

Predicting CFTR modulator clinical efficacy by preclinical biomarkers

I Sermet-Gaudelus

INSERM U 1151

Hôpital Necker-Enfants Malades



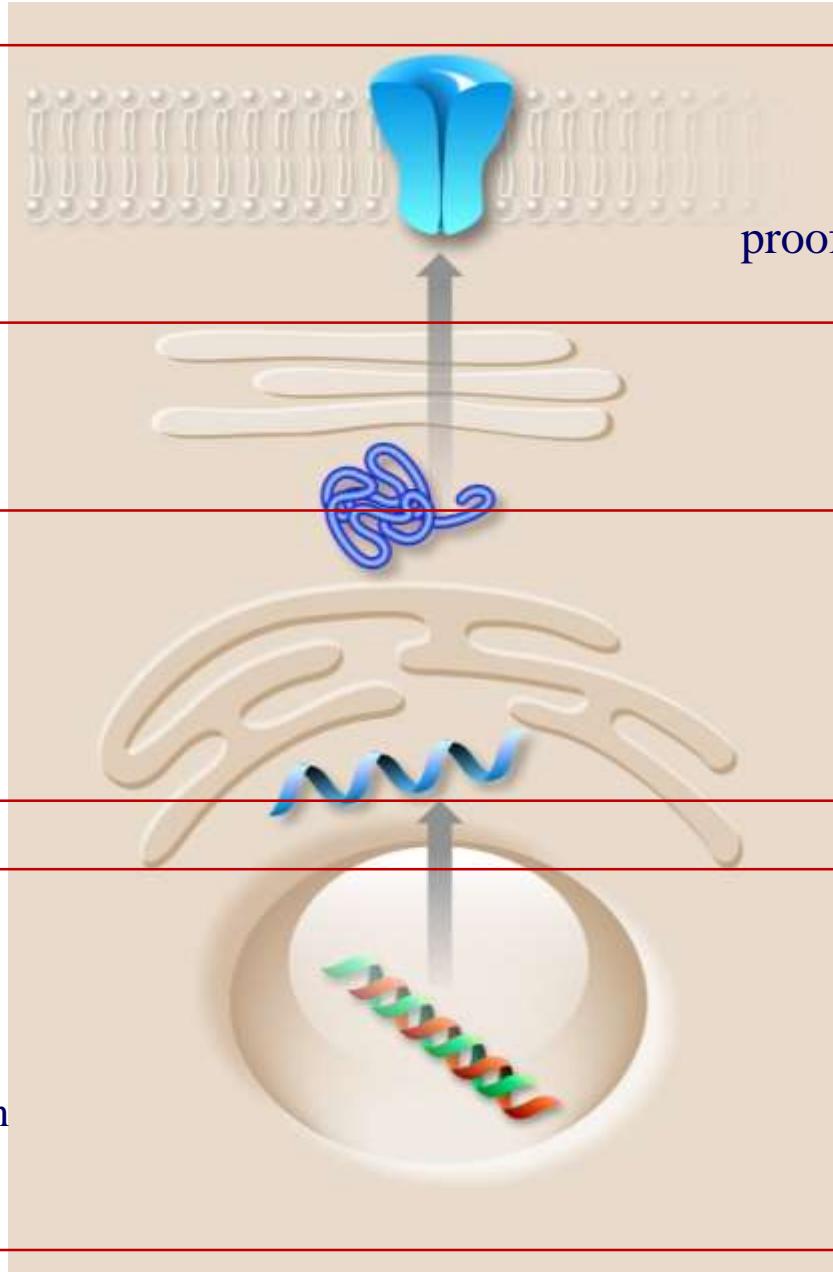
Centre de Ressources et de Compétences
de la Mucoviscidose

Hôpital Necker-Enfants Malades



Mutant CFTR biology and therapeutic susceptibility

Defective Function
(gating, conductance)



CFTR « potentiator »
(VX-770)
proof of concept for CFTR rescue
Ramsey et al. NEJM 2011

Defective Processing
(Decreased stability
Increased degradation)

Processing corrector
(VX-809, 661)
Limited clinical efficacy

Sermet-Gaudelus et al. ERJ 2014

Defective Synthesis

Premature termination codon

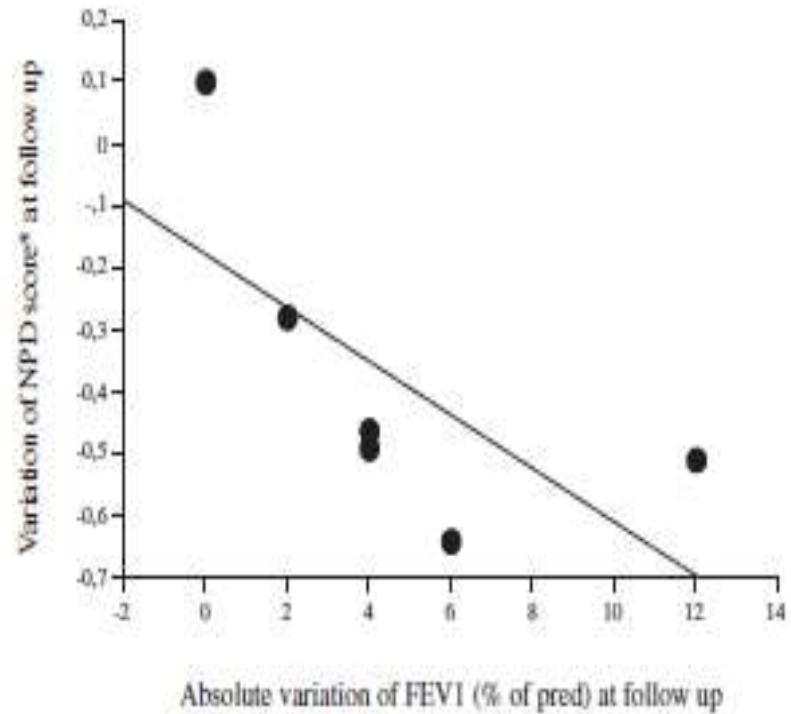
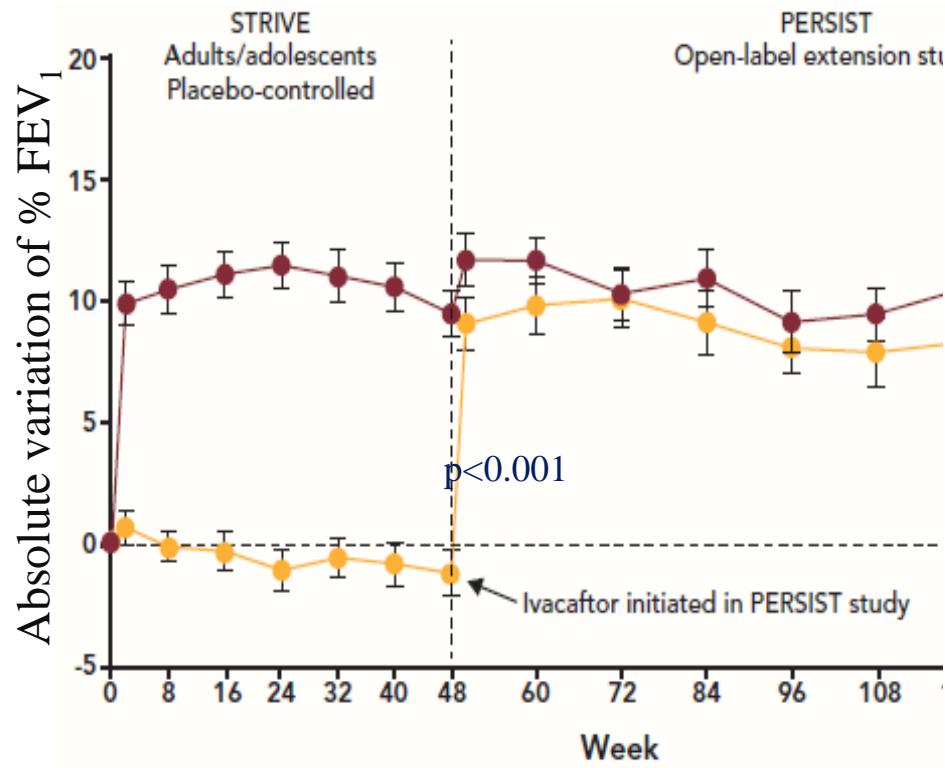
Readthrough agent
no clinical efficacy

Sermet-Gaudeluc et al. AJRCCM 2016

Gene Therapy (AAV)

Vidovic et al. AJRCCM 2015

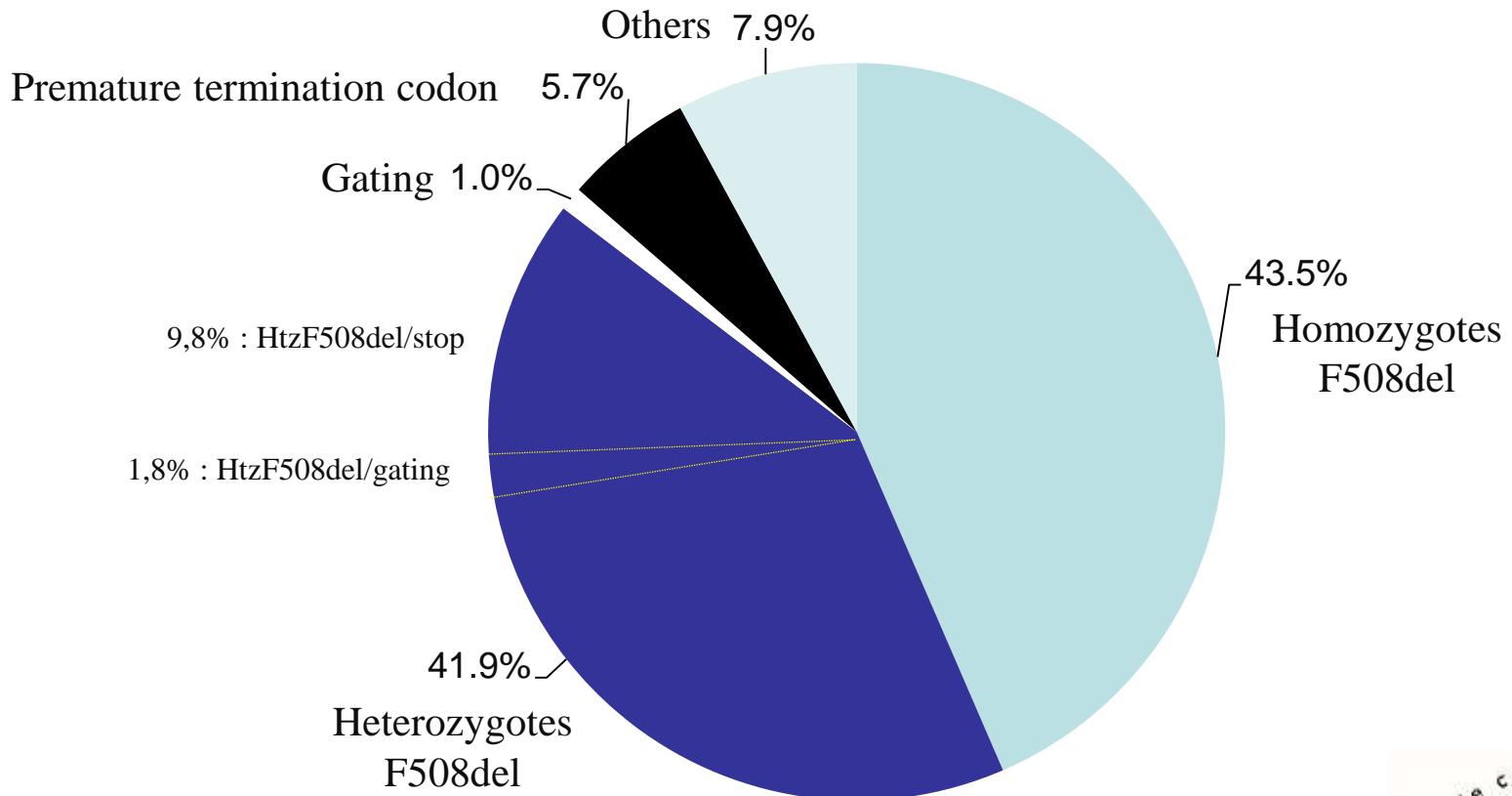
VX-770 – respiratory function improvement



Ramsey et al. N Engl J Med J 2011
Mc Kone et al. Presented at the NACFC 2013

Mesbahi and Steinberg. J Cyst Fibr 2016

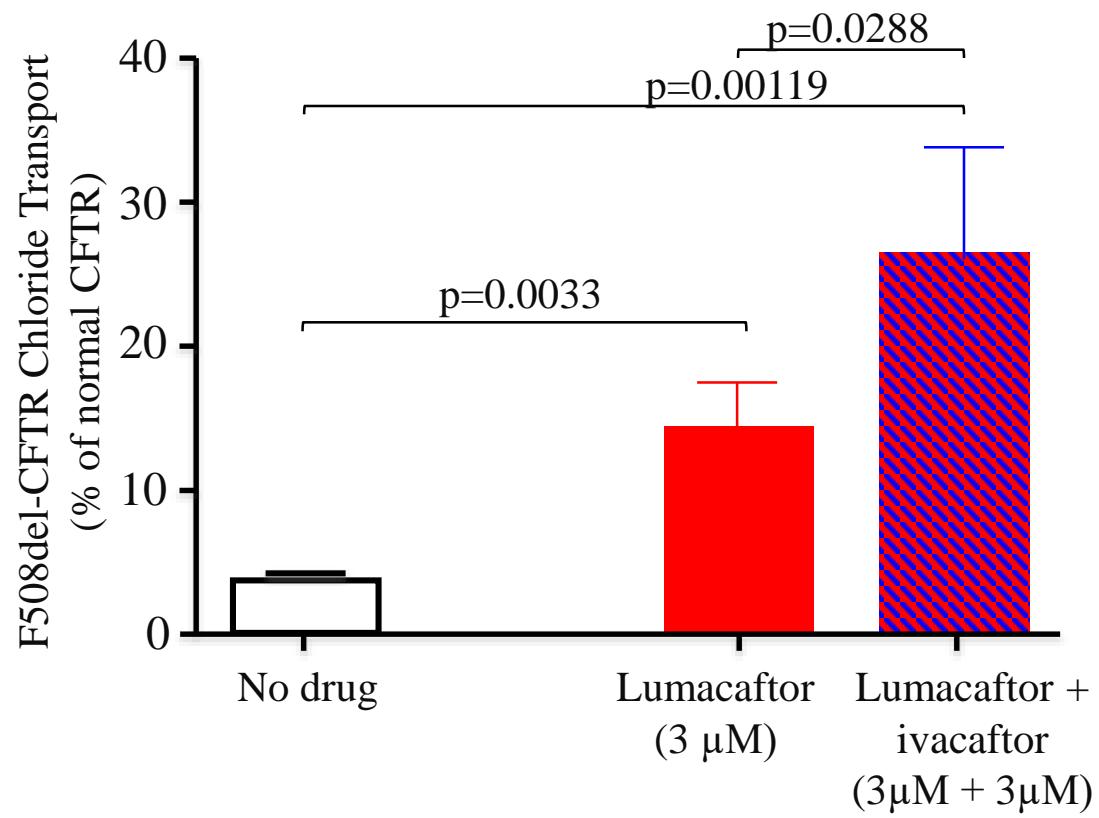
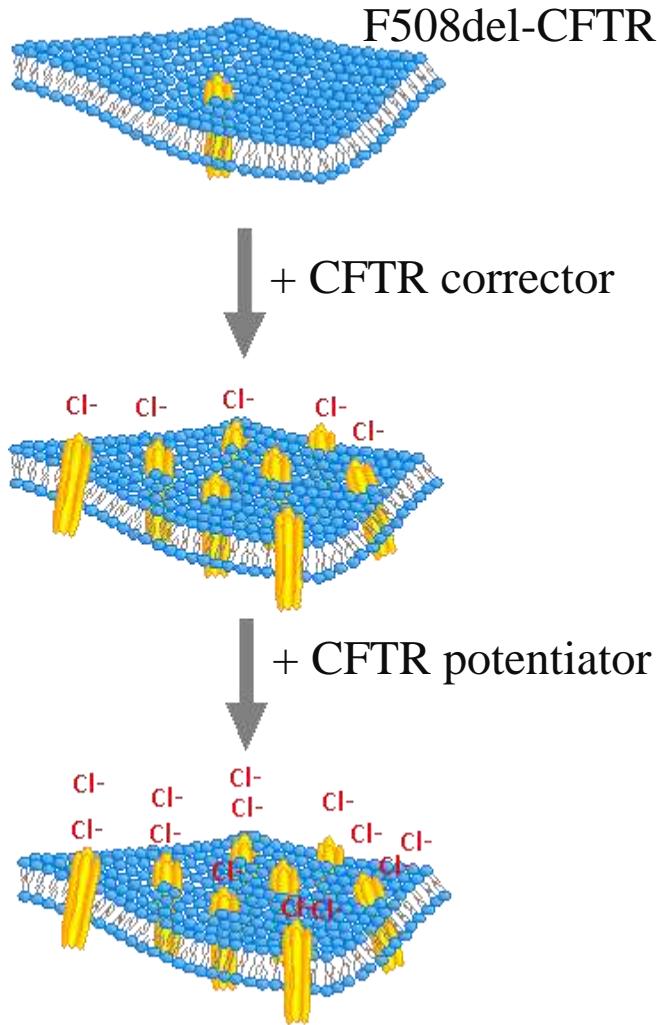
5970 patients in France with at least one known mutation



Données issues du Registre Français

Combination of correction and potentiation for F508del-CFTR

VX-809 corrector + VX-770 potentiator = Orkambi®



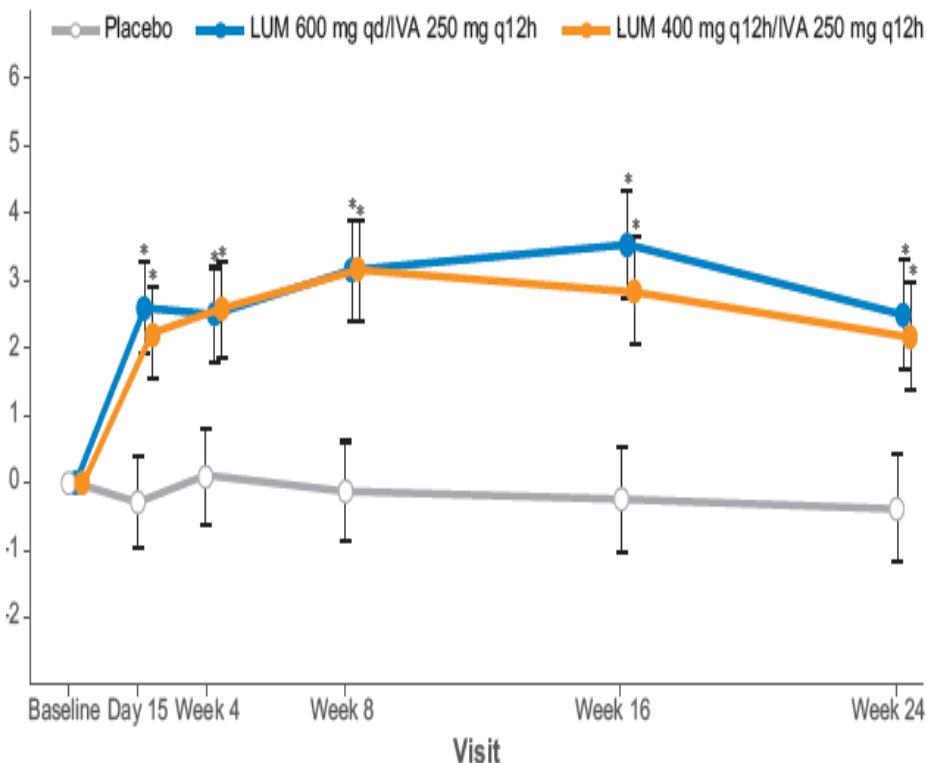
Modified from Van Goor et al. PNAS 2011

Heterogeneity of clinical responses to Orkambi®

25% of patients increase by more than 10% their FEV1

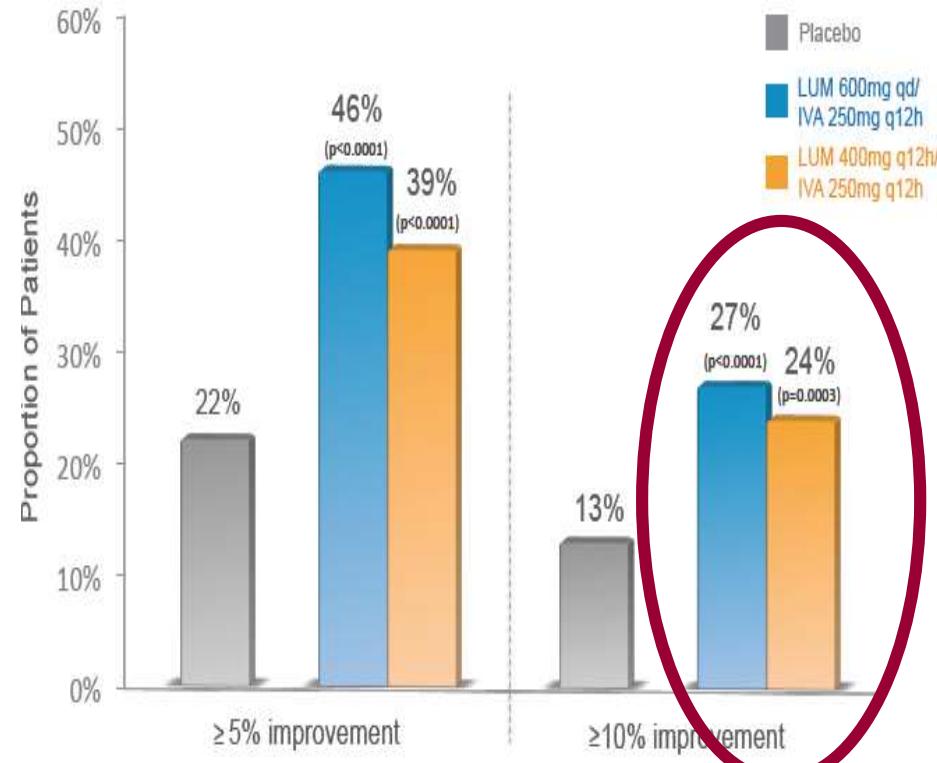
Absolute variation of % FEV₁

ppFEV1
(LS Means +/-95%CI)



% of patients improving their FEV₁ by
>5% >10%

Average relative change
in % predicted FEV₁ at week 24

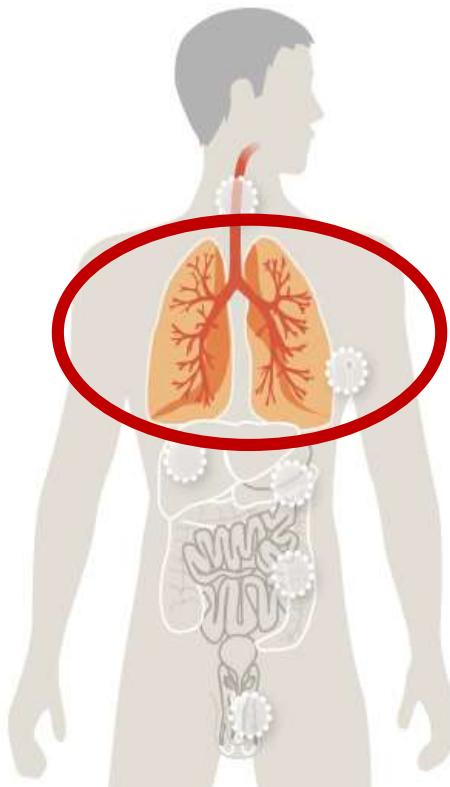


	M0 (n=37)	M6 (n=34)	P
Female sex no (%)	18 (48.64)		
Mean age (range) year	15 (12-22)		
Mean BMI Z-score (range)	-0.53 (-2.43-1.98)	-0.36 (-2.29-1.9)	NS
Number of exacerbations (range)	1.5 (0-6)	2 (0-5)	NS
% pred FEV1			
Mean (range)	73.8 (25.8-123.1)	77.9 (27.3-116.1)	
Subgroup no (%)			
<40%	4	2	NS
≥40 to < 70%	11	8	
≥70 to ≤90%	13	10	
>90%	9	14	
%pred FVC			
Mean range	85.7 (27.7-125.6)	91.5 (29.4-118.3)	NS
LCI 2.5 mean (SD)	10.25 (1.95)	10.06 (1.81)	NS
Sweat test mmol/l mean (range)	98.96 (81-118)	84.57 (44-123)	0.029

In vivo biomarkers do not differentiate clinical response Responder (FEV1 absolute variation by at least 10%)

	Responder (n=7)	Non-responder (n=27)	p
Sweat test variation			
Mean (SD)	-14.5 (13.43)	-14,33 (20.29)	NS
Betadrenergic response	0	0	
NPD variation (mean (SD))			
Delta Amiloride	5.9 (12.44)	-6,8 (16.02)	NS
Delta O-Chlorure	-2.56 (2.45)	-1.36 (7.98)	NS
Delta Isoprotérenol	-2,06 (5.68)	-0,549 (8.91)	NS
Delta O-Chlorure/Isoprotérénol	-2.98 (3.1)	-2.47 (9.87)	NS

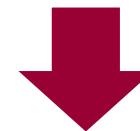
Preclinical biomarkers



Develop a patient – cell specific biomarker

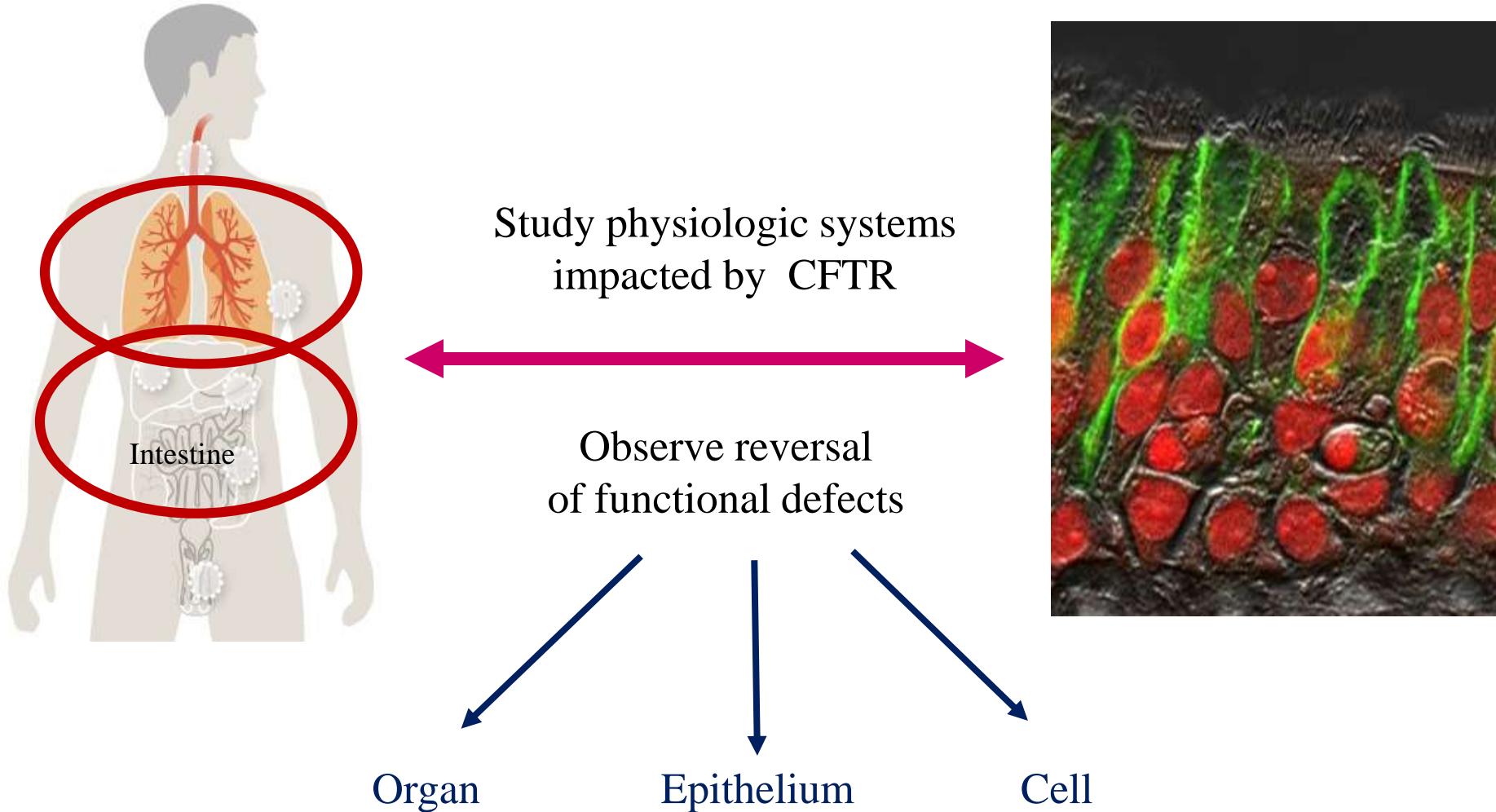


that can reliably predict respiratory improvement upon therapies in patients



to preselect responder patients predicted to benefit from a given treatment

Preclinical biomarkers related to the presence/function of CFTR



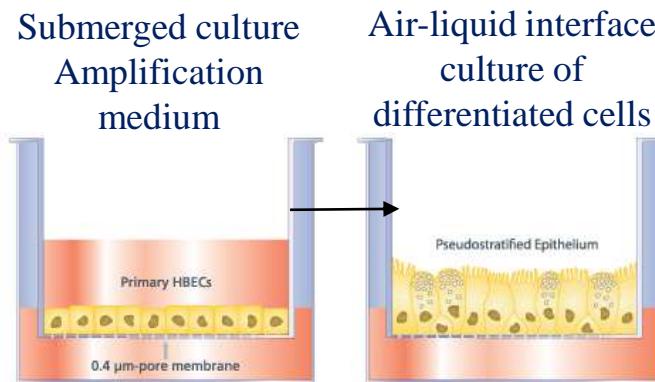
(1) Evaluation of CF patient's derived primary nasal epithelial cell cultures aiming to predict clinical efficacy of CFTR modulators

Primary human nasal epithelial cells

- isolated by nasal brushing of medial wall and the inferior turbinate of each nostril

Primary human bronchial epithelial cells

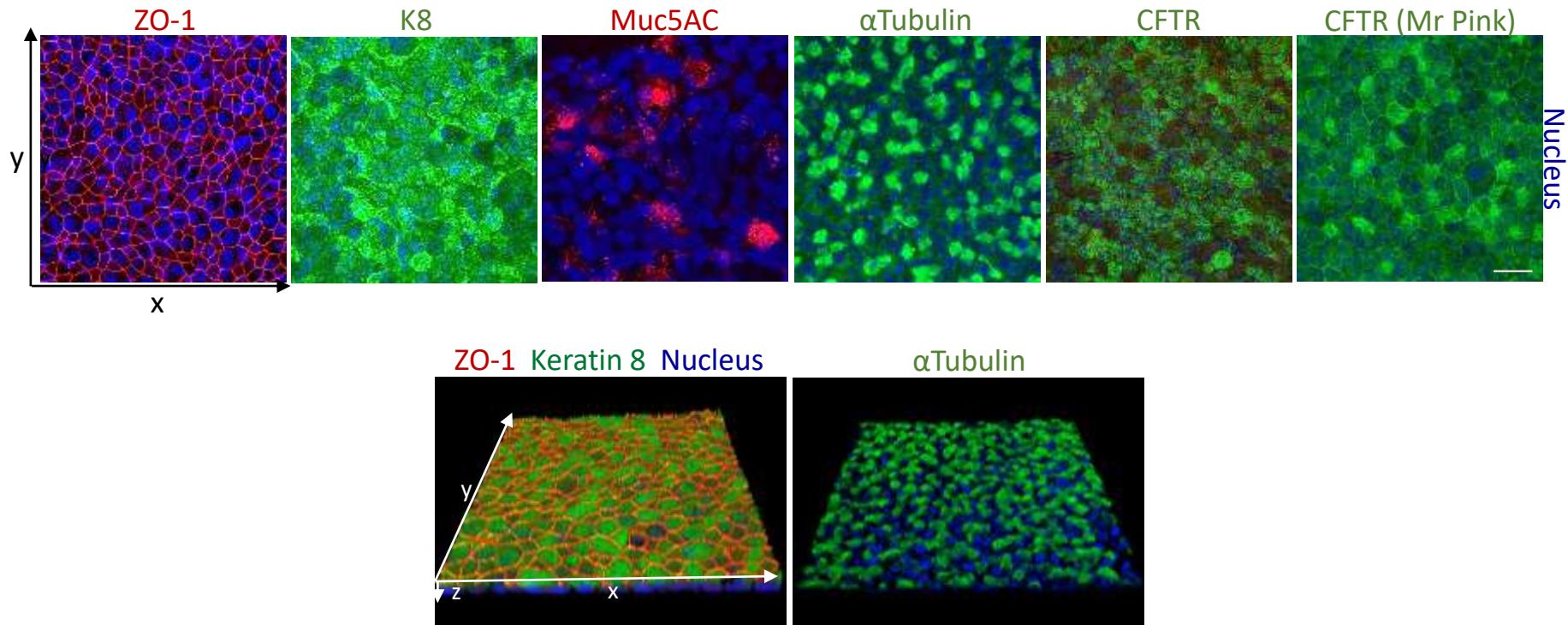
- isolated from bronchial explants after lung transplantation or lobectomy



Human nasal epithelial (HNE) cell cultures

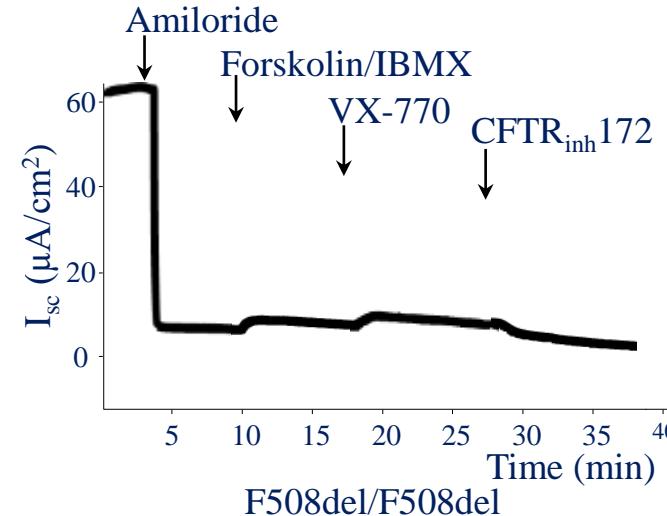
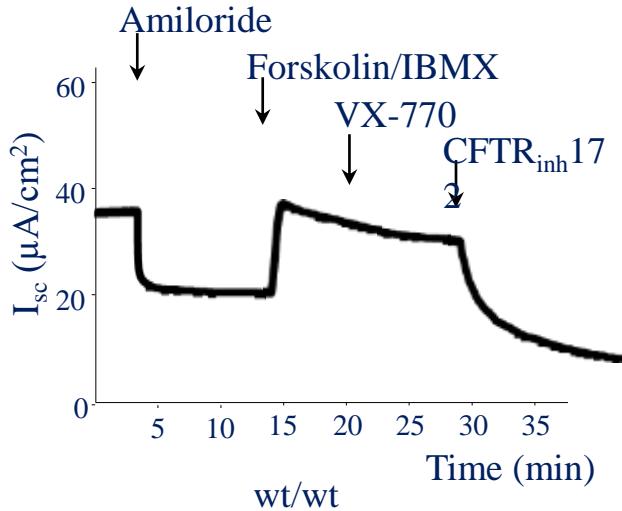
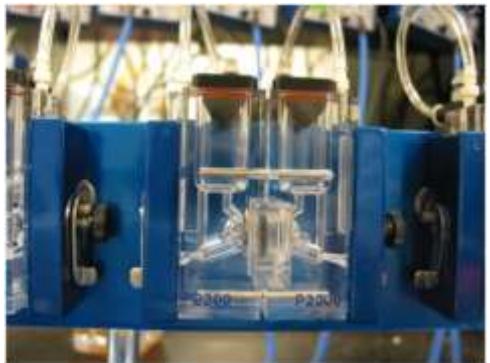
Human bronchial epithelial (HBE) cell cultures

Cell culture differentiation

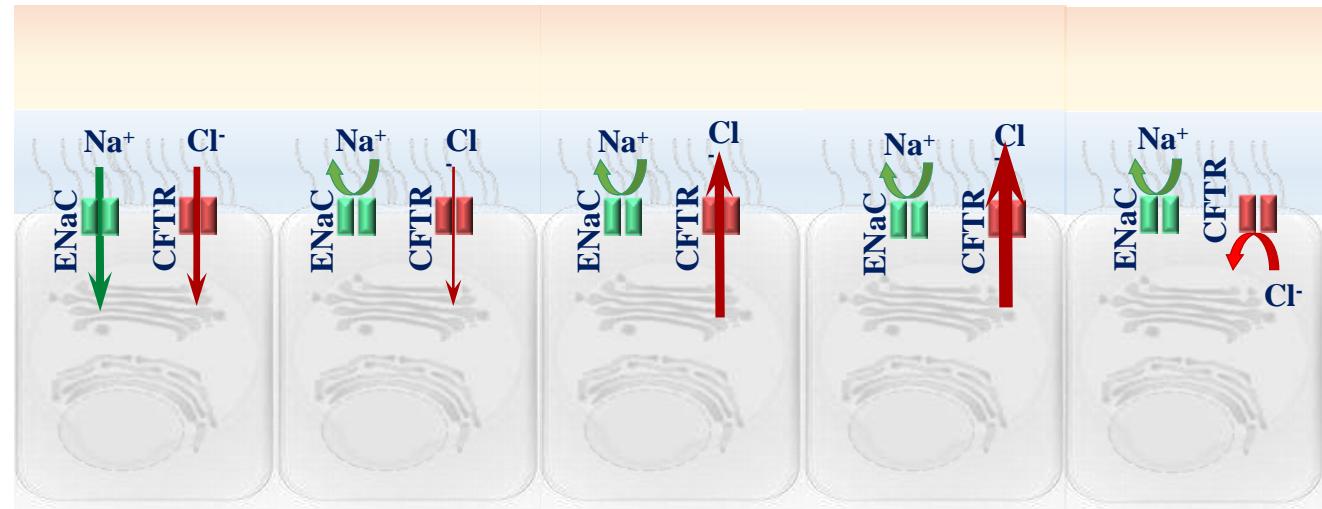


- Compare the primary human nasal epithelial (HNE) cell cultures with human bronchial epithelial (HBE) cell cultures
 - CFTR function : Short-circuit-current (Isc, index of CFTR activity)
 - CFTR expression and localization : Cytoimmunochemistry
- Establish correlation between ion transport in primary HNE cell cultures and nasal epithelium in patients
 - Isc vs. nasal potential difference (CFTR activity)
- Test correction of mutated CFTR by VX-809 in HNE and HBE cell cultures
 - Towards personalized therapy
- Correlate CFTR activity in VX-809-treated HNE cell cultures with clinical status of CF patients treated with the same corrector
 - Isc vs. FEV1

Isc – short circuit current - measurement

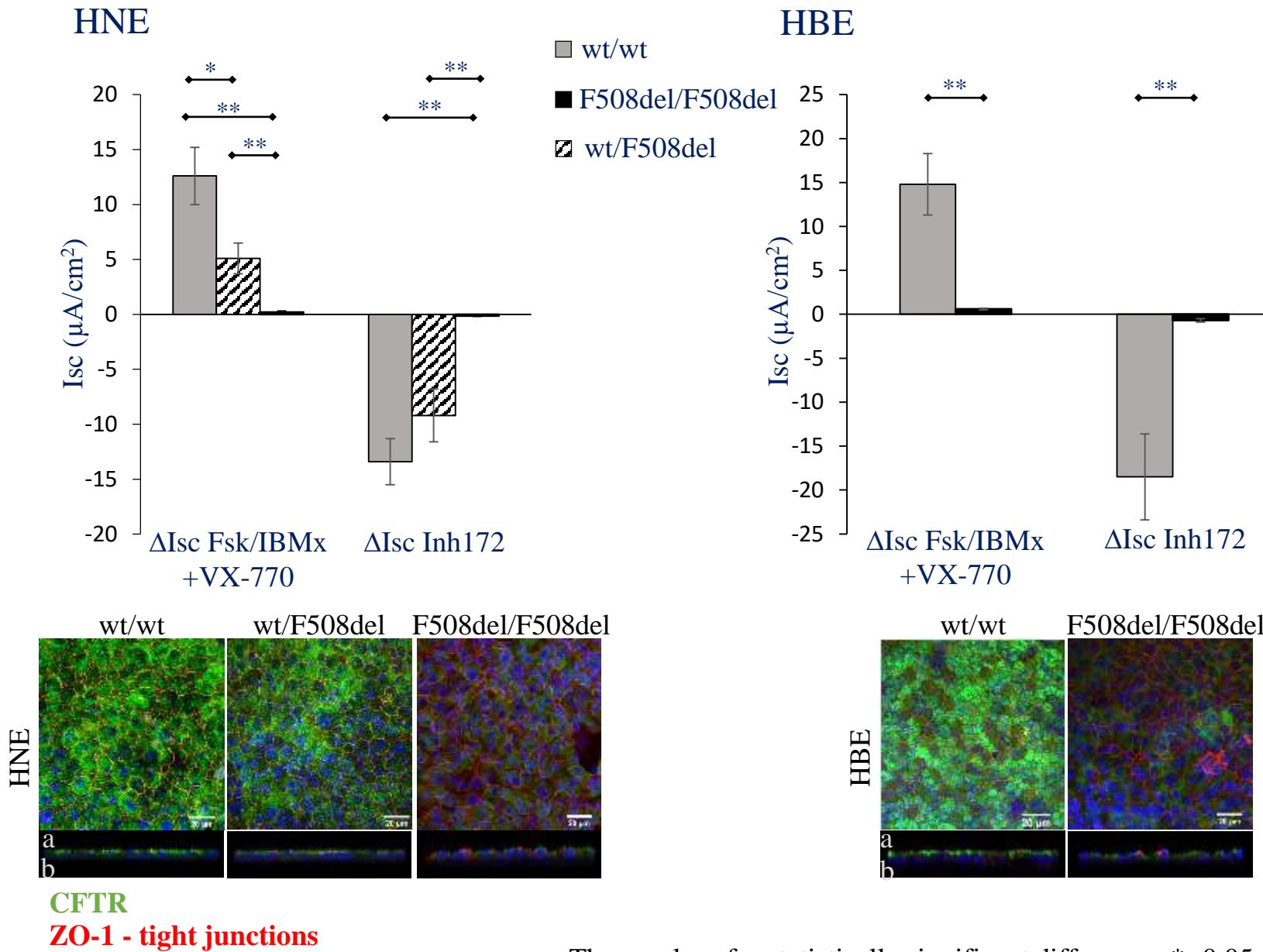


	Baseline	Amiloride 100 μM	Forskolin/IBMx 10/100 μM	VX-770 ivacaftor 10 μM	CFTR Inh172 5 μM
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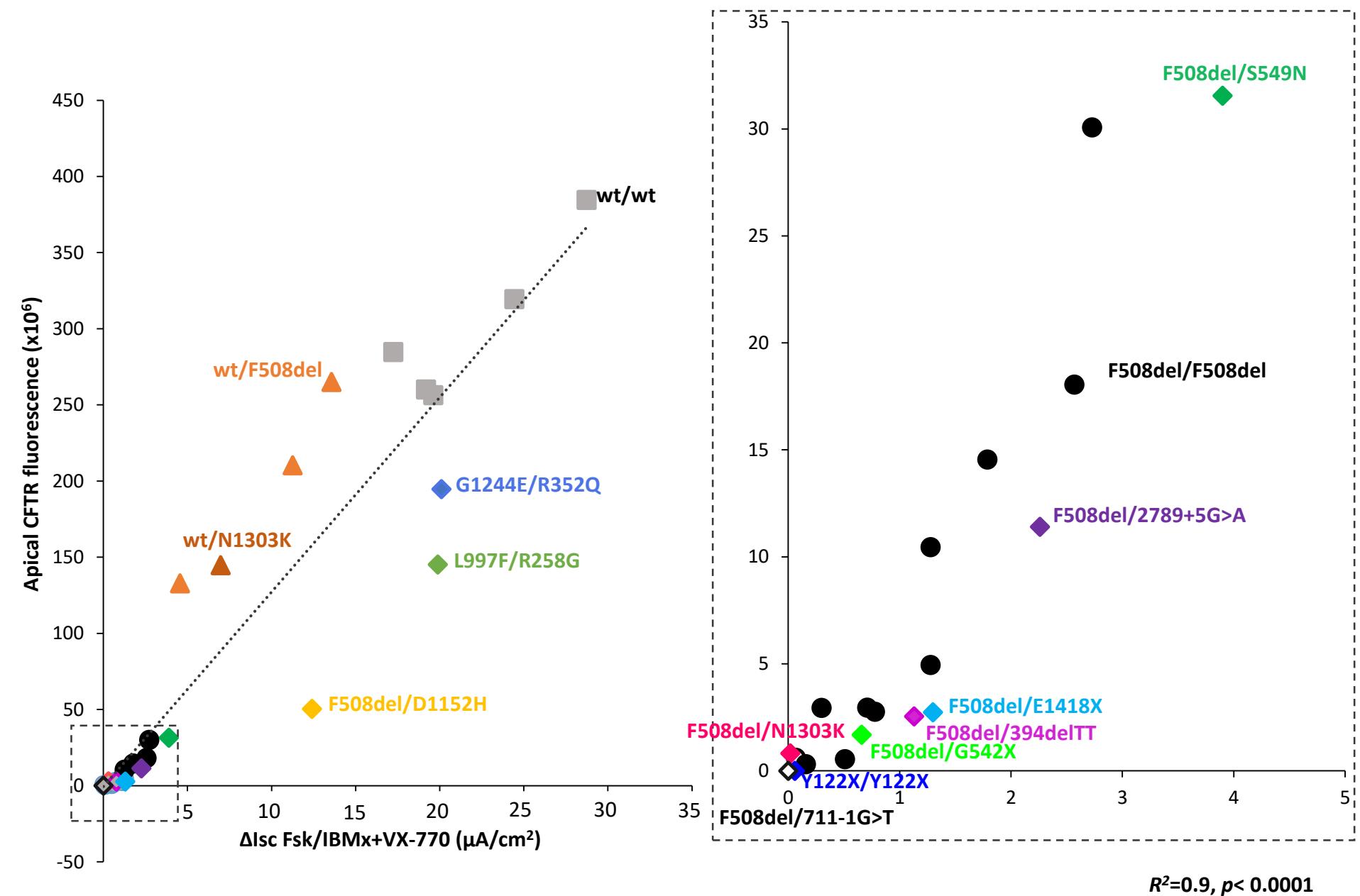
Measures the current that is continuously adjusted and injected across the epithelium in response to ion transport to keep trans-epithelial voltage at 0 mV

CFTR function and expression in HNE vs. HBE cell cultures



The p-values for statistically significant differences * <0.05 and ** <0.01

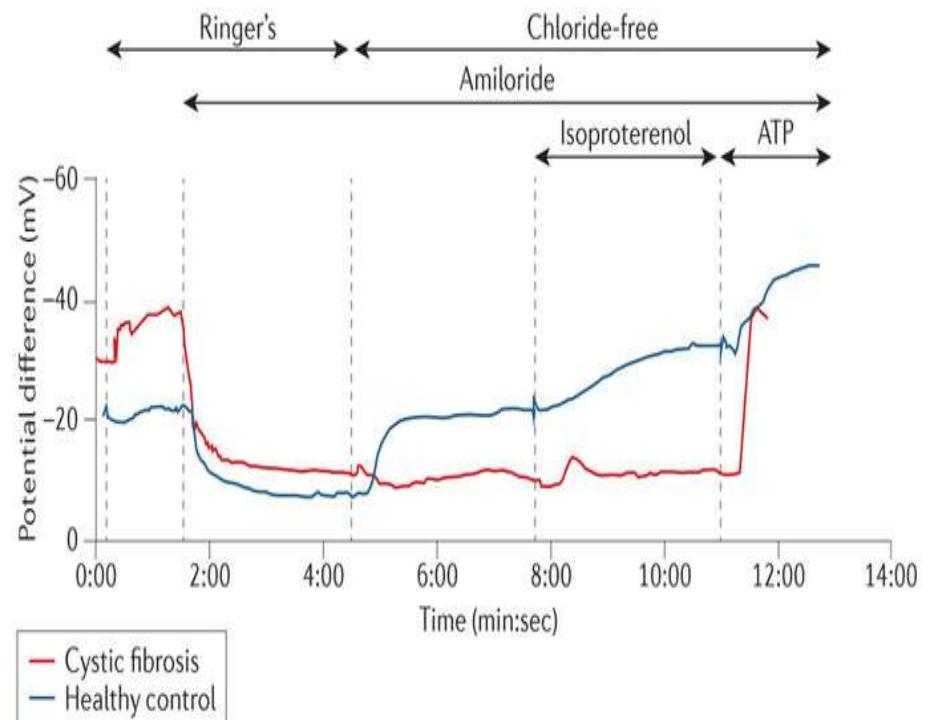
Correlation of CFTR activity and apical expression in cell cultures



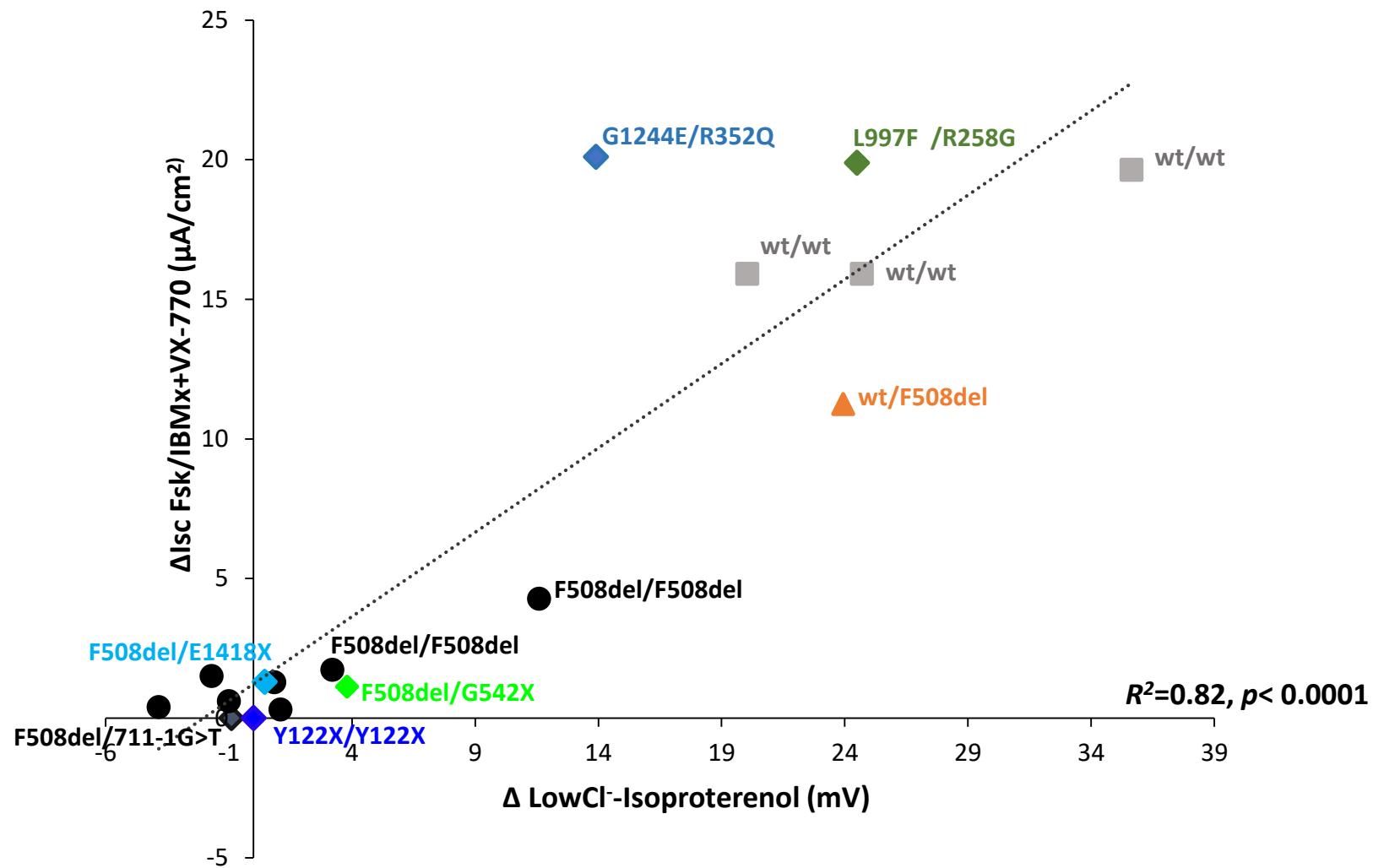
- Compare the primary human nasal epithelial (HNE) cell cultures with bronchial (HBE) cell cultures
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NPD – nasal potential difference - measurement

Measures the potential difference (voltage) across the nasal epithelium, which results from trans-epithelial ion transport



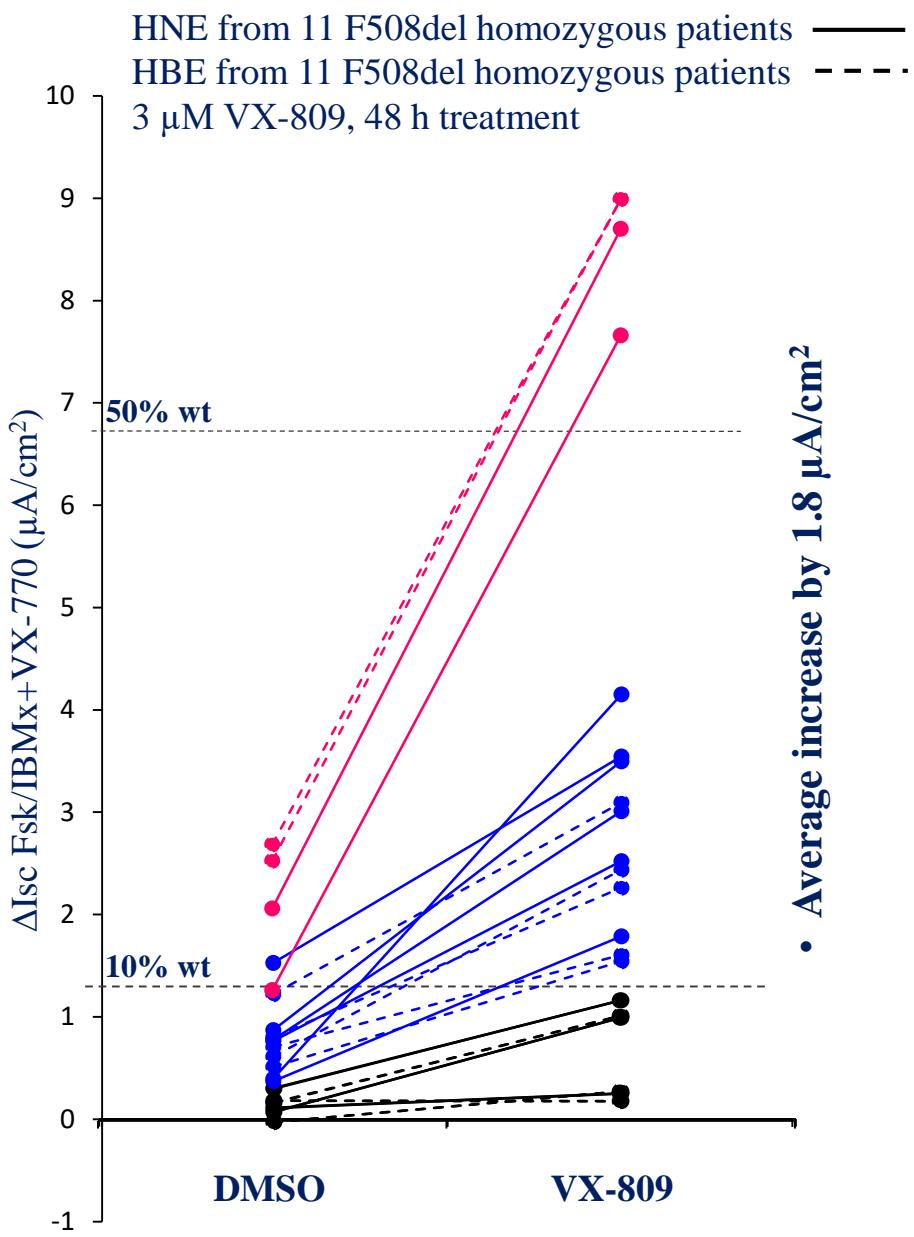
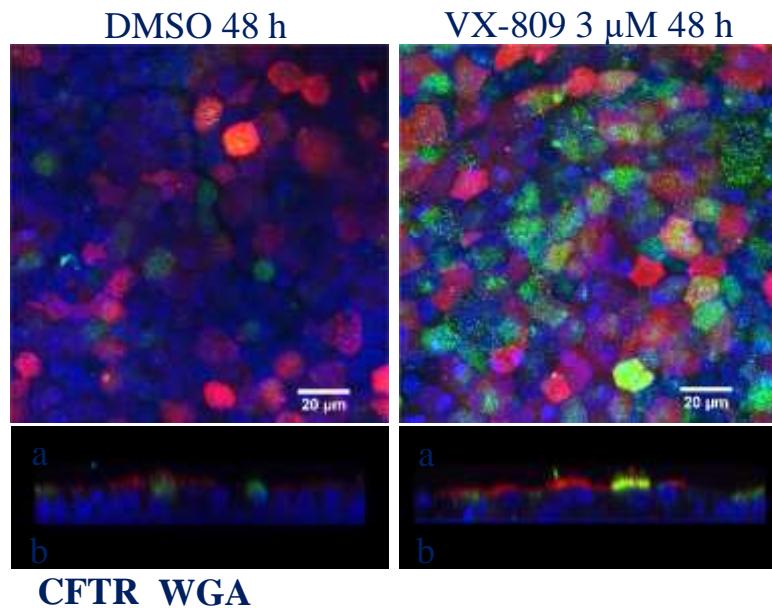
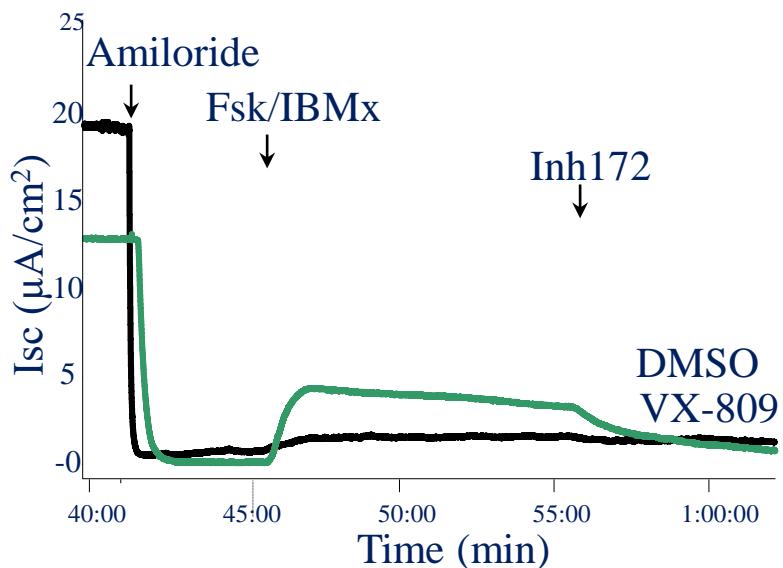
CFTR activity in nasal cells measured *in vitro* and *in vivo*



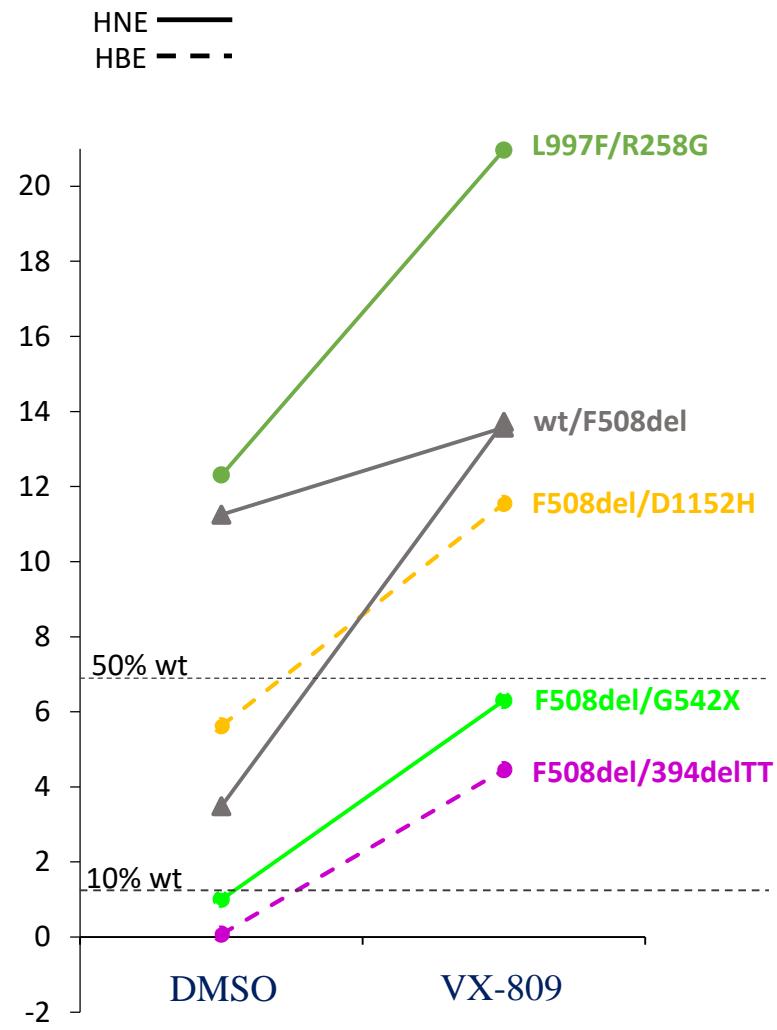
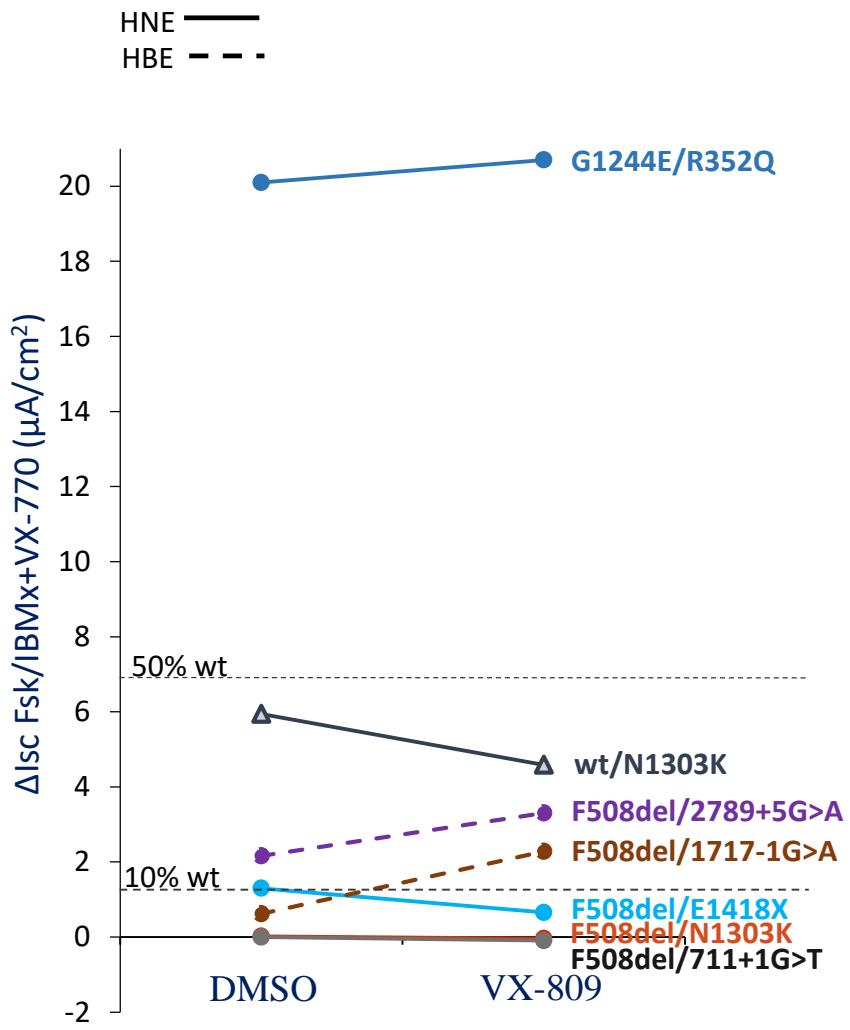
Primary HNE cells reproduce the *in vivo* detected differences in the CFTR activity

- Compare the primary human nasal epithelial (HNE) cell cultures with bronchial (HBE) cell cultures
 - Short-circuit-current (I_{sc} , index of CFTR activity)
 - Cytoimmunochemistry (CFTR expression and localization)
- Establish correlation between ion transport in primary HNE cell cultures and nasal epithelium in patients
 - I_{sc} vs. nasal potential difference (CFTR activity)
- Test correction of mutated CFTR by VX-809 in HNE and HBE cell cultures
 - Towards personalized therapy
- Correlate CFTR activity in VX-809-treated HNE cell cultures with clinical status of CF patients treated with the same corrector
 - I_{sc} vs. FEV1

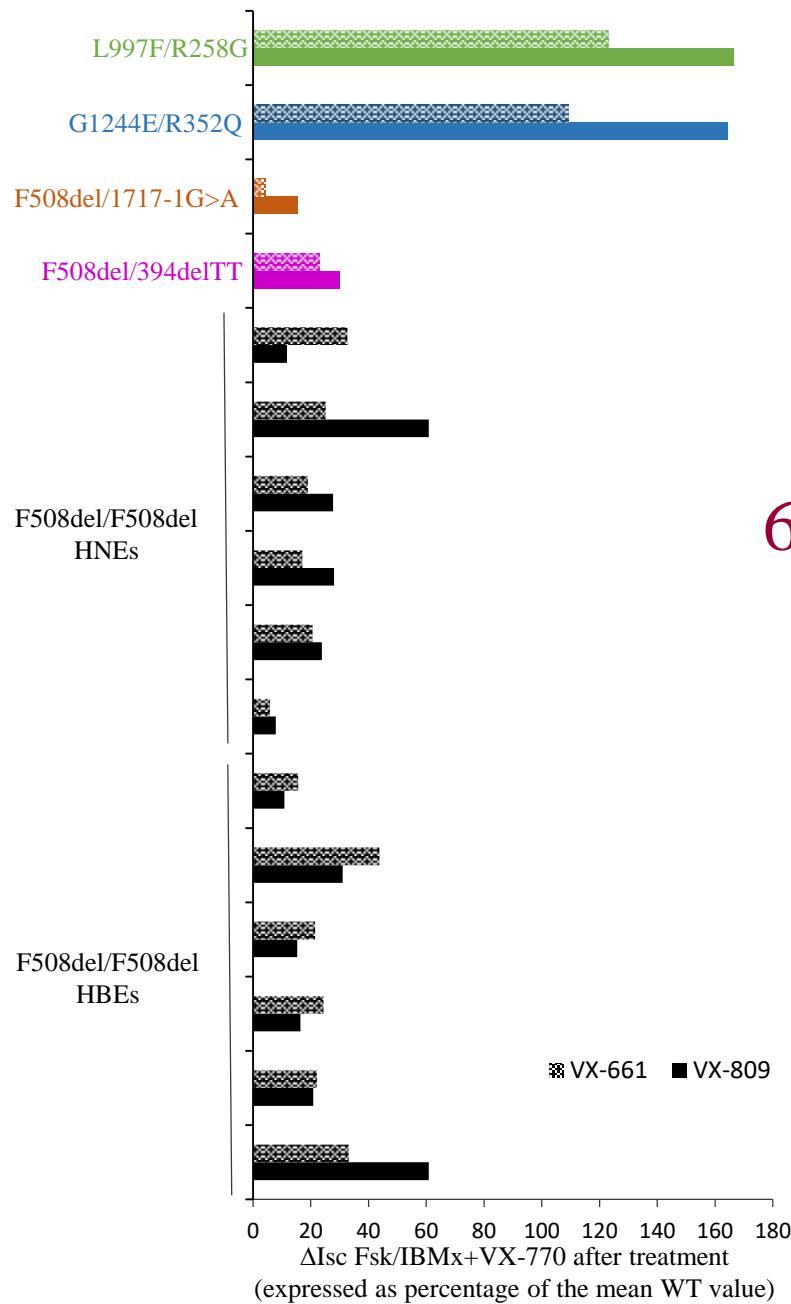
VX-809 stimulated correction of CFTR-dependent Cl⁻ secretion and CFTR apical expression (patient specific)



Correction of CFTR-dependent Cl⁻ secretion in composite heterozygote cells (mutation specific)

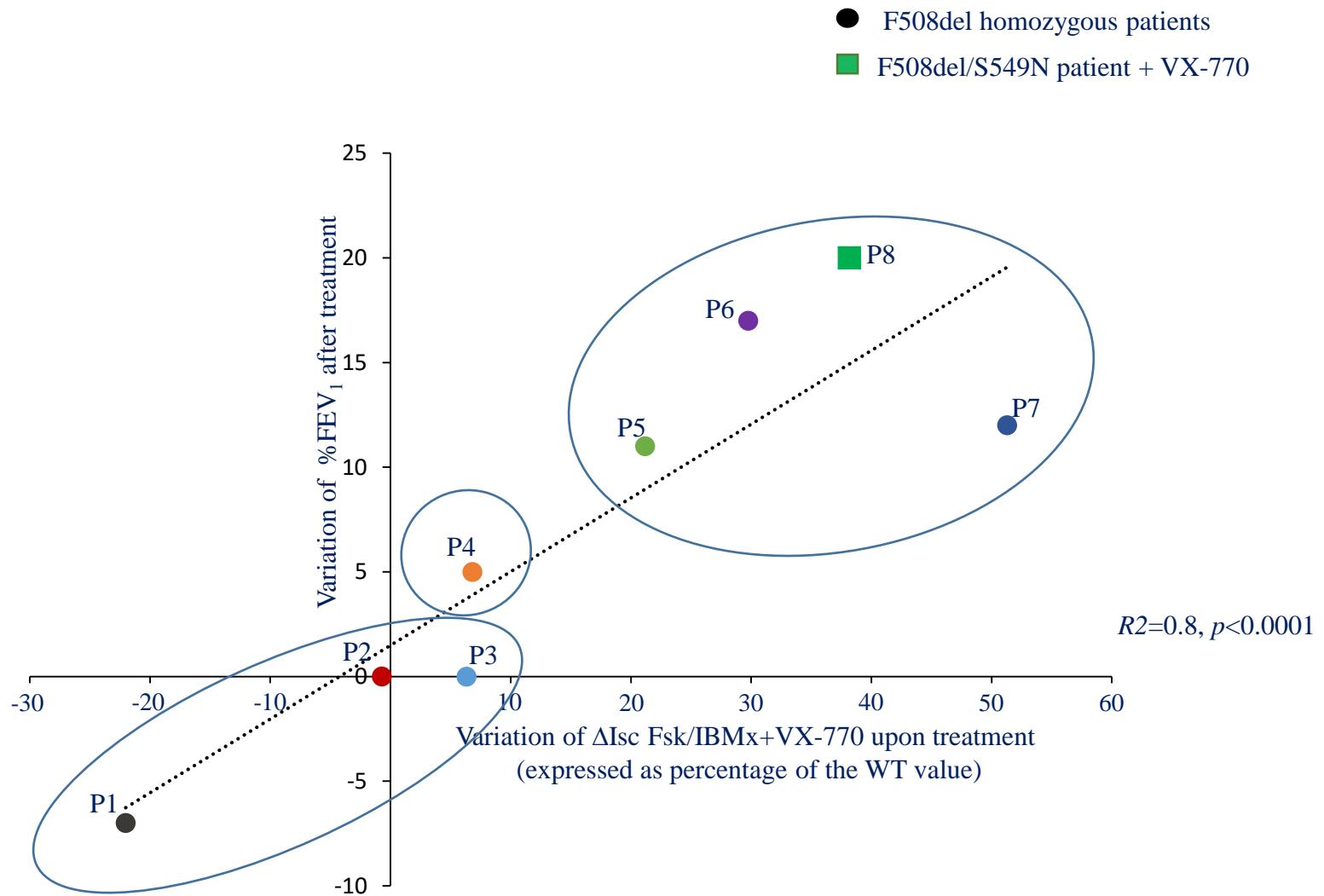


661 versus 809 correction (drug specific)



- Compare the primary human nasal epithelial (HNE) cell cultures with bronchial (HBE) cell cultures
 - Short-circuit-current (I_{sc} , index of CFTR activity)
 - Cytoimmunochemistry (CFTR expression and localization)
- Establish correlation between ion transport in primary HNE cell cultures and nasal epithelium in patients
 - I_{sc} vs. nasal potential difference (CFTR activity)
- Test correction of mutated CFTR by VX-809 in HNE and HBE cell cultures
 - Towards personalized therapy
- Correlate CFTR activity in Orkambi-treated HNE cell cultures with clinical status of CF patients treated with the same molecule
 - I_{sc} vs. FEV1

Correlation of CFTR activity in VX-809-treated HNE cultures with clinical status of CF patients – Isc vs. FEV1



Correction of CFTR function in HNE cell cultures predicts respiratory status in CF patients

(2) Evaluation of CF patient's fresh cells

PFA_L12B4_Frozen cells from nasal brushing

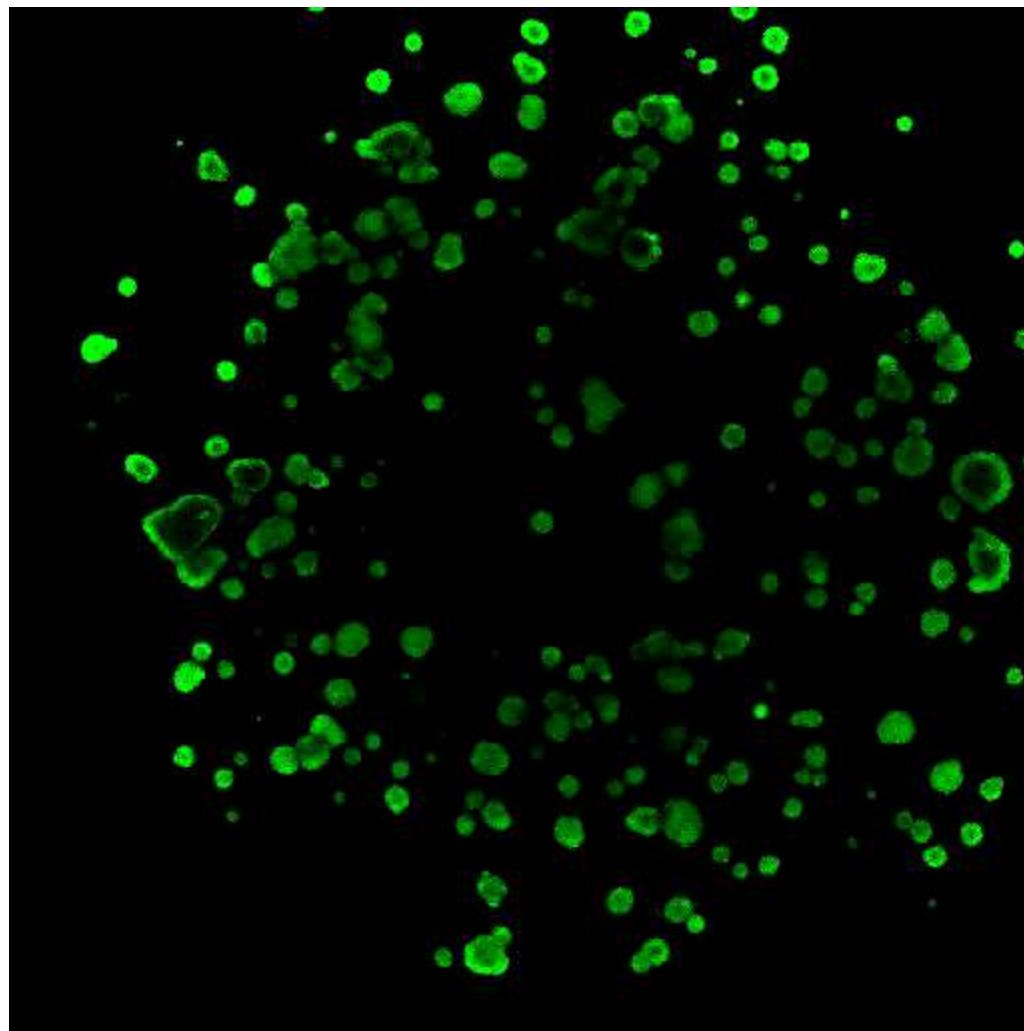


Red: CFTR
Green: β -tubulin
Blue: DAPI

(3) Evaluation of CF patient's derived primary
intestinal cell cultures
organoids

Organoids from intestinal biopsy





Conclusions

- Primary HNE cell cultures recapitulate the properties of HBE cultures and constitute physiologically relevant model to evaluate correctors efficacy
- CFTR activity in Primary HNE cell is correlated to in vivo activity
- Primary HNE cell cultures allow to differentiate different levels of CFTR activity
- More data about
 - intrapatient variability/reproducibility
 - the clinical relevant threshold

Perspectives

- Large study to test whether correction of CFTR function and expression in HNE cell cultures reliably predict respiratory status in CF patients, and the clinical relevant threshold
- Comparison of in vitro drug responses in HNE cultures and rectal organoids
- CFTR gene sequencing to study
 - Additional variant(s) in the CFTR gene in *cis* or *trans* position to the primary mutation
 - Exon skipping (9T)
 - Silent mutations – which do not change final amino acids, but might impact on CFTR function
 - Epigenetic changes (DNA methylation, histone modification)
- Proteomics/transcriptomics to study additional factors that modulate disease severity and response to treatment (modifiers genes)

Basic research

Translational research

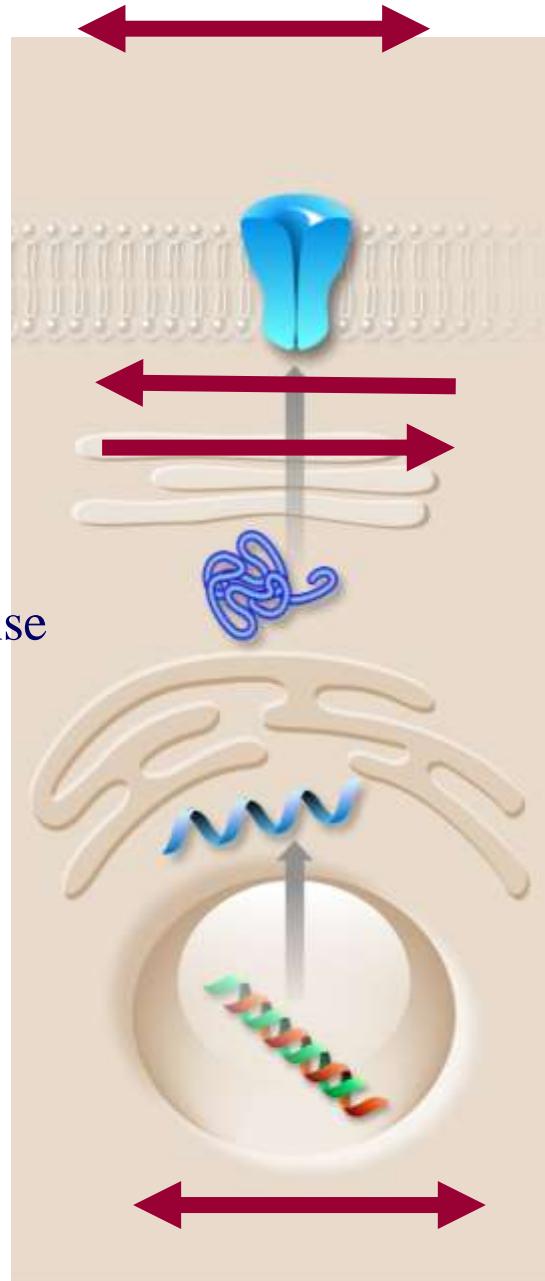
Molecular basis for response
Known CFTR correctors



Modifying factors of the response



Precision therapy



Patient derived tissue



“ Theratype”



Personalized medicine

Institut Necker Enfants Malades

Aleksander Edelman

Iwona Pranke

Aurélie Hatton

Juliette Simonin

Alexandre Hinzpeter

Myriam Mesbahi

Alix Vignole-Vidoni

Lucile Vignaud

Valerie Urbach

Monika Hollenhorst

Charles-Henry Cottart

Danielle Tondelier

Nathalie Servel

Hôpital Necker-Enfants Malades

Sebastien Pierrot

Naziha Dunlop



Institut Cochin

Emanuelle Girodon

Institut Imagine

Jean Philippe-Jais

Hôpital Foch

Pierre Bonnette

Dominique Grenet

Hôpital de la Timonne

Nathalie Stremler-Le Bel

Julie Mazenq

Ania Garcin

Microscopy platform

Nicolas Goudin

Meriem Garfa-Traoré



**Patients, their families
and volunteers**

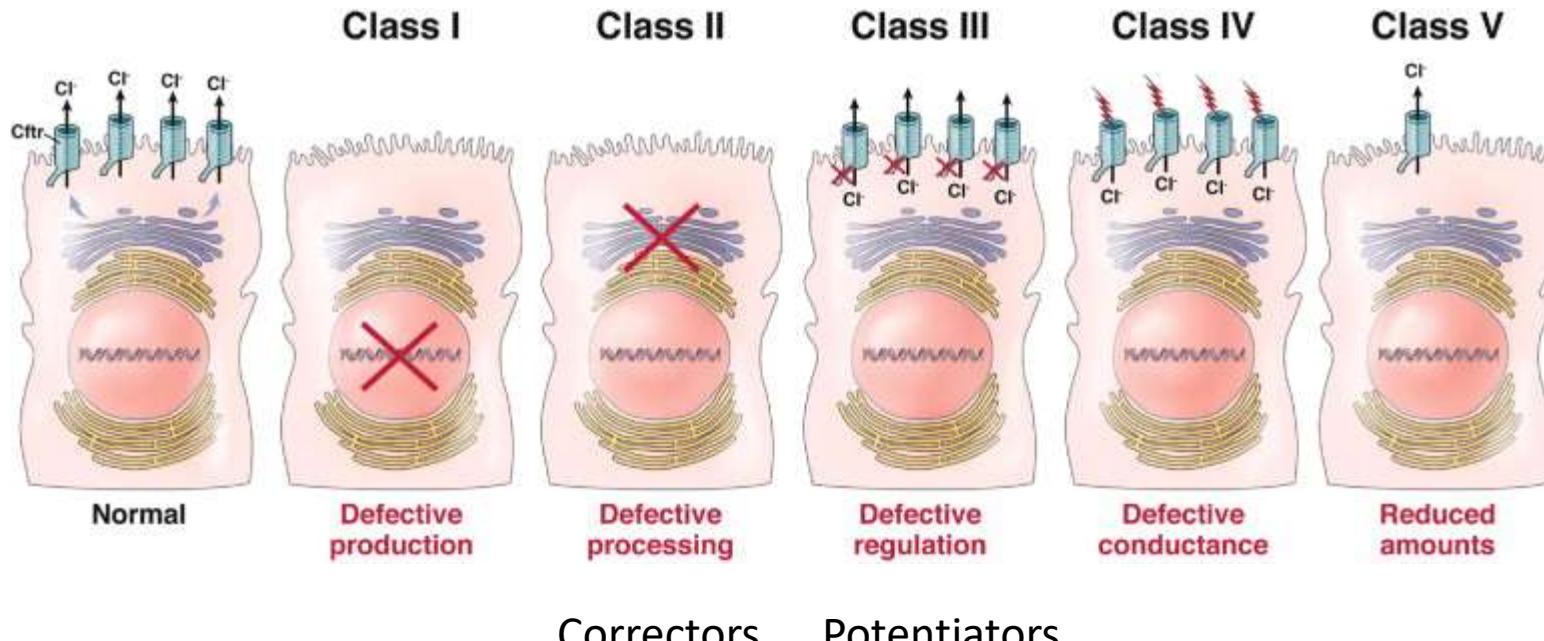


Hôpital Européen Georges Pompidou

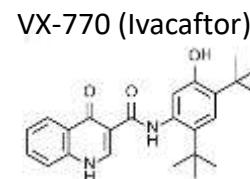
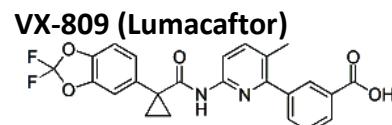


Françoise Le Pimpec-Barthes

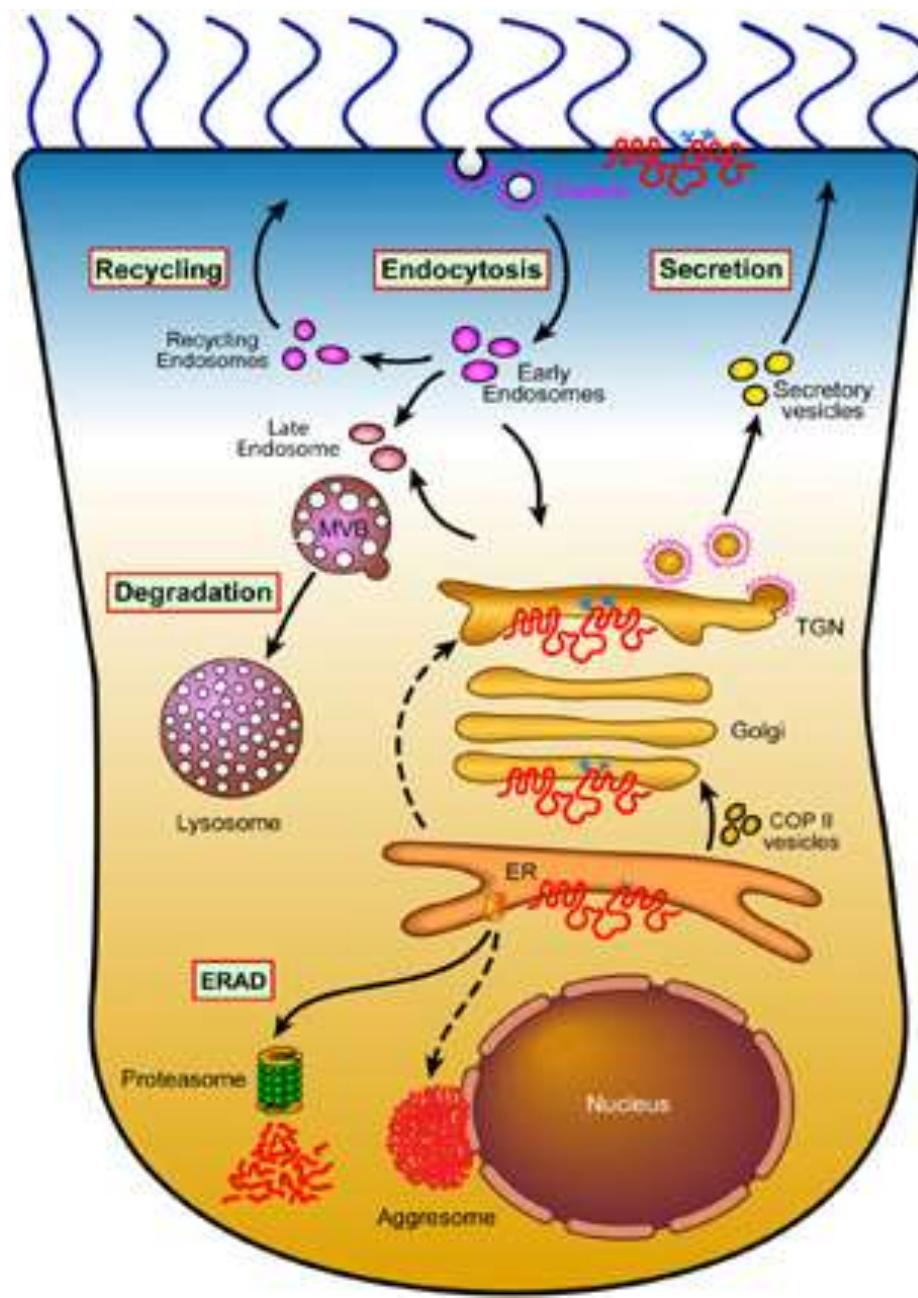
Mutations that cause CF



F508del mutation – processing and gating defect

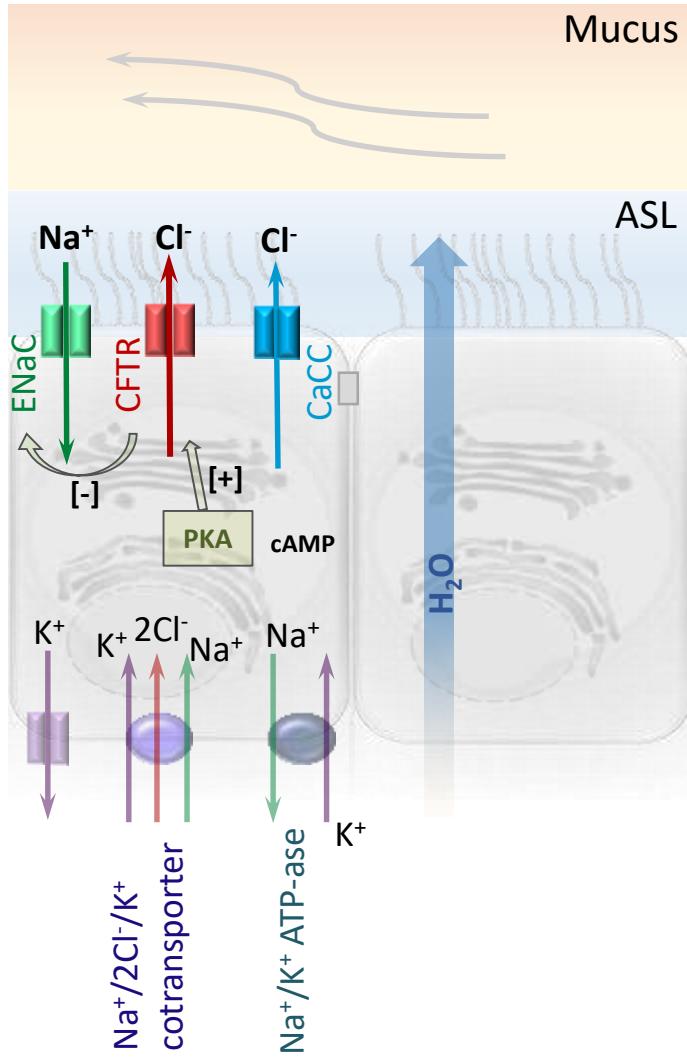


CFTR protein biosynthesis

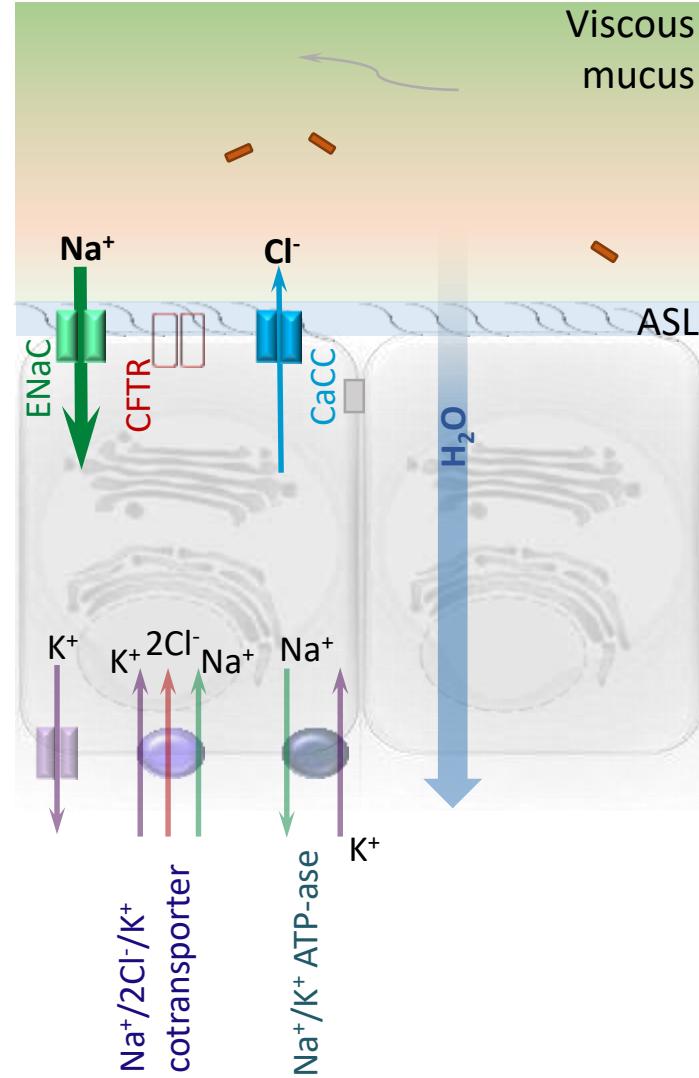


Defect of ion transport through respiratory epithelium in CF

Normal airway



CF airway



Mutations of CFTR and therapeutic strategies for basic defects

Function defects

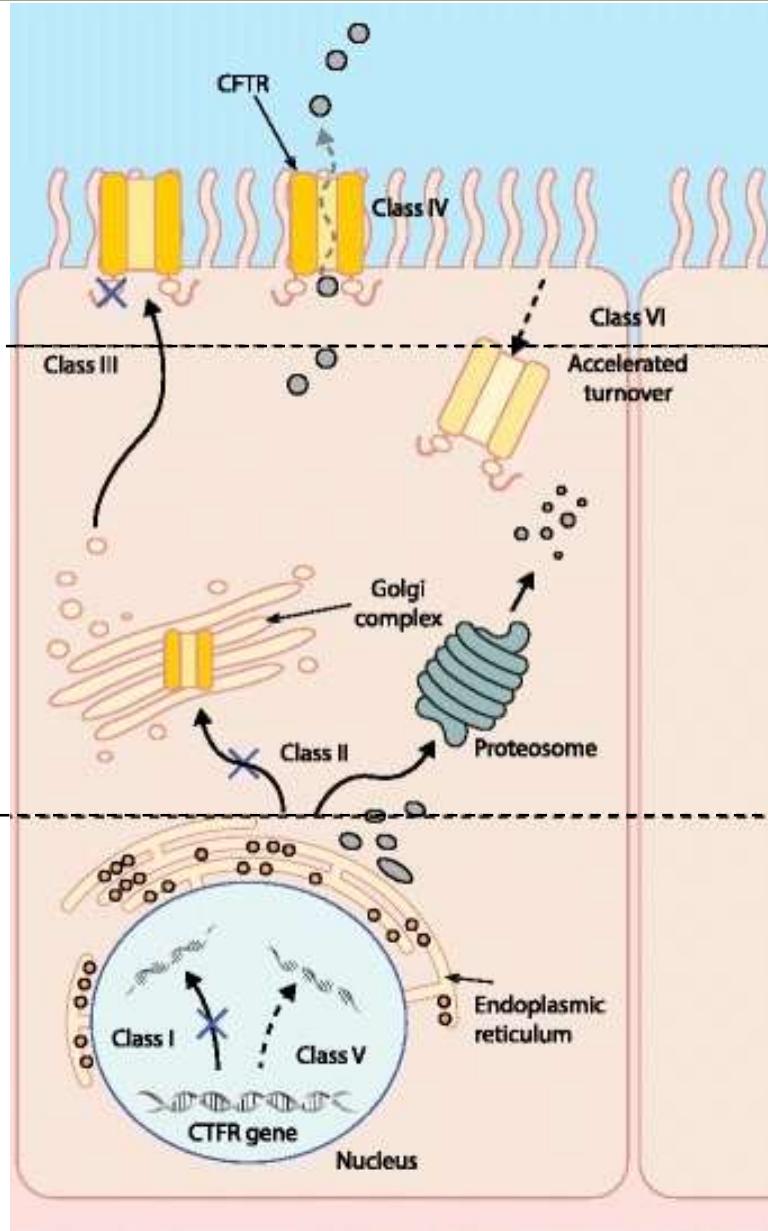
Gating
Conductance
G551D
F508del

Trafficking defects

Decreased stability
Increased degradation

F508del

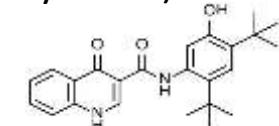
Synthesis defects



Potentiators –

potentiation of the plasma membrane CFTR

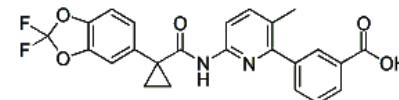
VX-770 (ivacaftor, Kalydeco®)



Correctors –

correction of CFTR trafficking defects

VX-809 (lumacaftor)

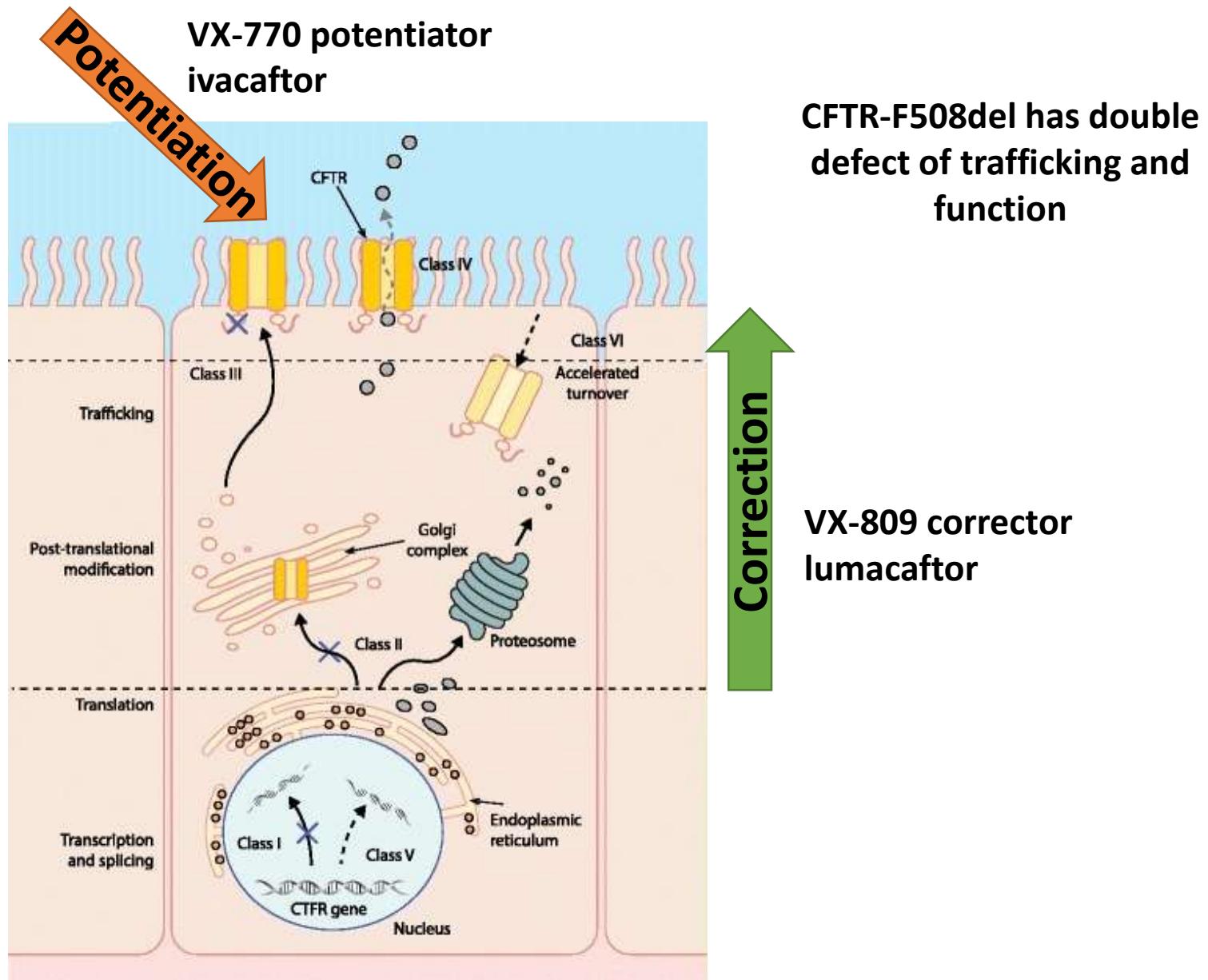


c407 – disrupts interactions with K8

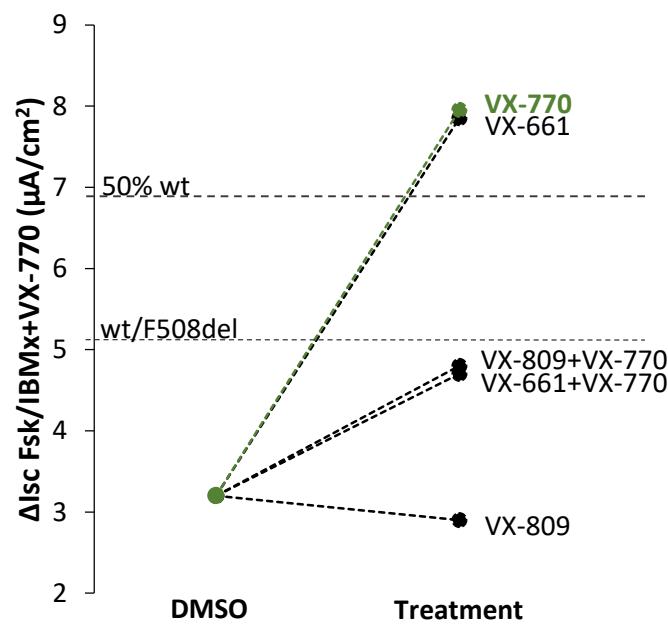
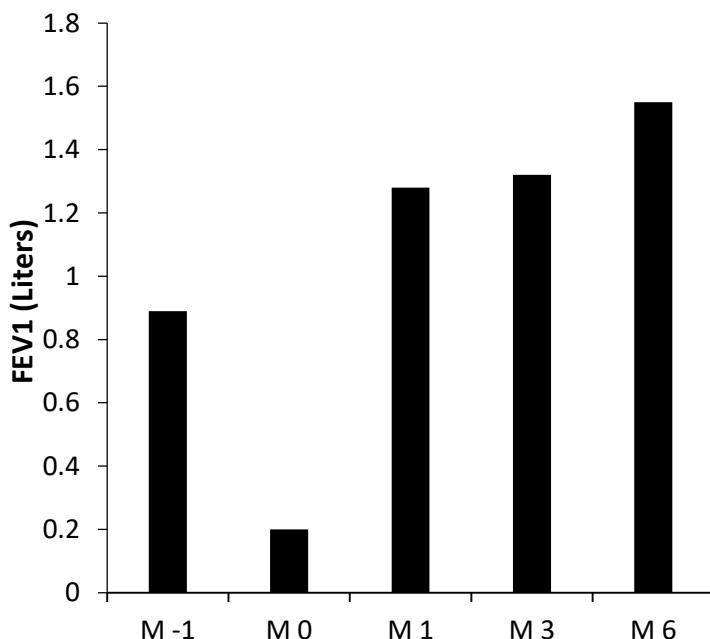
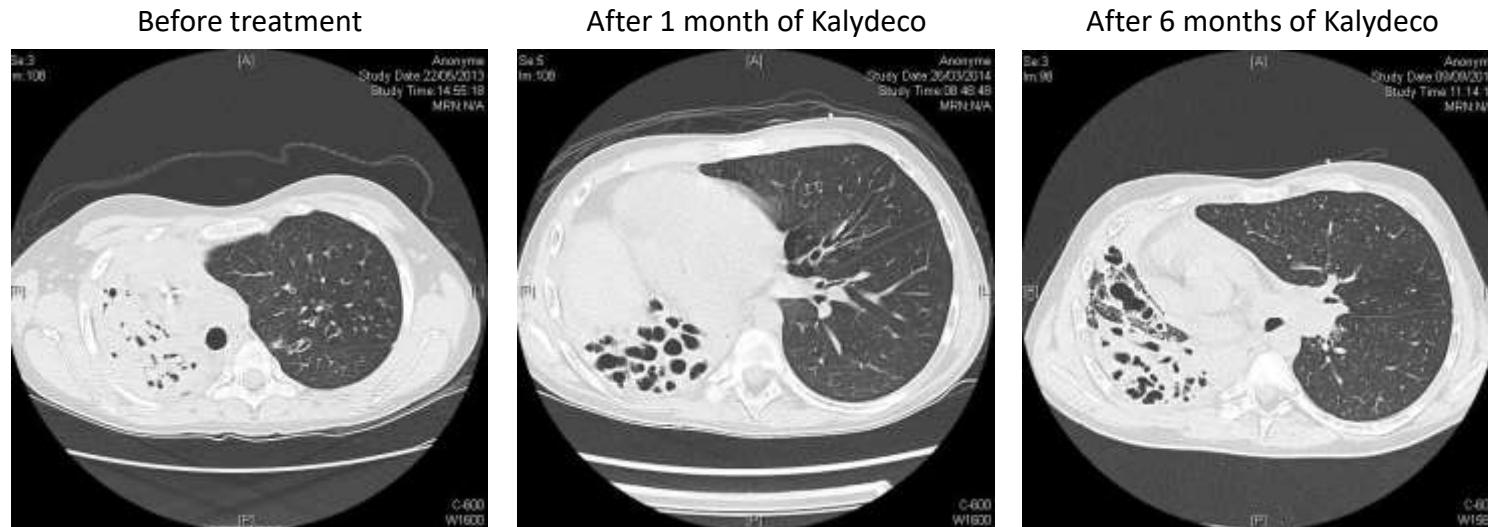
Transcription and translation correctors

Combination of correction and potentiation for CFTR-F508del

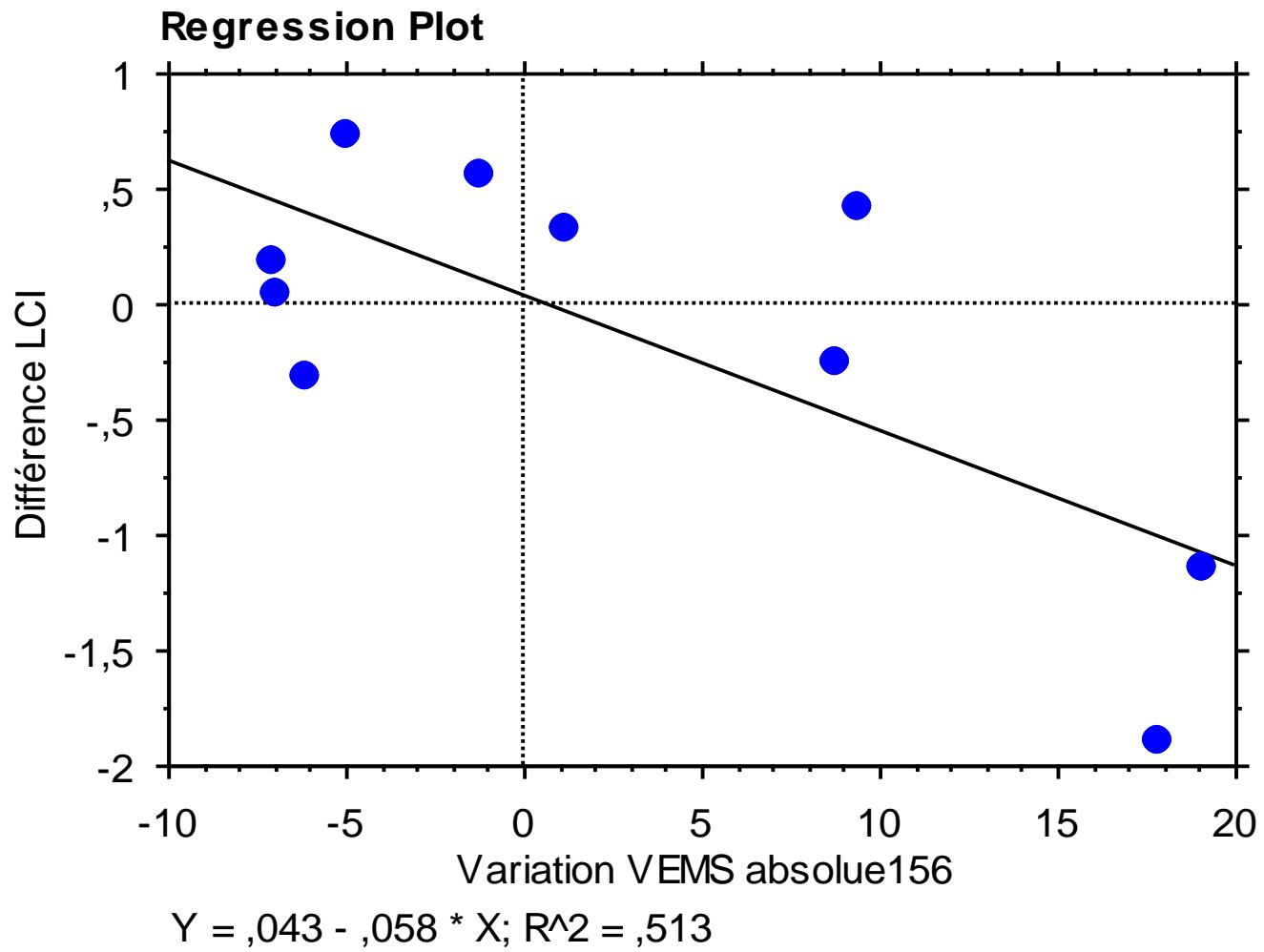
VX-809 corrector + VX-770 potentiator = Orkambi®



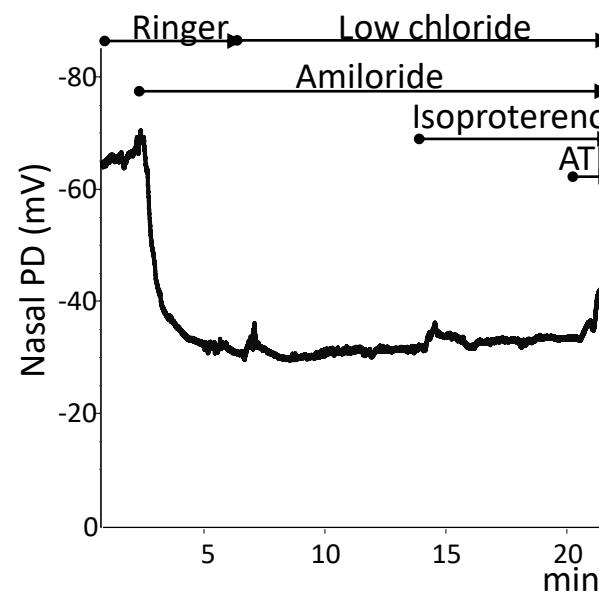
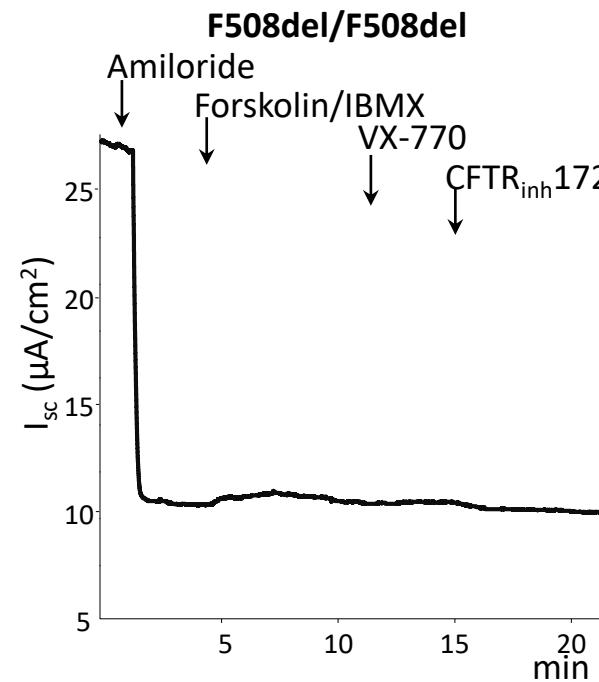
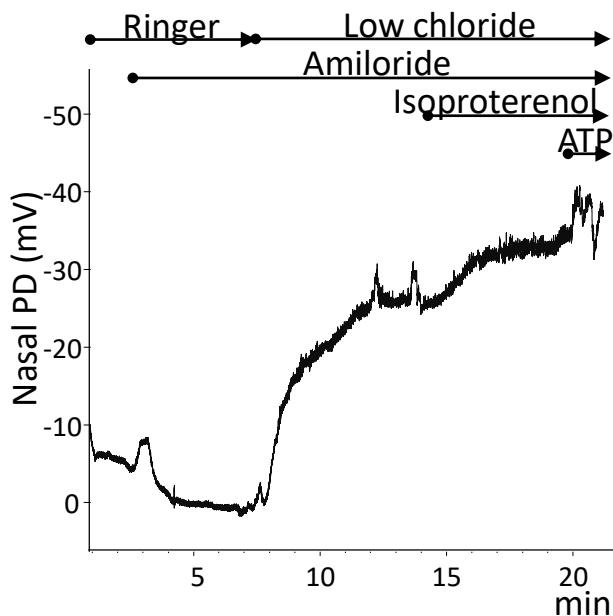
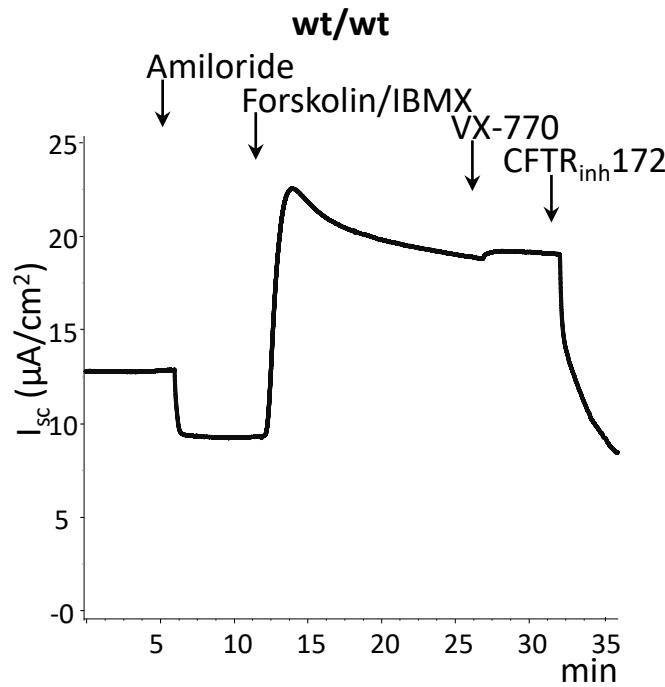
The F508del/S549N patient and potential effects of combination therapy



	Responder (n=7)	Non-responder (n=27)	p
Sweat test			
Mean (SD)	-14.5 (13.43)	-14,33 (20.29)	0.99
LCI 2.5	n=2	n=7	0.25
Mean (SD)	-0.36 (1.1)	0.18 (0.39)	
BMI Z-score	n=7	n=17	0.22
Mean (SD)	0.32 (0.34)	0.12 (0.35)	
NPD n, mean (SD)			
Delta Amiloride	n=3 5.9 (12.44)	n = 11 -6,8 (16.02)	0.23
Delta O chlorure	n=3 -2.56 (2.45)	n = 12 -1.36 (7.98)	0.80
Delta Isoprotérenol	N=3 -2,06 (5.68)	N=11 -0,549 (8.91)	0.78
Delta O chlorureIsoprotérénol	n=3 -2.98 (3.1)	n=11 -2.47 (9.87)	0.93



CFTR activity in nasal cells measured *in vitro* and *in vivo*



Changes of CFTR functional measurements and clinical improvements in cystic fibrosis patients with non p.Gly551Asp gating mutations treated with ivacaftor



Myriam Mesbahi ^{a,1}, Michal Shtenberg ^{b,c,1}, Michael Wilschanski ^d, Aurelie Hatton ^a, Thao Nguyen-Khoa ^a, Hannah Friedman ^d, Michael Cohen ^d, Virginie Escabasse ^e, Muriel Le Bourgeois ^a, Vicenzina Lucidi ^f, Isabelle Sermet-Gaudelus ^{f,*}, Laurence Bassinet ^{g,2}, Galit Livnat ^{b,c,2}

