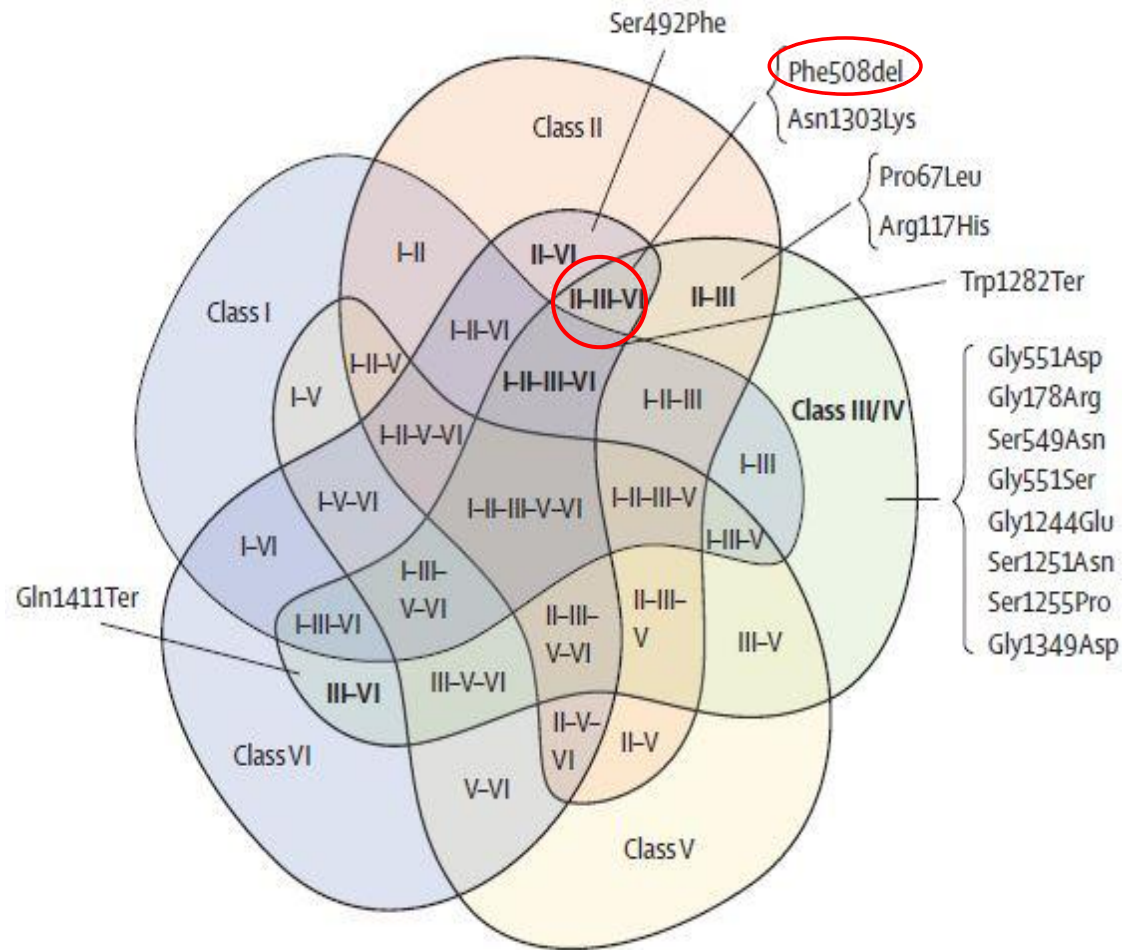


Examples of theratyping using rectal organoids of rare CFTR variants: a clinical, regulatory and organizational challenge

Claudio Sorio, University of Verona

Limitations of CFTR classification system and the concept of theratyping



Veit G et al 2016

Amaral MD. et al 2021

F508del/F508del + F508del/other (~74%)



Corrector
(Luma/Tezacaftor/Tezacaftor
+ Elexacaftor) + **Potentiator**
(Ivacaftor)

G542D +9 Gating + 23 RD*
mutations (~8%)



Potentiator
(Ivacaftor)

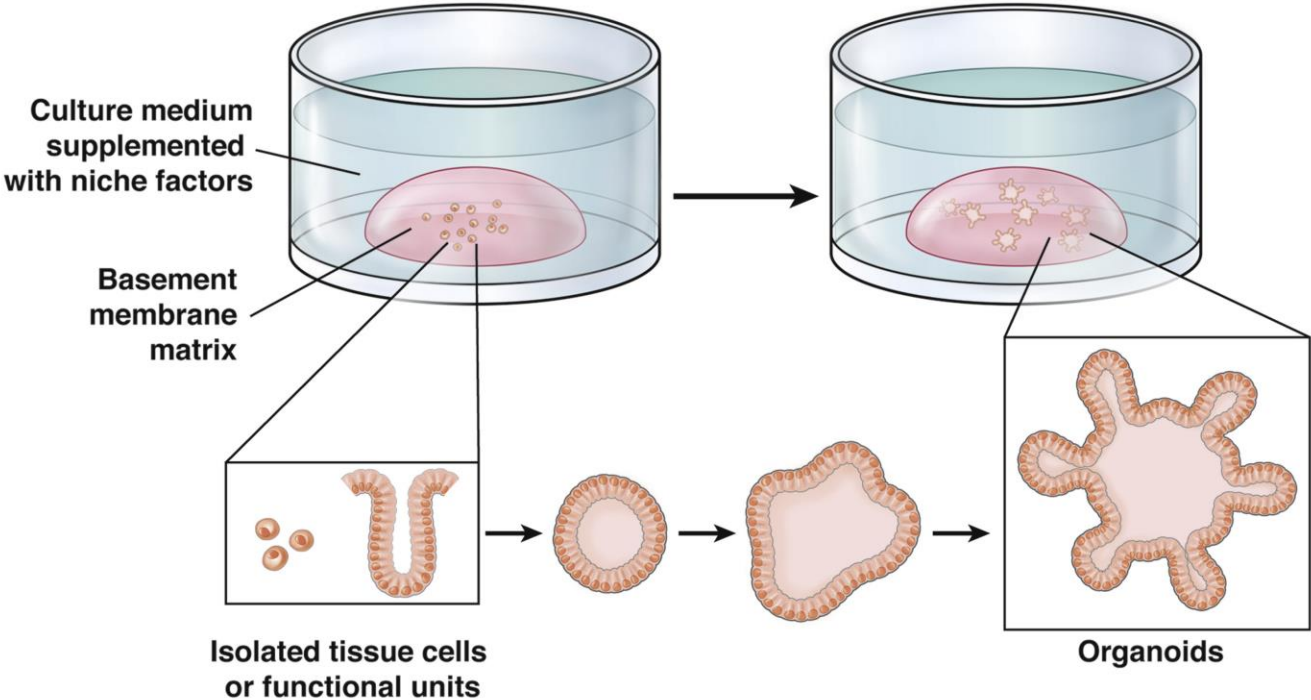
Other mutations (~18%**)



No drug

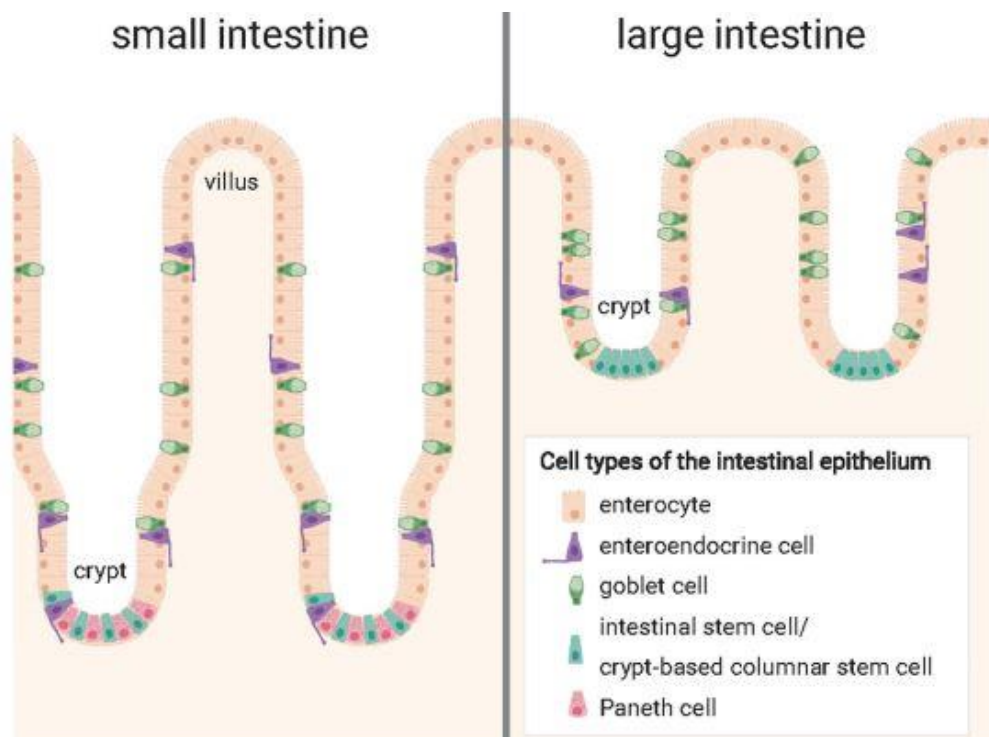
*RD – residual function mutations
** In Europe

What is an organoid?

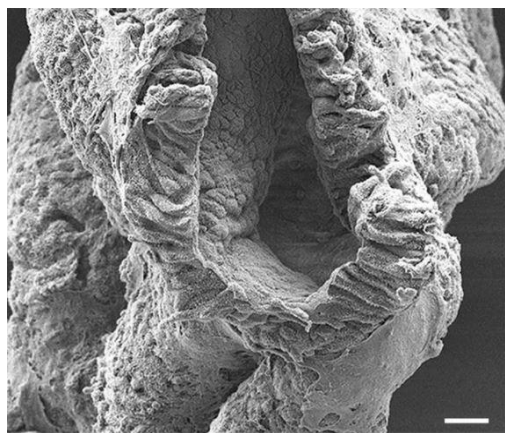


Tissue	Growth factor condition
Esophagus	Wnt-3A, R-spondin, EGF, FGF10, Noggin, TGF-beta1, p38i
	EGF
Liver	R-spondin, EGF, FGF10, HGF, TGF-beta1, forskolin
Stomach	Wnt-3A, R-spondin, EGF, FGF10, Noggin, TGF-beta1,
Extrahepatic bile duct	DKK1, R-spondin, EGF
Pancreas	Wnt-3A, R-spondin, EGF, Noggin, TGF-beta1
Intestine	Wnt-3A, R-spondin, EGF, Noggin, TGF-beta1, p38i

Colonoids resemble colon epithelium morphology and functionality

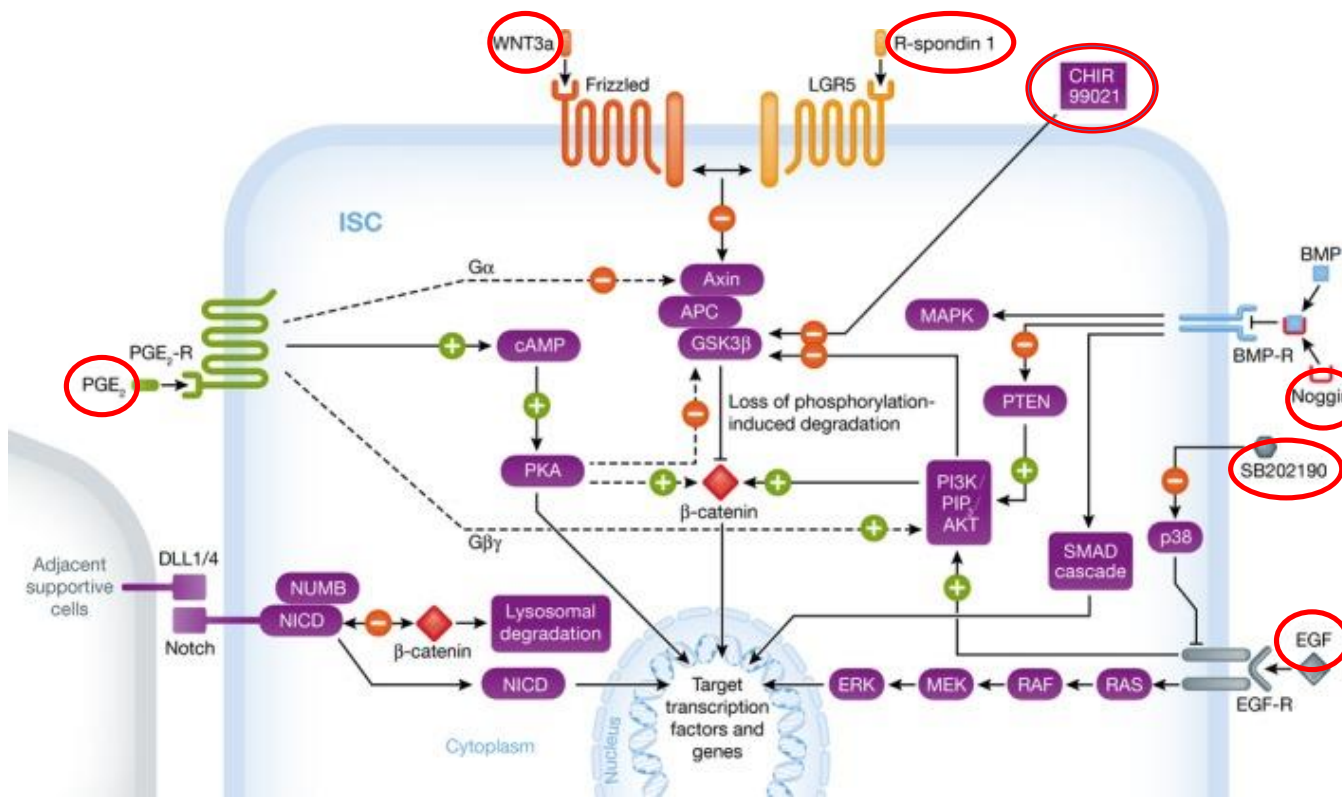


Seeger B. et al 2020



Magnification bars = 20 μ m

Varani J, et al 2021



Holmberg FE, et al 2017

Intestinal mucosa current measurements (ICM) is one of the approved diagnostic tests for CF, together with NPD

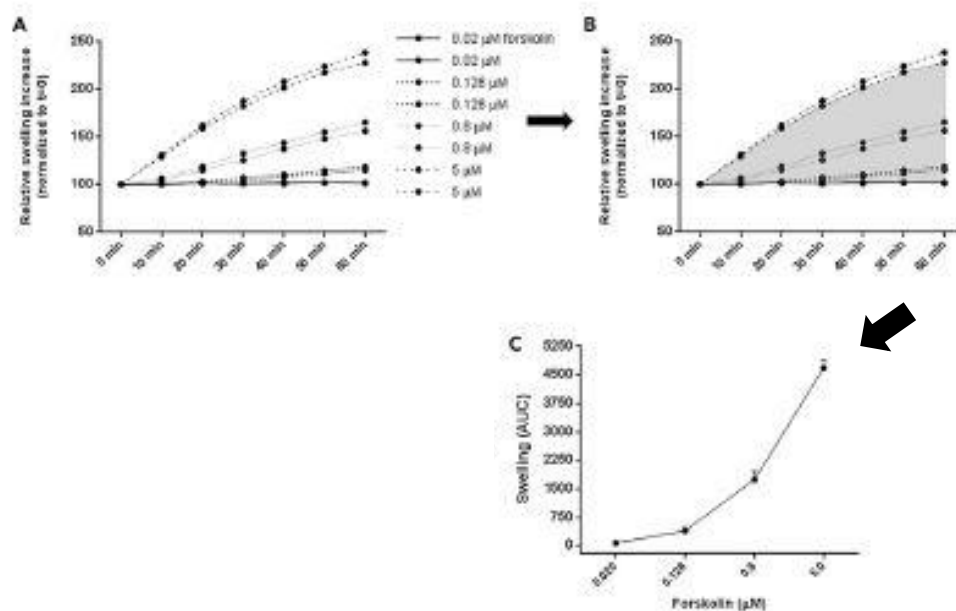
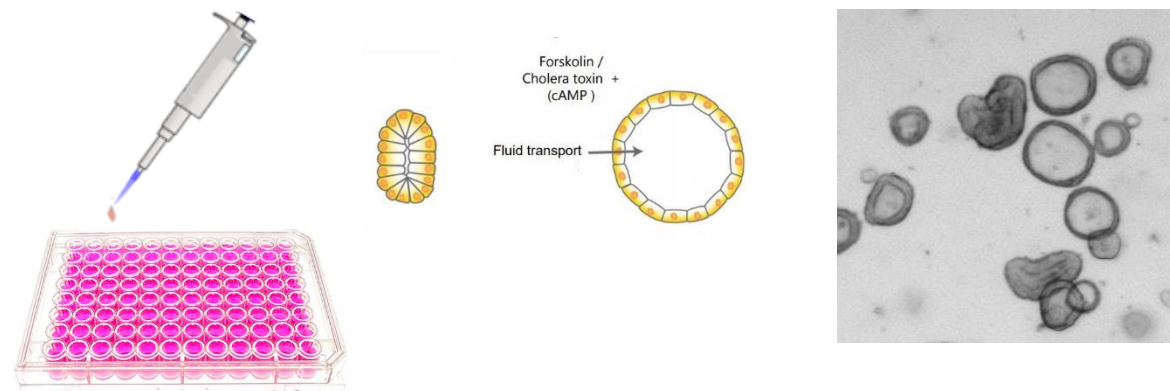
Cystic fibrosis

Intestinal current measurement for diagnostic classification of patients with questionable cystic fibrosis: validation and reference data

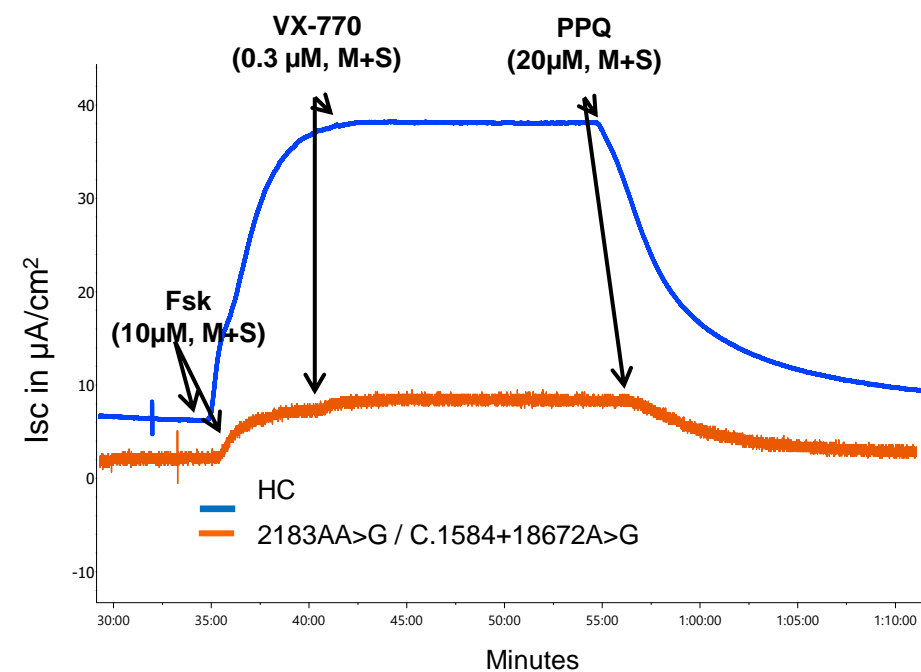
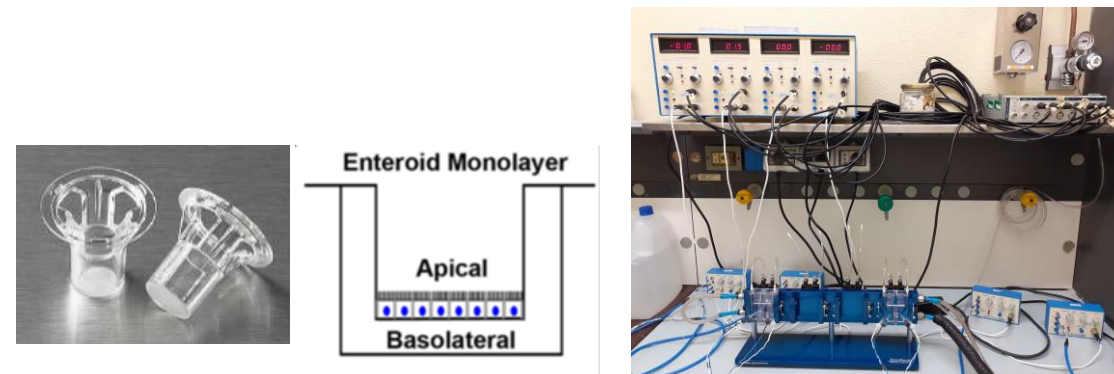
Nico Derichs,¹ Javier Sanz,² Thomas Von Kanel,² Cornelia Stolpe,¹ Antonia Zapf,³ Burkhard Tümmler,¹ Sabina Gallati,² Manfred Ballmann¹

Techniques currently applied for theratyping applications

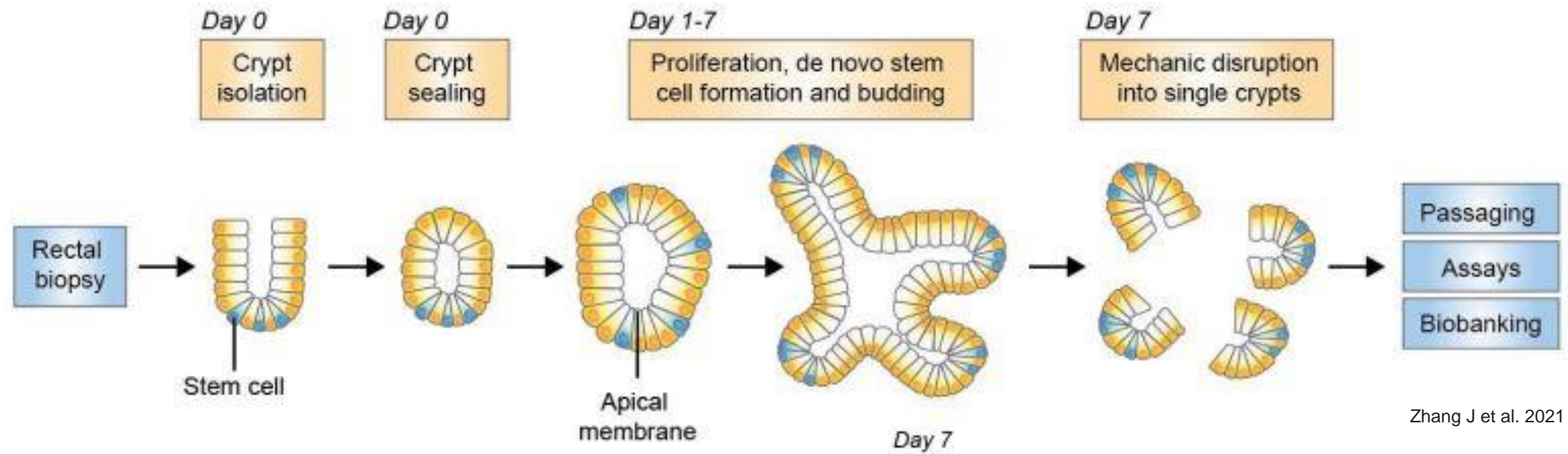
- **FIS = Forskolin Induced Swelling**



- **TCM = Transepithelial Current Measurement short circuit current (Isc) in Ussing chambers**



From patient to organoids culture establishment and maintenance

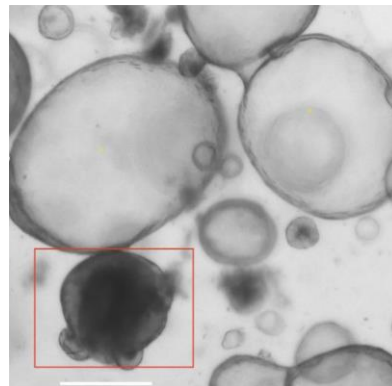


Zhang J et al. 2021

Dekkers JF et al 2013



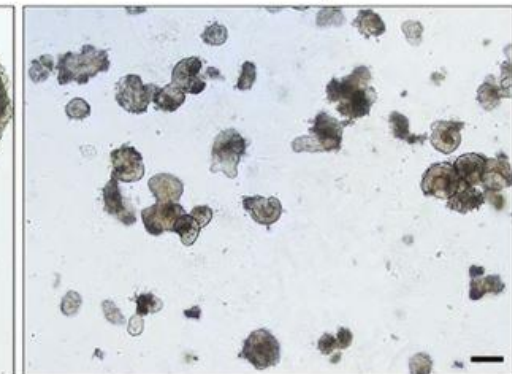
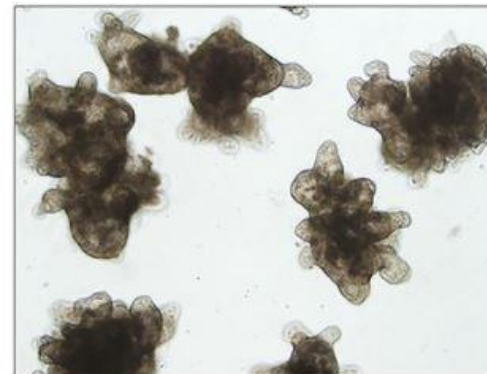
OG6619
(CFTR^{dele} 17a-18/IVS8 T5/T12)



WT organoids
Scale bar, 100 μ m (?).

Pre-split

Post-split



Magnification bar = 200 μ m

Varani J et al 2021

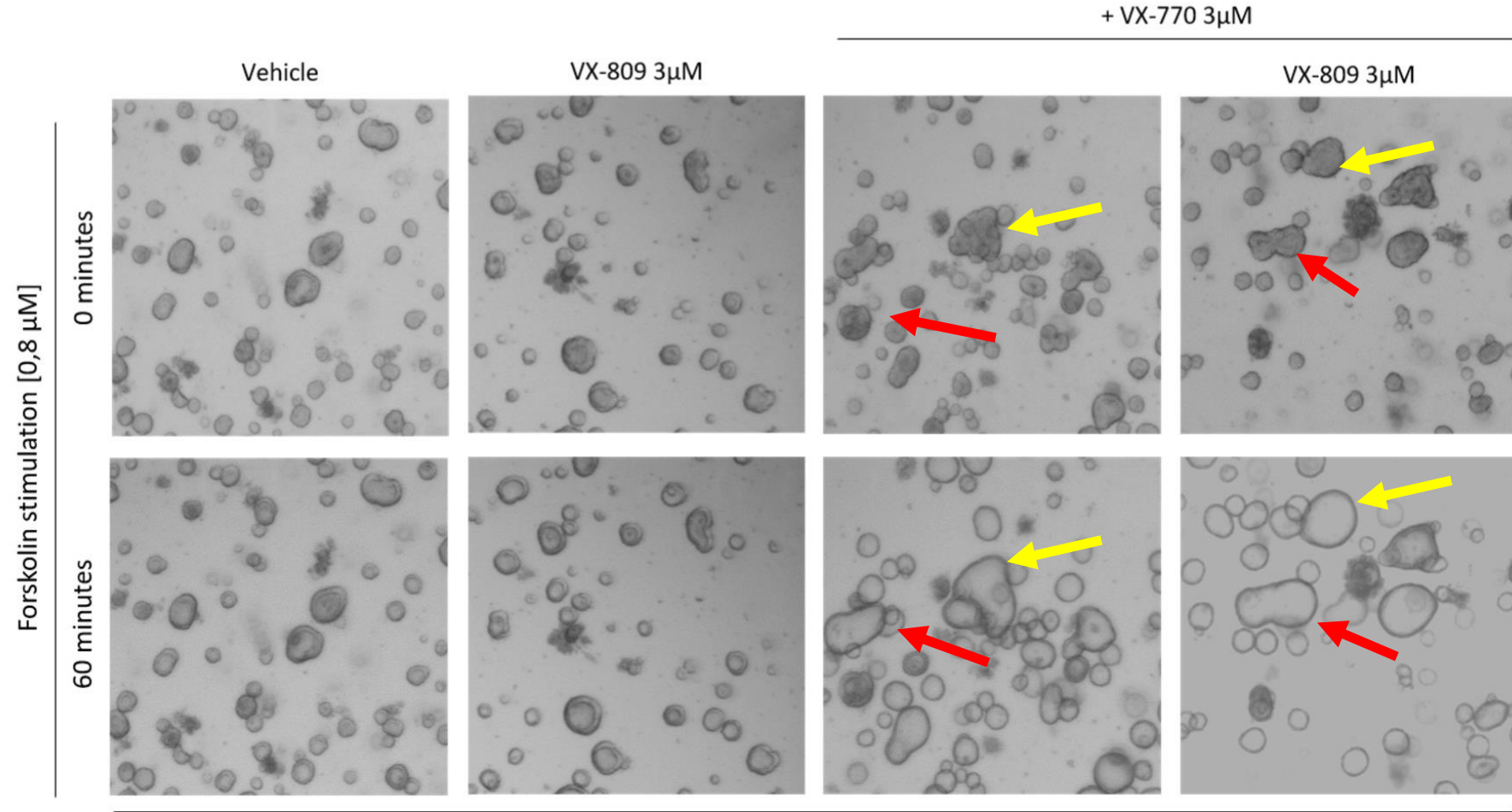
37 years-old, female, with confirmed diagnosis of cystic fibrosis (W57G/A234D genotype)

- GCST: 112mmol/L (Normal < 60)
- FEV1:65% of predicted value
- bilateral bronchiectasis, kidney stones, recurrent sinusitis
- lung infection (*Pseudomonas aeruginosa*)
- pancreatic sufficiency
- Intestinal Current Measurement (ICM), Nasal Potential Difference (NPD) and beta adrenergic optical sweat test were consistent with diagnosis of Cystic Fibrosis

NOTE:

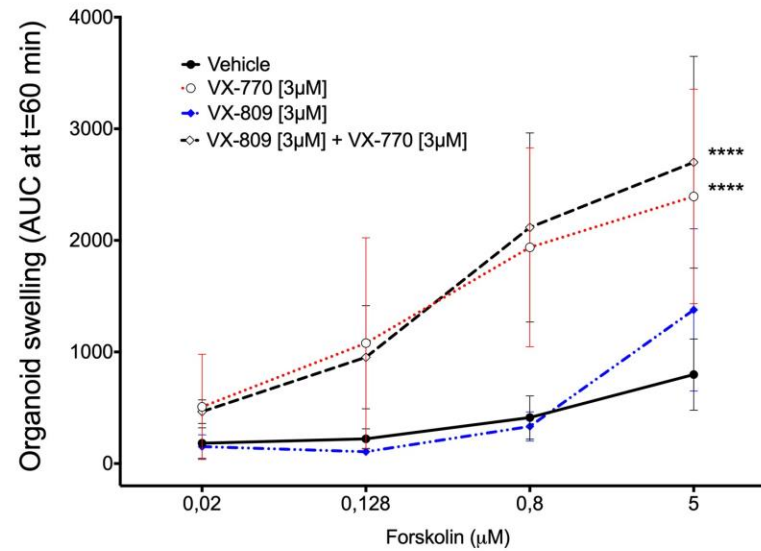
- Very rare, unclassified variants

Forskolin Induced Swelling (FIS) assay

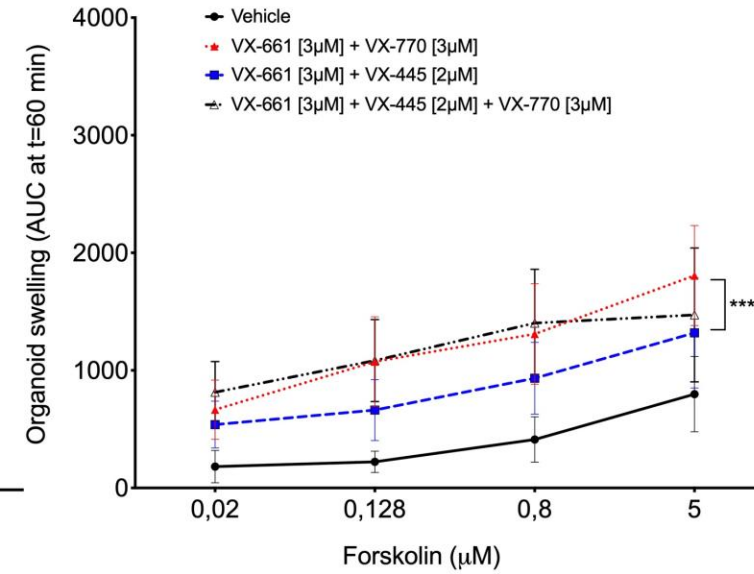


Summary of FIS assay results

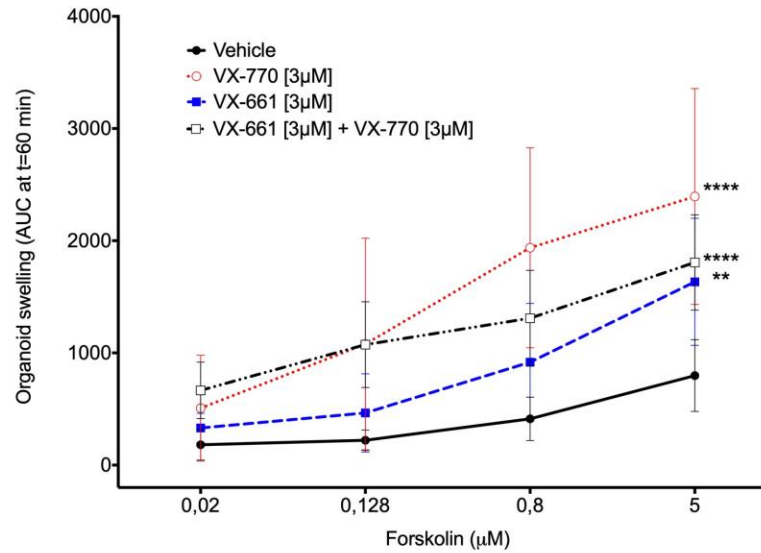
A



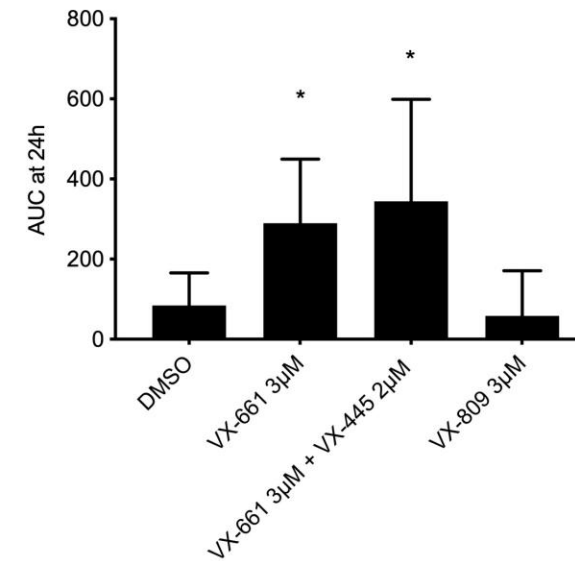
C



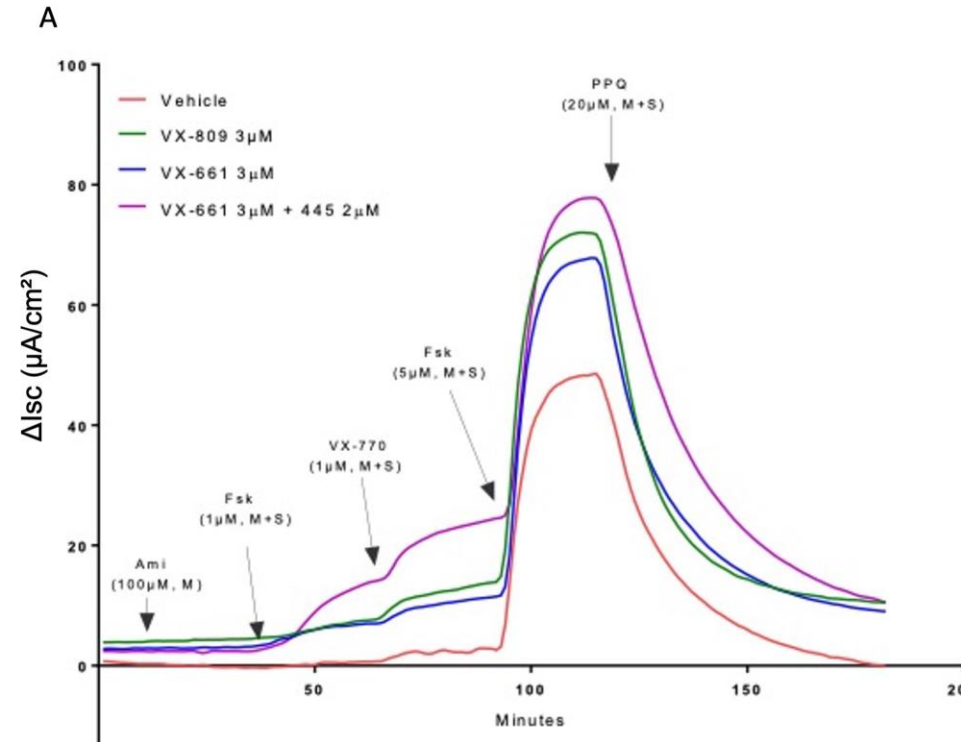
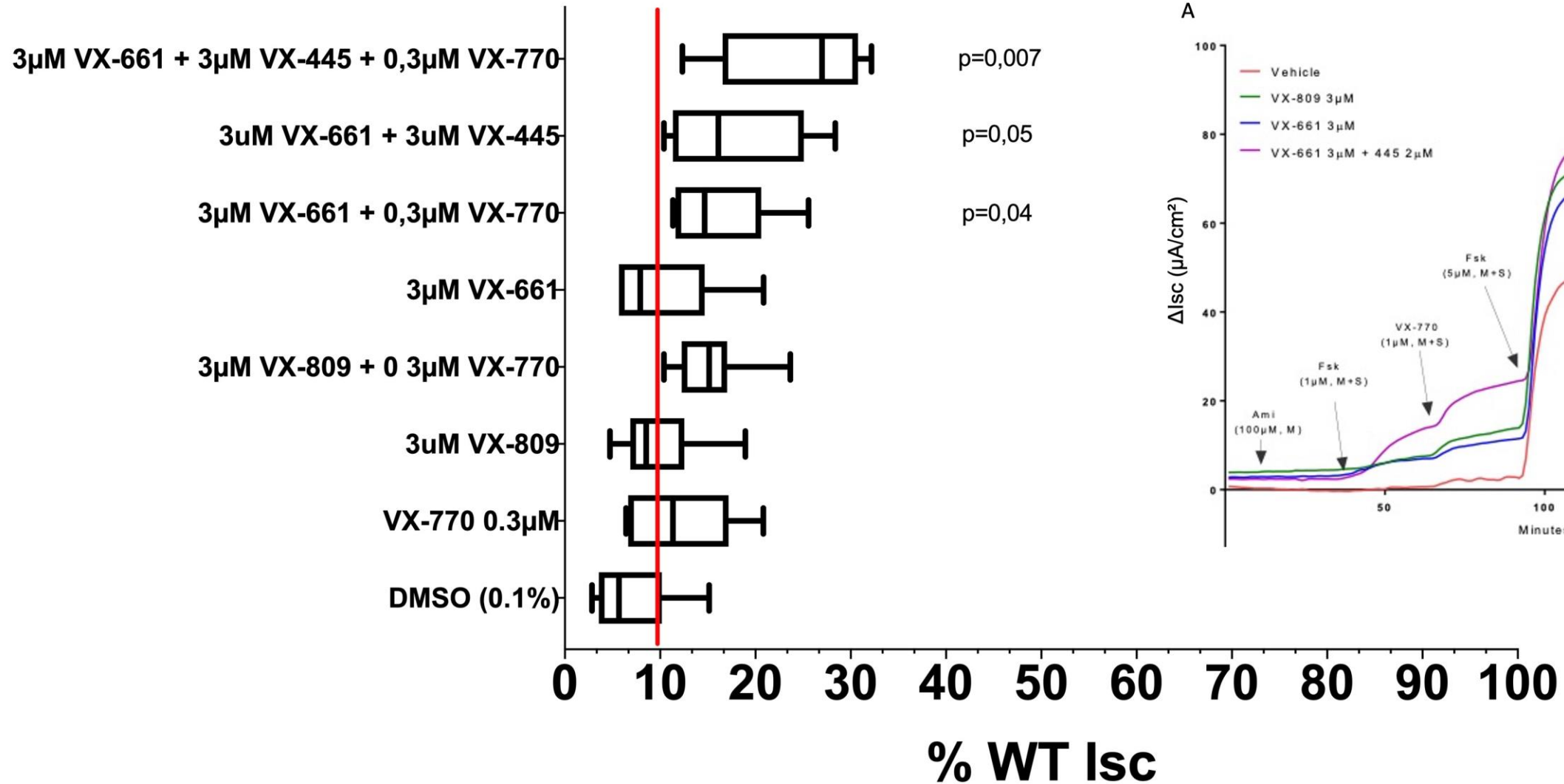
B



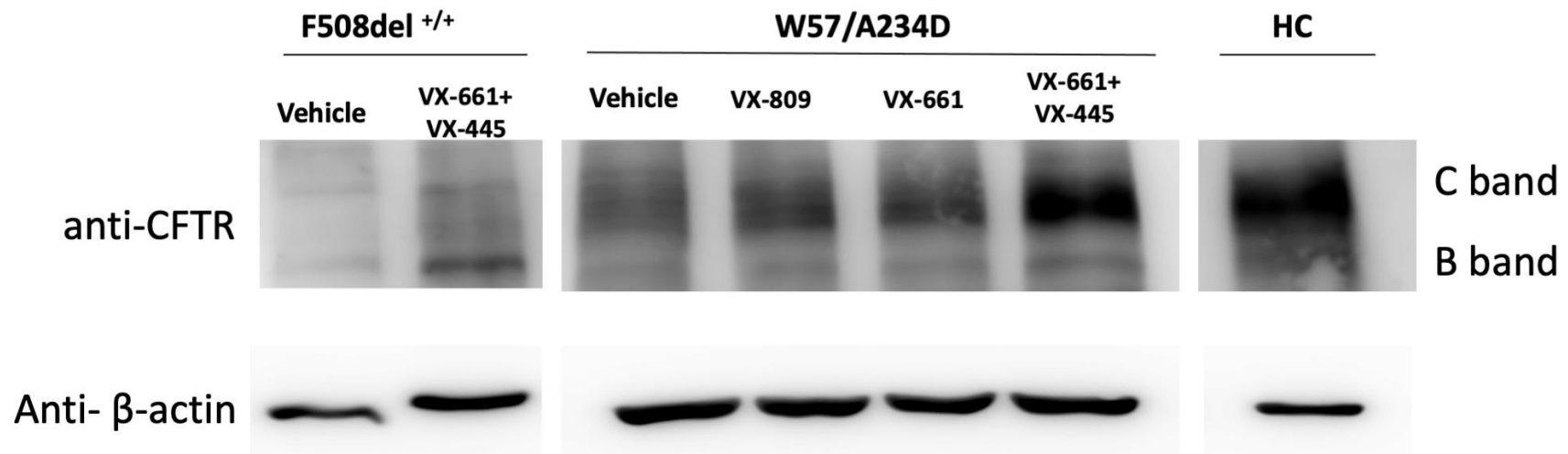
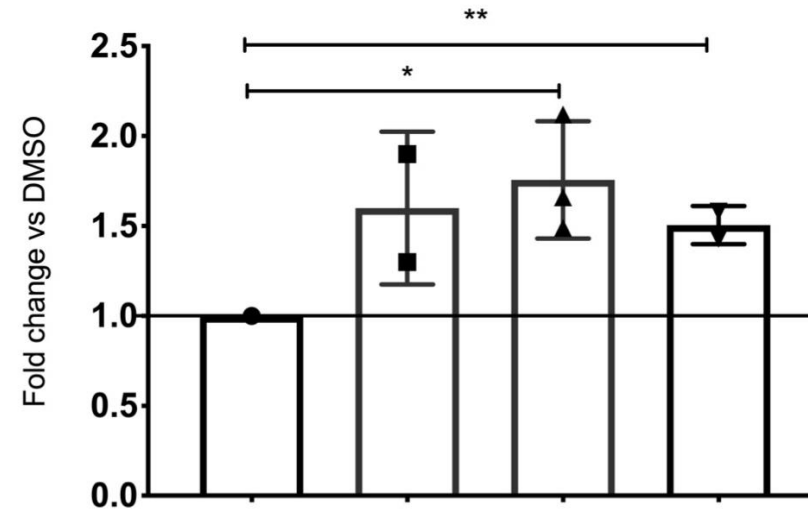
D



Summary of Ussing chamber data



Protein expression: Western blot



Other genotypes (% of WT I_{sc} value)

% recovery vs WT									
Treatments	NON-CF	F508del/F508del	R74W+V201M+D1270 N/ CFTRdele22-24	L227R/ L227R	3849+10kbC->T /3849+10kbC->T	W1282X / W1282X	711+5G>A / R1162X	G542X/ G85E	W57G/A234D
Vehicle	100,00	1,83	2,93	0,73	7,35	2,09	2,26	2,36	7,00
VX-770	110,08	2,55	4,51	1,25	9,03	3,42	5,21	14,15	12,00
VX-661 +VX-445	-	19,39	10,58	2,04	27,80	-	-	-	17,00
VX-661 +VX-445 + VX-770	-	26,80	11,71	3,80	28,21	-	-	-	24,40
VX-809	-	8,87	9,21	2,18	-	-	-	-	9,70
VX-809+VX-770	-	15,58	8,37	4,47	-	-	-	-	16,00
VX-661	-	-	11,34	-	11,32	-	-	-	10,20
VX661+770	-	-	14,18	-	12,95	-	-	-	
PTC124	-	-	-	-	-	1,12	4,76	3,78	
PTC124 + VX-770	-	-	-	-	-	2,50	9,29	11,85	
G418	-	-	-	-	-	2,27	1,18	11,82	
G418 + VX-770	-	-	-	-	-	5,53	3,18	14,19	
Elx-02	-	-	-	-	-	1,37	2,55	-	
Elx-02 + VX-770	-	-	-	-	-	2,03	6,29	-	

5-10%

>10%

CONCLUSIONS

- FIS assay, Isc assay and western blot confirm that W57G/A234D genotype is responsive in vitro to **all CFTR modulators**, with a predicted maximal response to Trikafta combination.
- Interpretation of FIS assay needs to be adapted to the high efficacy of new CFTR modulators combination that can induce a **forskolin-independent swelling before FIS assay**
- This combined approach is (at this stage) **costly but feasible** and is being applied to **other genotypes**
- The CF community should strongly promote the conditions for a **fast approval of experimental treatments based on in vitro data** to permit at least to quickly evaluate the predicting value of approaches based on individual patient's primary cells

Open questions

- respiratory-GI-leukocyte cells: is there a specific advantage for some model? Should we use...one...two out of three...?
- Lab certification?
- Cutoff values?
- Standardized protocols?
- Reference centers?

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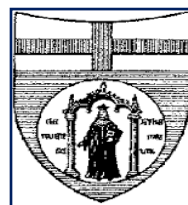


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