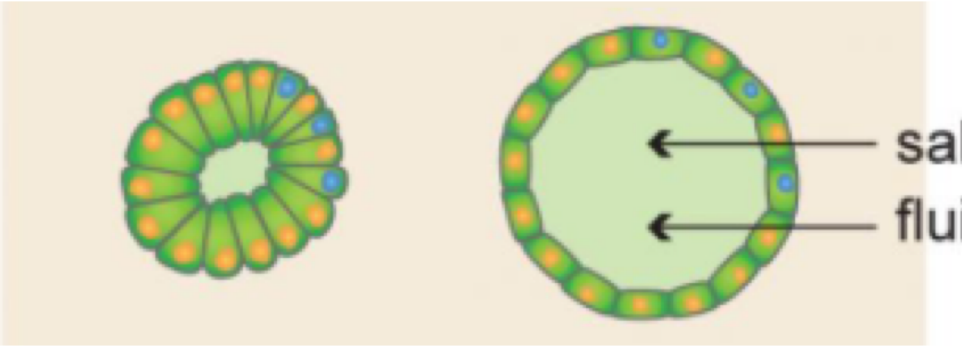
Alternative all'uso delle cellule epiteliali bronchiali per approcci di medicina personalizzata:

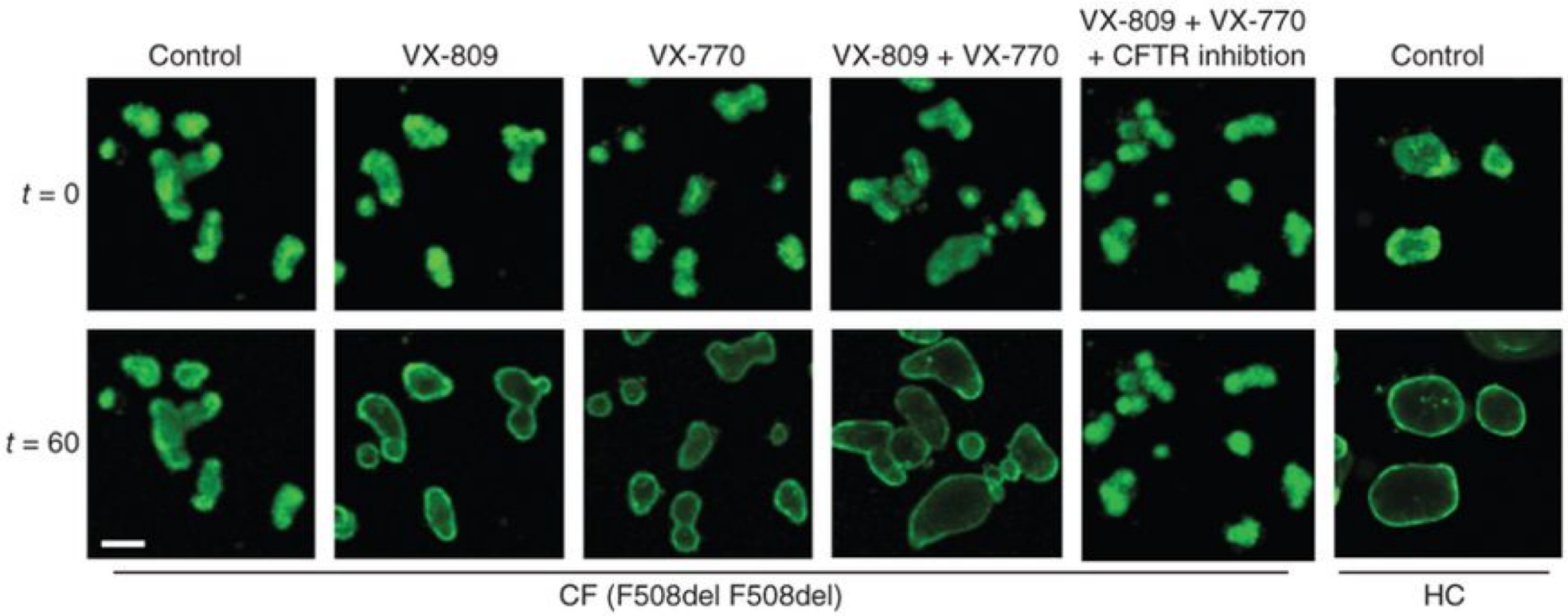
Organoidi intestinali

Cellule epiteliali nasali

ORGANOIDI INTESTINALI

Gli organoidi vengono utilizzati per misure di "rigonfiamento"
(Forskolin-Induced Organoid Swelling Assay)





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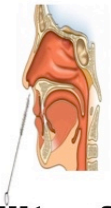
EPITELI DA CELLULE NASALI

Mediante un brushing nasale vengono recuperate delle cellule epiteliali che vengono coltivate

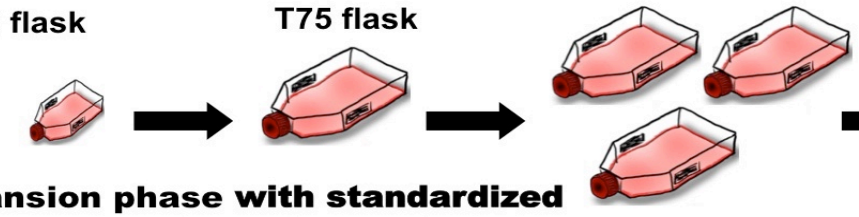


Availability of different genotypes

Primary human nasal epithelial cells (HNECs) are collected by nasal brushings of both nostrils

T25 flask

T75 flask



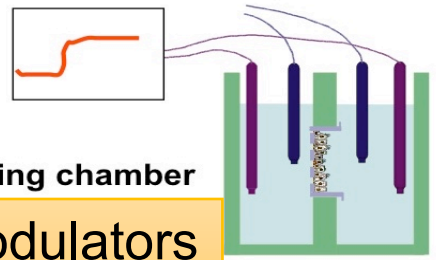
expansion phase with standardized anti-SMAD protocol

(multipotent basal cells)

generation of nasal epithelia

Plating on porous support in air liquid interface (ALI) condition

(conditional reprogramming)



Ussing chamber

Analysis of transepithelial ion transport by short-circuit current (Isc) recordings

+ CFTR modulators

Per info e suppo

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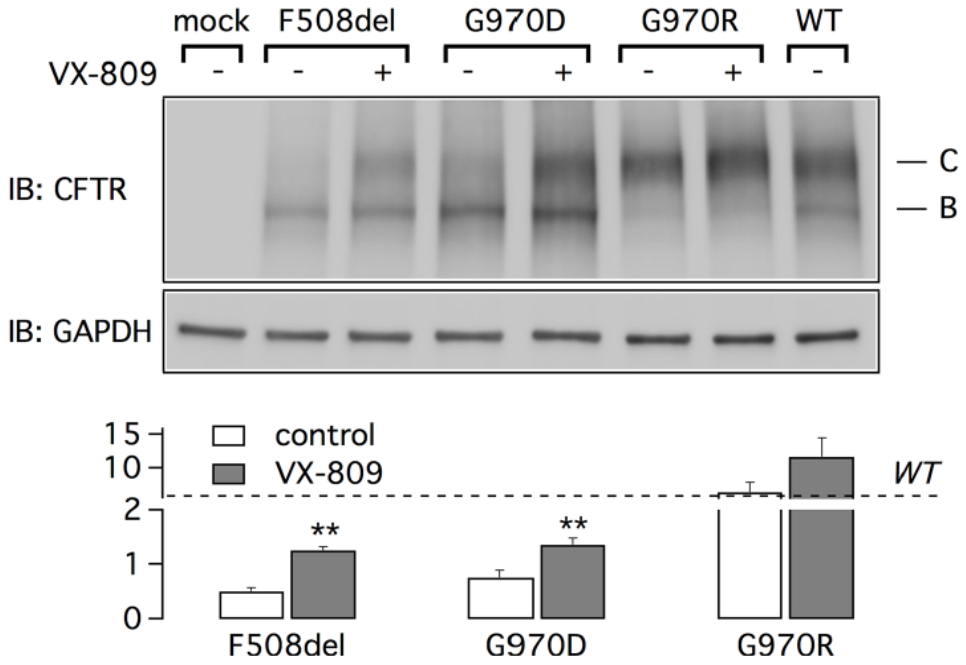
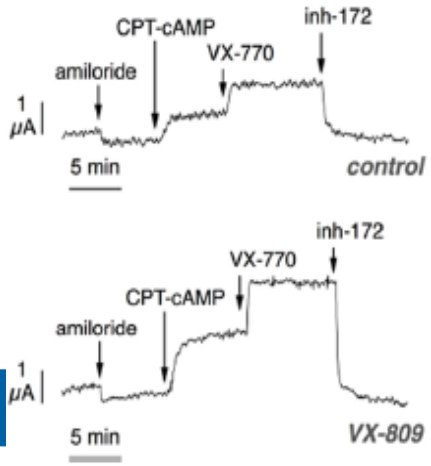
CONTRIBUTO PREDITTIVO DELLE CELLULE NASALI E INTESTINALI DA PAZIENTE

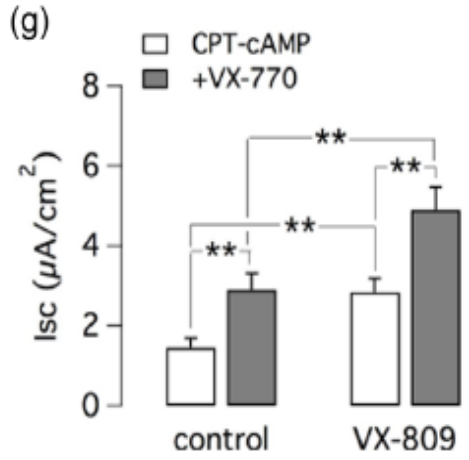
- **Per studiare il tipo di difetto causato della mutazione**
- **Per determinare la responsività ai nuovi farmaci**

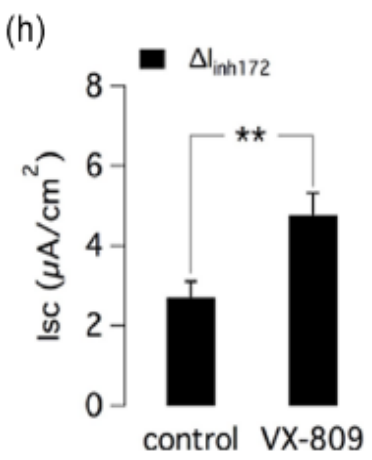
TERATIPO

TERATIPO DI MUTANTI RARI DI CFTR









Paziente con G970D

- La mutazione G970D è sensibile al VX-770
- Presenta anche un parziale difetto di maturazione sensibile al VX-809

Si prevede un potenziale beneficio terapeutico dalla combinazione potenziatore + correttore (F508del sul secondo allele)

Amato et al, Hum Mutat. 2019; 40:742-8

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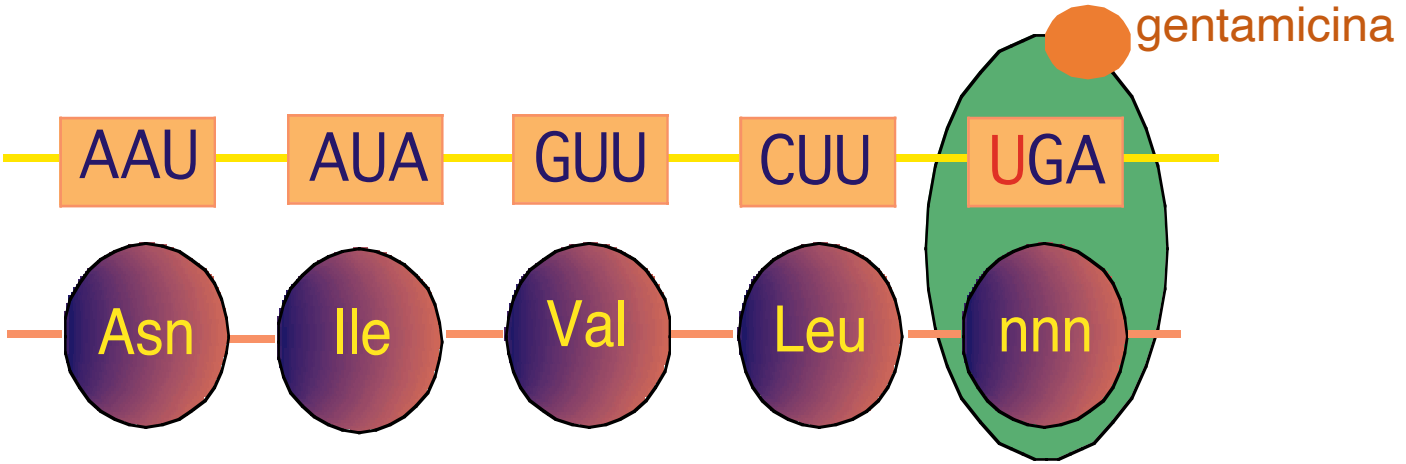
Alcuni pazienti FC (circa il 10-15%) rimangono esclusi dalle terapie con i modulatori di CFTR:

- **Mutazioni Classe I (Stop prematuro)**
- **Mutazioni di Splicing con mRNA assente**
- **Delezioni**
- **….**

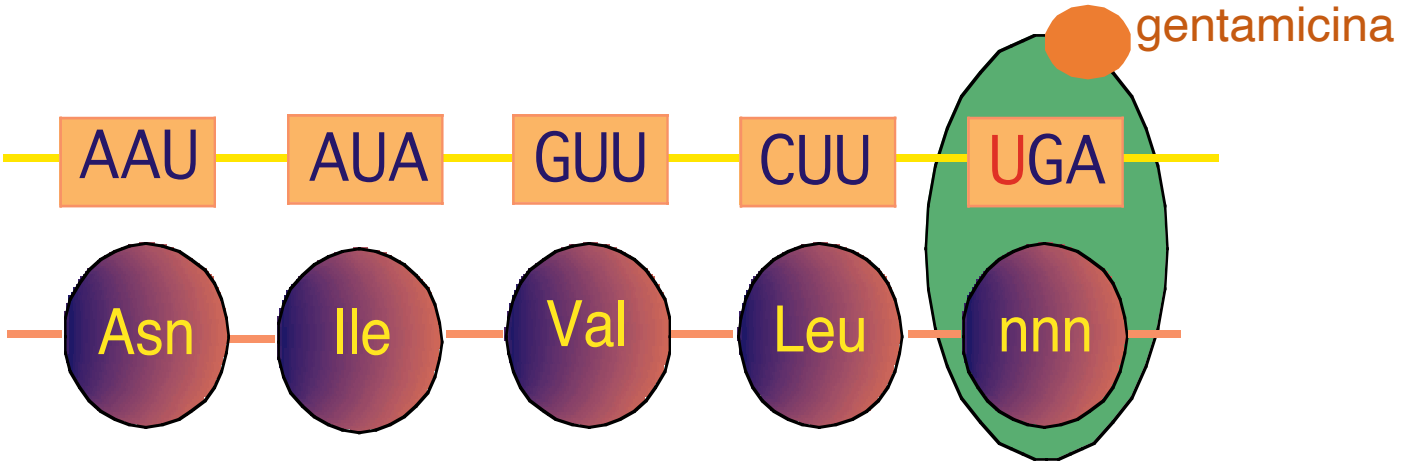
Modulatori per mutanti di classe I

FARMACI READTHROUGH



Modulatori per mutanti di classe I

FARMACI READTHROUGH



SI PRODUCE UNA PROTEINA CON UN AMINOACIDO "SBAGLIATO":

...un potenziatore potrebbe aumentarne la funzione

...un correttore potrebbe migliorarne la maturazione

Modulatori per mutanti di classe I

FARMACI READTHROUGH

Mutation-Induced Alternative Splicing and Alternative Polyadenylation May Be a Liability for Nonsense Readthrough Therapeutics

Normand E. Allaire, M.S., Head of Genomics
CFFT Lab, Cystic Fibrosis Foundation
Lexington, MA, USA

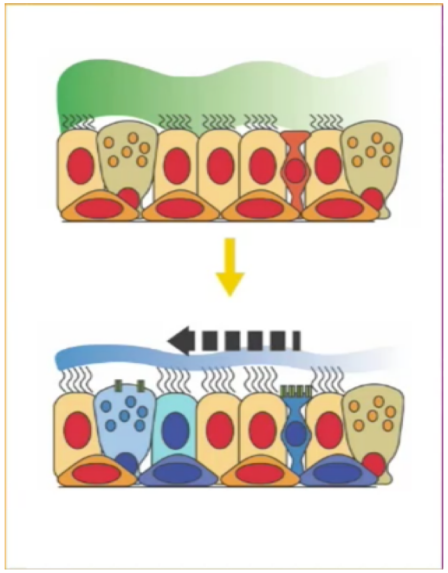
PTC	New Donor Site	New Exonic Splice Silencer (ESS)	Exonic Splice Enhancer (ESE) Broken
W1282X	+	+	+
R1162X	+	-	+
R553X	-	-	+
G542X	-	-	-
Y122X	-	-	-

- G542X and R553X exert additional effects on splicing including exon 12 skipping and reduction of full-length, readthrough competent mRNA in both 16HBEge and primary cells from donors with CF.
- R1162X and W1282X induce a significantly increased use of alternative pA site past exon 22, resulting in truncated and likely non-functional protein and reduction of full-length readthrough competent mRNA in 16HBEge cells.

Taken together, these results suggest that PTCs (and likely other mutations) can have additional effects on alternative splicing and alternative polyadenylation and that these effects should be considered in the development of therapeutics for these mutations.

PATH TO A CURE - CFF

NextGen Gene Therapeutics to Treat ALL People with CF



Brian R. Davis Ph.D.
UTHealth
Maria Limberis Ph.D.
Univ. of Pennsylvania

Gene delivery

- Restores CFTR protein irrespective of mutation.
- *CFTR* DNA delivered via certain vectors (e.g., AAV, nonviral) will not be integrated into the chromosomal DNA.
- *CFTR* DNA delivered via integrating vectors (e.g., lentivirus) will be integrated into chromosomal DNA. Long-term benefit would require delivery to basal cells.
- Level of CFTR protein is unlikely to be regulated in its normal cell-type specific manner.

mRNA delivery

- Restores CFTR protein irrespective of mutation.
- Since mRNA has a limited half-life in the cell, restoration of CFTR protein will last only so long; also, airway cells will slough off with aging/injury. Will need to re-administer.
- Desire delivery to ionocytes/secretory cells, perhaps to ciliated cells. Do not require delivery to basal cells.
- Level of CFTR protein is unlikely to be regulated in its normal cell-type specific pattern.

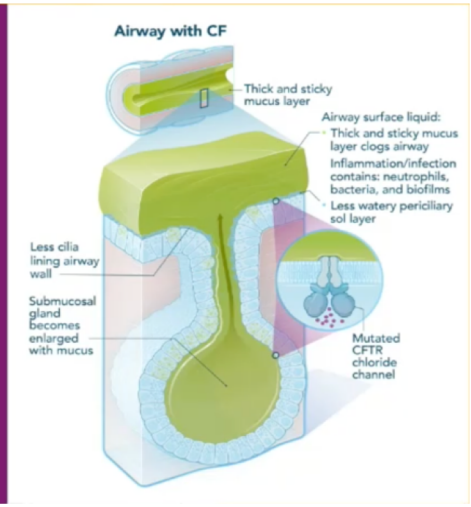
Gene editing

- Editing may be performed in either a mutation-specific or mutation-independent manner.
- Level of CFTR protein is regulated in its normal cell-type specific manner.
- Editing of basal cells may result in long-term correction of basal cells and their progeny.
- Editing of basal cells may be possible via targeting of basal cell-specific surface proteins.
- Delivery to the basal cell layer may require transient depletion of secretory/ciliated cells.

OSTACOLI…

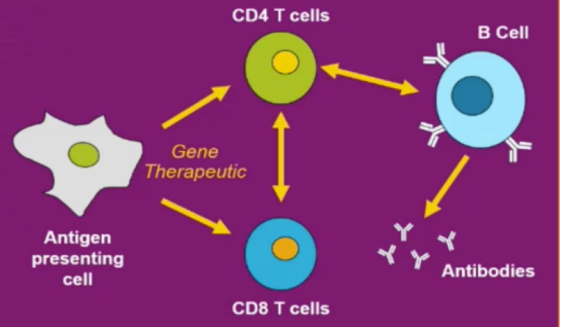
Physical Barriers to Effective Airway-Directed Gene Therapeutics

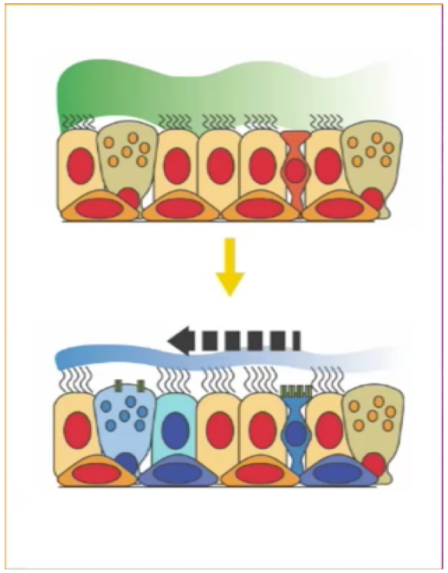
- Mucus layer
- Cilia and MCC
- Glycocalyx
- Tight junctions
- No receptors on apical surface



Biological Barriers to Effective Airway-Directed Gene Therapy

- Immune responses
  - Cellular & Humoral
- Antibodies to gene therapeutic
- T cells to CFTR & gene therapeutic

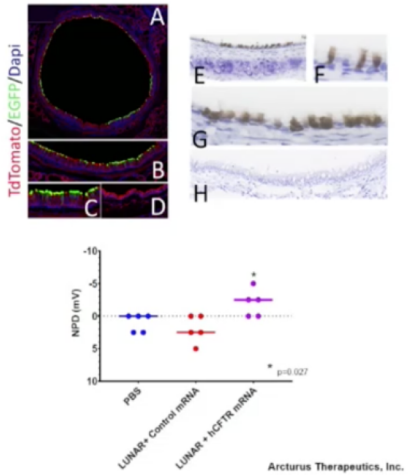




… E SOLUZIONI

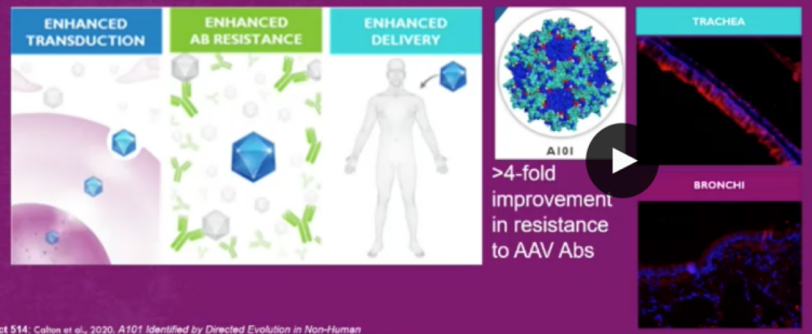
Development of an mRNA Therapeutic That Targets the Airway

Transduction profile of the LUNAR-CFTR mRNA in animal models



Abstract 422: Perez-Garcia et al., 2020. *LUNAR-CF, an MRNA replacement therapy for Cystic Fibrosis lung disease.* Arcturus Therapeutics, Inc., San Diego, CA.

Development of an Airway Efficient AAV That Evades Humoral Immunity



Abstract 514: Colton et al., 2020. *A101 Identified by Directed Evolution in Non-Human Primates Demonstrates Robust Gene Delivery Both In Vitro and In Vivo.* 4D Molecular Therapeutics, Emeryville, CA. University of California, Berkeley, CA

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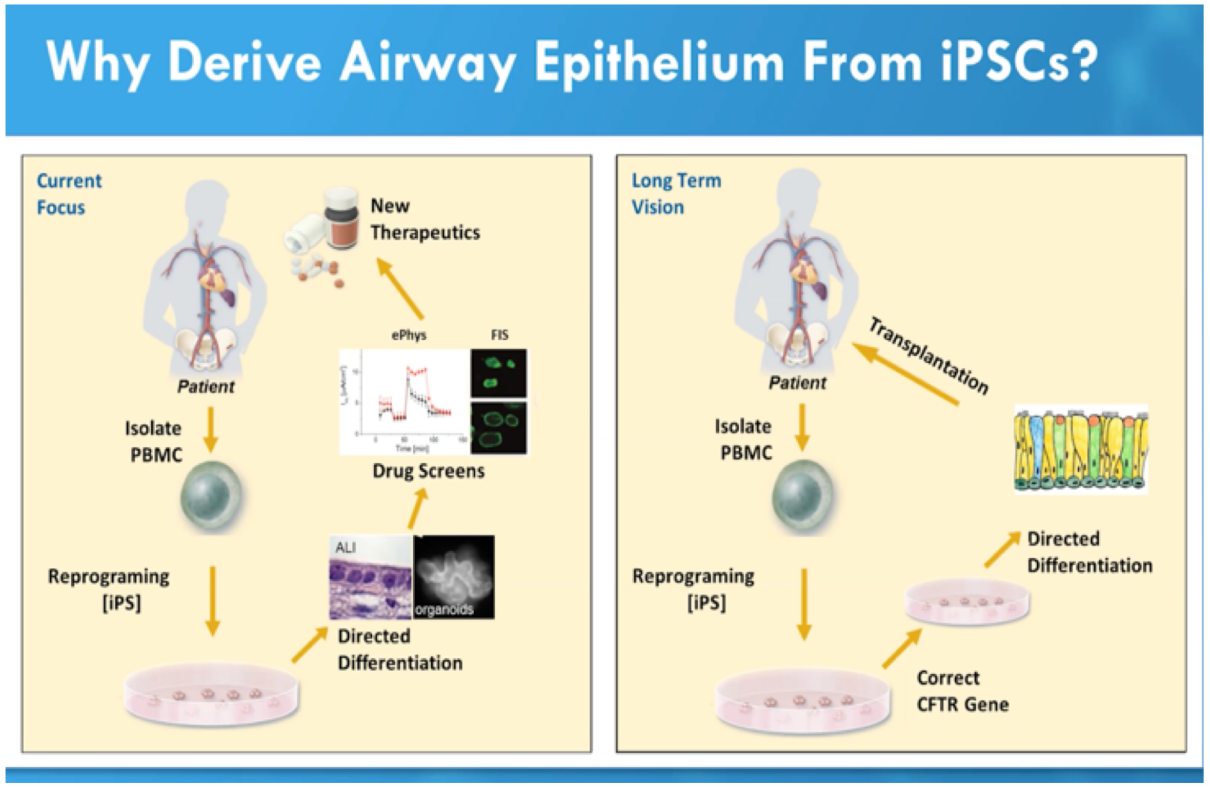
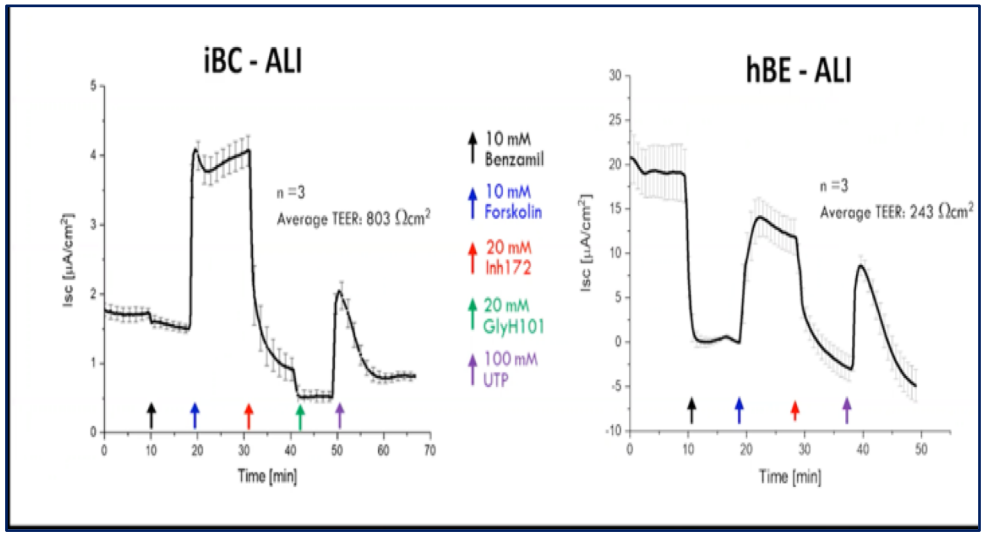
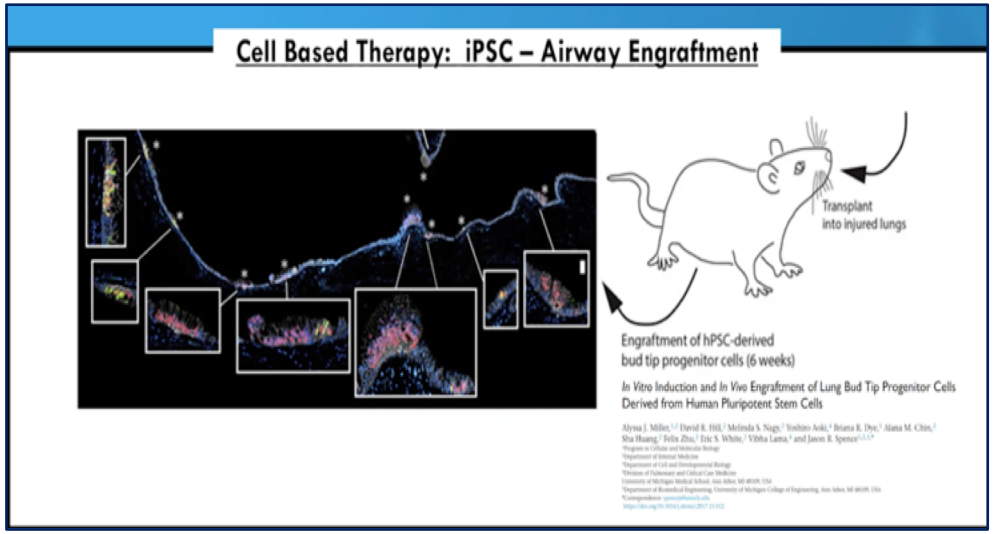
TERAPIA BASATA SU CELLULE

John Mahoney Ph.D., Cystic Fibrosis Foundation





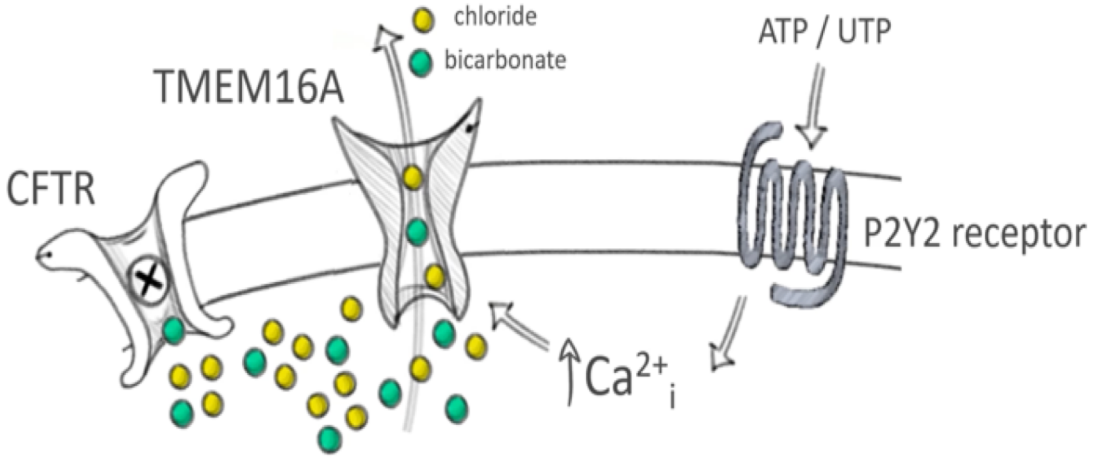


TARGET ALTERNATIVI: TMEM16A

Henry Danahay Ph.D., Enterprise Therapeutics

TMEM16A: calcium activated chloride channel

Conducts both chloride & bicarbonate



- Potentiating TMEM16A function
  - An opportunity to treat ALL CF patients irrespective of CFTR mutation

Will TMEM16A potentiators increase airway hydration & mucus clearance?

- In vitro & in vivo data consistent with mucosal hydration mechanism

What are the consequences of enhancing TMEM16A function in other organs?

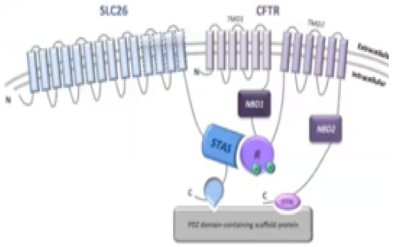
- No evidence of effects of TMEM16A potentiators on lung function, mucus production / release or cardiovascular function

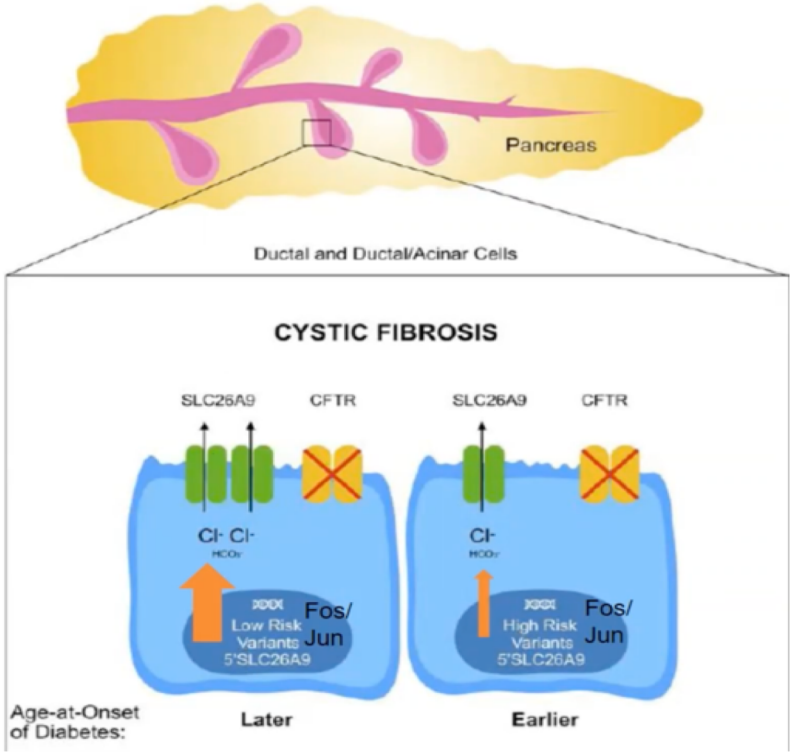
TARGET ALTERNATIVI: SLC26A9

Garry Cutting MD, Johns Hopkins

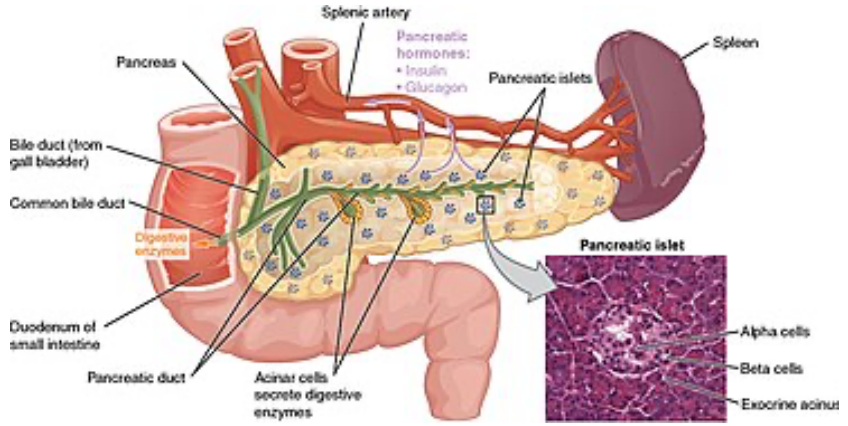
SLC26A9 facilitates chloride transport and interacts with CFTR

- Functions as chloride channel and as bicarbonate/chloride transporter
- SLC26A9 shown to interact with CFTR via its C-terminal PDZ-domain binding motif and Sulfate Transporter and Anti-Sigma factor antagonist (STAS) domain





SLC26A9 and CFTR are co-expressed in a population of pancreatic cells with ductal characteristics



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GRAZIE PER L'ATTENZIONE!