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Discovery of a potent pharmacological corrector of F508del-CFTR chloride channel

Tiziano Bandiera, PhD

tiziano.bandiera@fibrosicisticaricerca.it

Fondazione per la Ricerca sulla Fibrosi Cistica - Verona



Università Milano Bicocca
Dipartimento di Biotecnologie e Bioscienze

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Cystic Fibrosis

Cystic Fibrosis (CF) is an **autosomal recessive, rare genetic disease** affecting approximately 1 in ca. 5000 live births in the US¹ and 1 in ca. 2500 to 1 in ca. 6000 in European countries².

Worldwide, **people with CF are estimated at ca. 188,000** of which ca. 111,000 are diagnosed³ ca. 57,000 in Europe, ca. 34,000 in USA, ca. 4,500 in Canada, ca. 4,500 in Australia + New Zealand, and ca. 5,500 in Brazil.

CF is caused by mutations in the **Cystic Fibrosis Transmembrane conductance Regulator (CFTR)** gene that lead to loss-of-function or loss-of-expression of the CFTR protein.

As of end 2025, **2,121 CFTR variants** are known (<http://genet.sickkids.on.ca/StatisticsPage.html>)⁴.

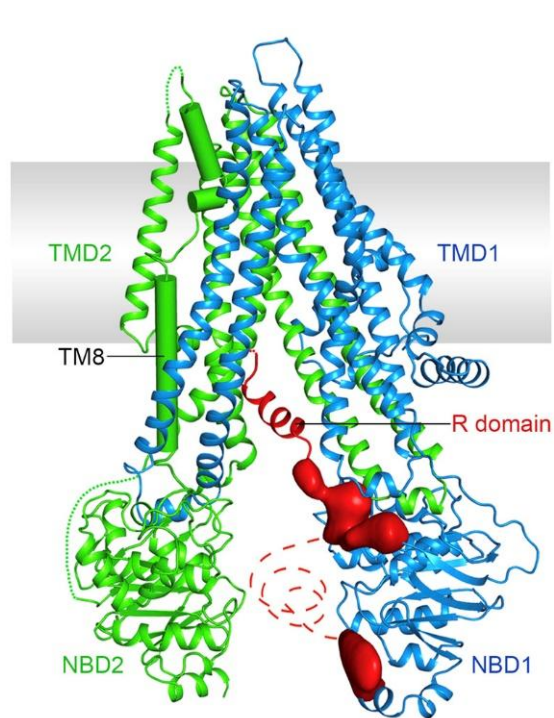
1) Stephenson et al., *J. Cystic Fibrosis* **2023**, 22, 443-449; 2) P. M. Farrell, *J. Cystic Fibrosis* **2008**, 7, 450-453; 3) L. Naehrlich, *Pediatric Pulmonology*, **2026**; 61:e71445

4) De Boek et al., *J. Cystic Fibrosis* **2026**, in press

Certain mutations in the CFTR gene cause Cystic Fibrosis

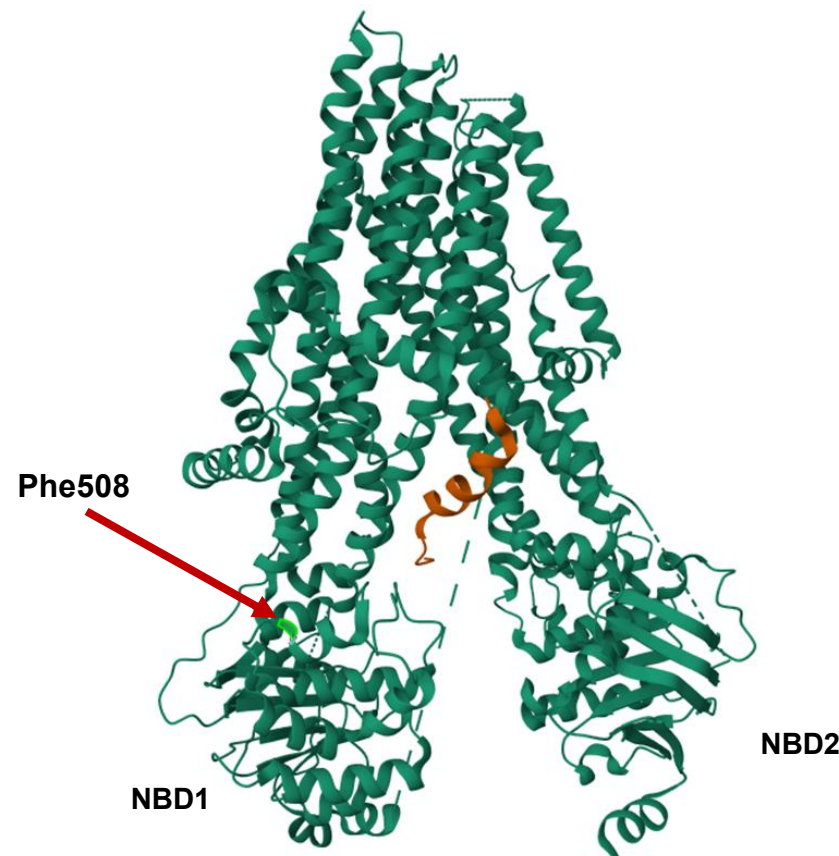
Deletion of the Phe508 (**F508del**) in the nucleotide binding domain 1 (NBD1) is the **most prevalent CFTR mutation** causing CF.

F508del mutation is present in ca. 80% of CF patients in Europe and ca. 85% of CF patients in USA¹.



Molecular structure of human CFTR determined in the dephosphorylated, ATP-free form

Liu et al., *Cell*, 2017, 169, 85–89



PDB structure: 5UAK

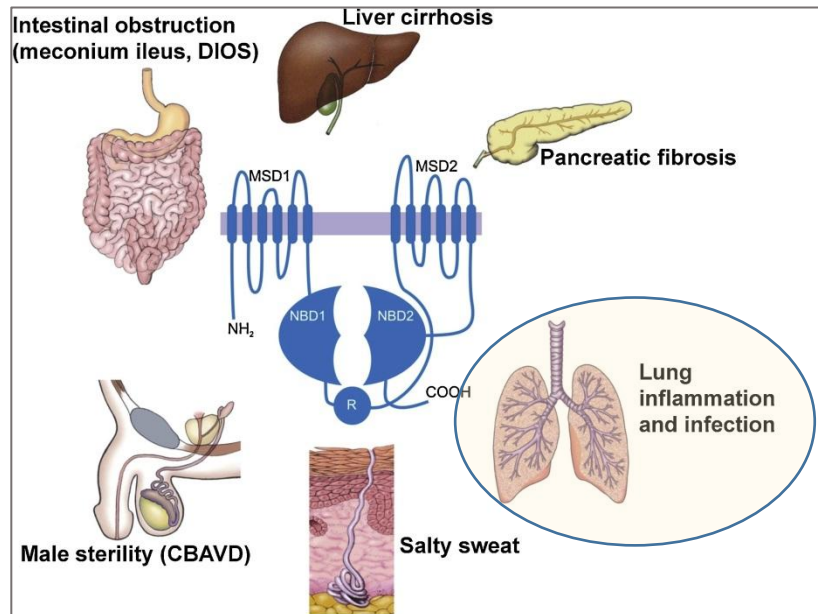
1) Data from: *European Cystic Fibrosis Society*, Patient Registry, Annual Data Report 2021, and *Cystic Fibrosis Foundation*, 2021 Patient Registry

CF is a systemic disease

CFTR mutations result in poor chloride (Cl^-) and bicarbonate (HCO_3^-) transport.

This causes dehydration of secretions with viscous mucus and leads to inflammation and chronic airway obstruction, pancreatic and digestive insufficiency, bowel obstruction, diabetes, hepatic damage, and male infertility.

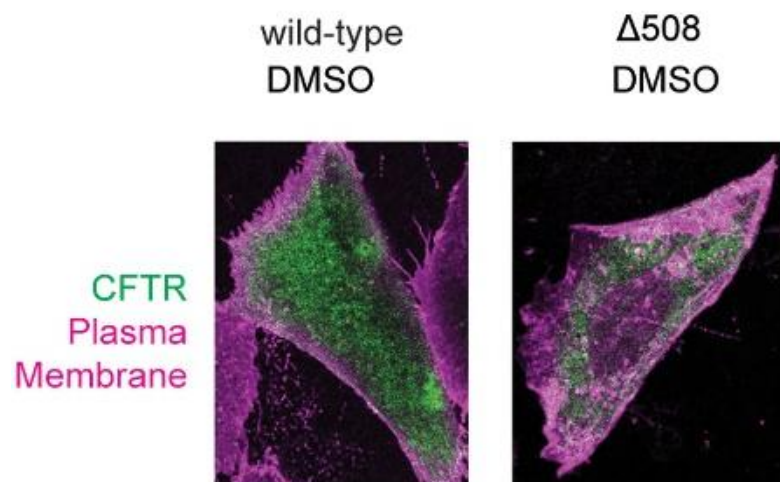
Effect on organ function of CFTR mutations causing severe disease.



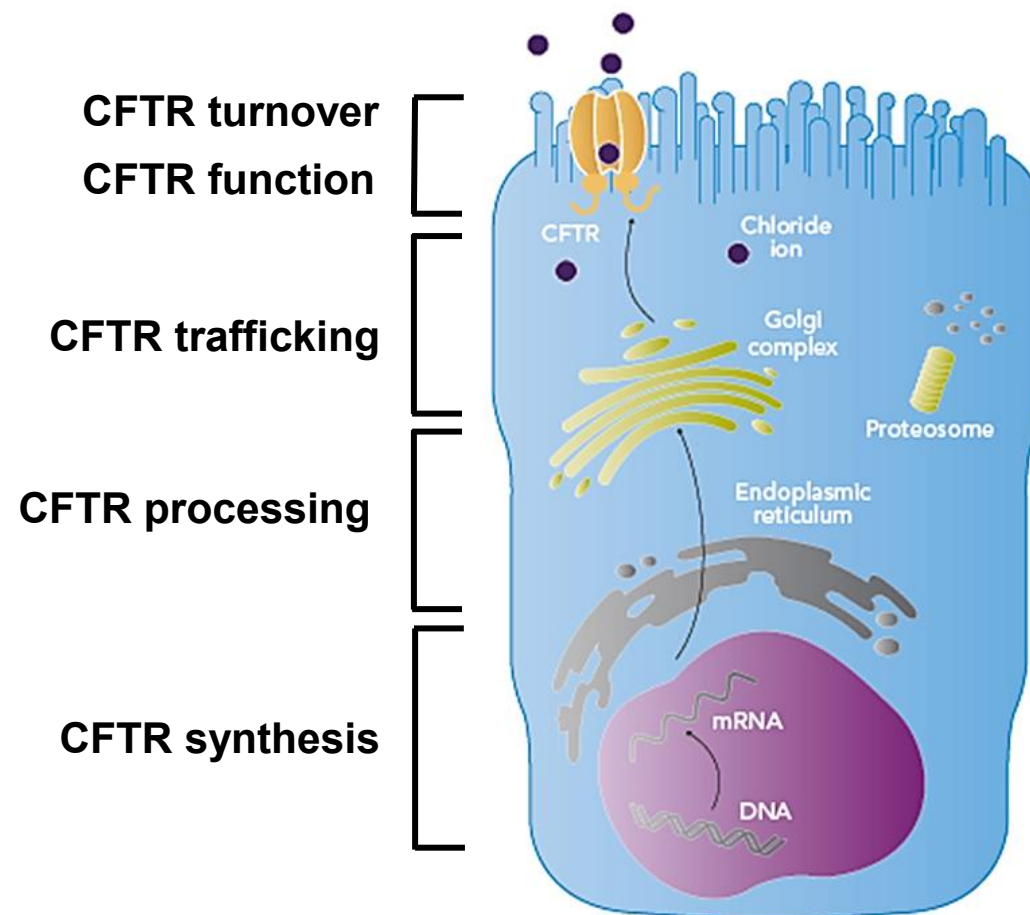
While many organs are affected in CF, pulmonary disease is the major cause of morbidity and mortality.

Mutations in the CFTR gene affect different processes

Only small amounts of Phe508del-CFTR protein can reach the plasma membrane.

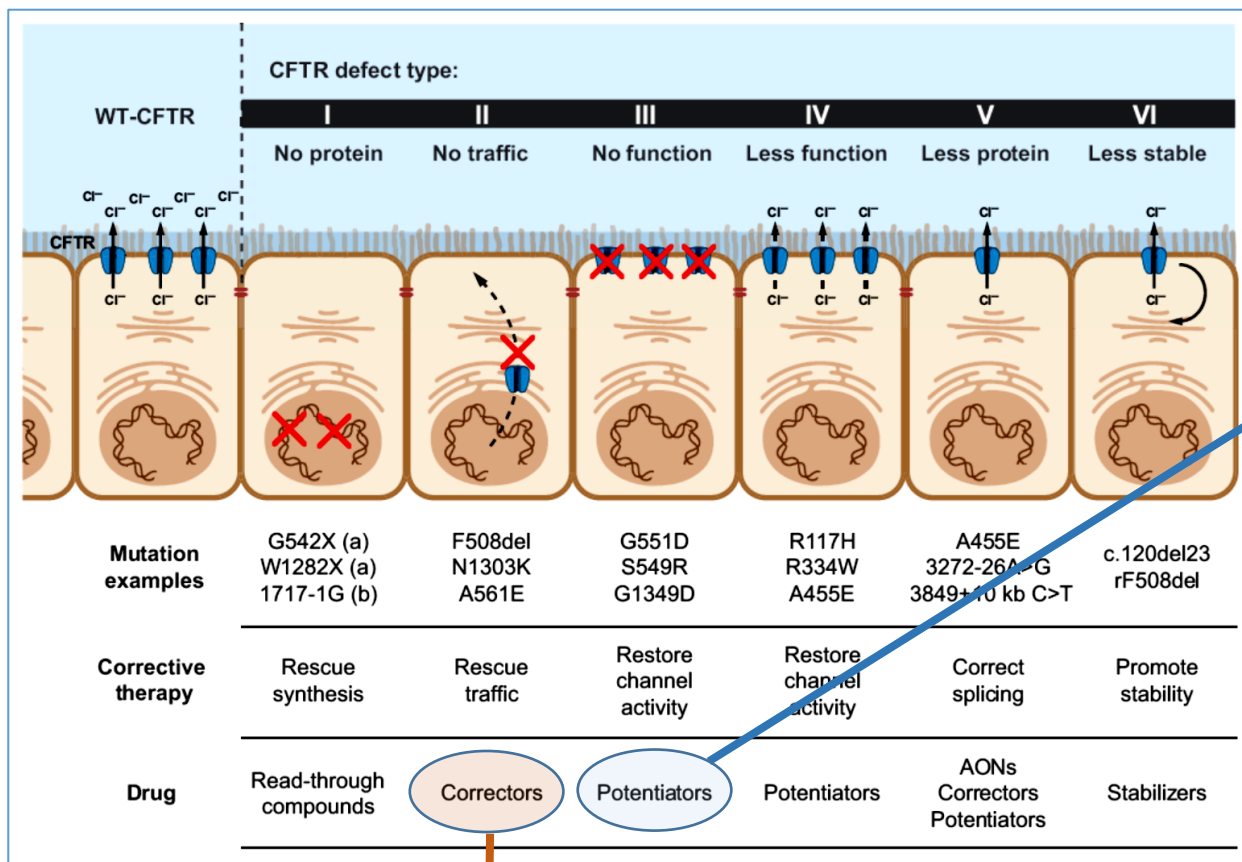


Chinese hamster ovary (CHO) cells expressing CFTR variants. Plasma membrane (magenta) was visualized by exciting Alexa Fluor 647–conjugated wheat germ agglutinin stain. CFTR (green) was visualized by exciting enhanced GFP (eGFP)-tagged CFTR.

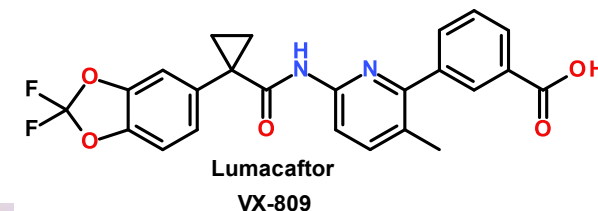
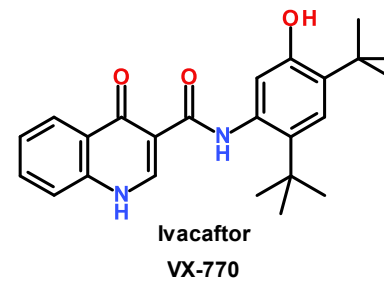


Credit: Vertex Pharmaceuticals Inc.

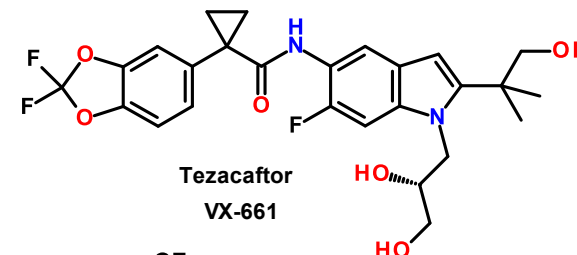
CFTR mutations are classified in 6 classes based on their phenotypic consequences.



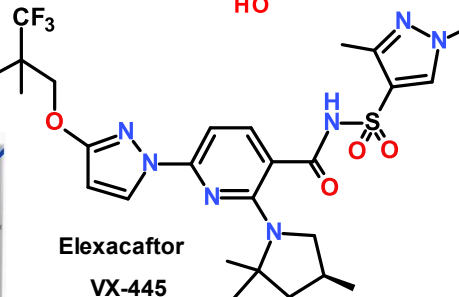
Iva
(2012)



Iva + Luma
(2015)



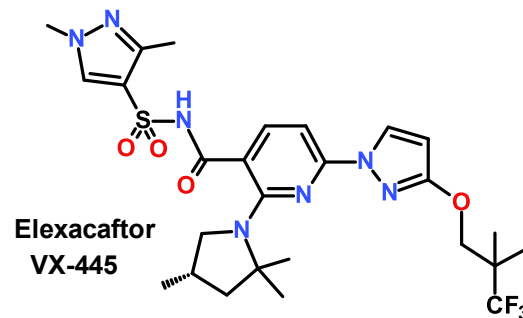
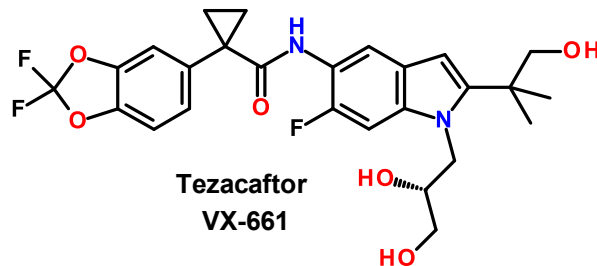
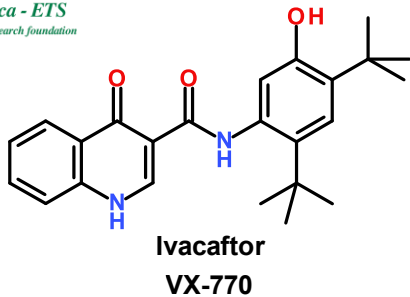
Iva + Teza
(2018)



Iva + Teza + Elexa
(2019)

CF disease-modifying therapy:

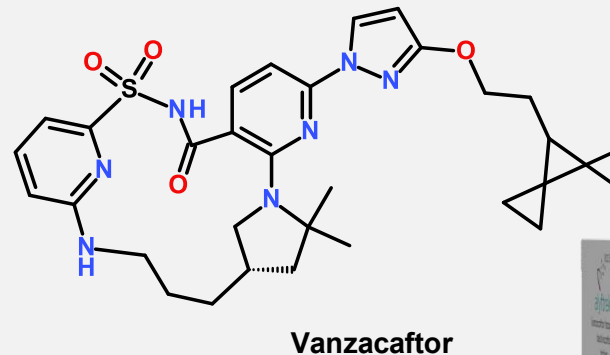
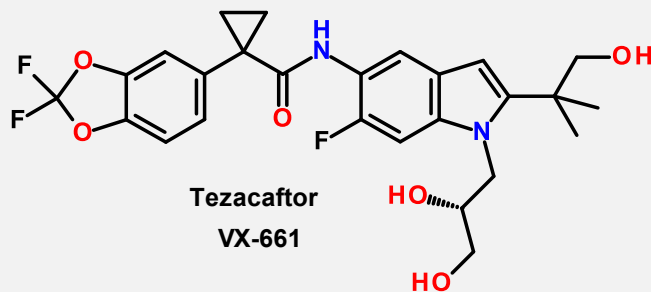
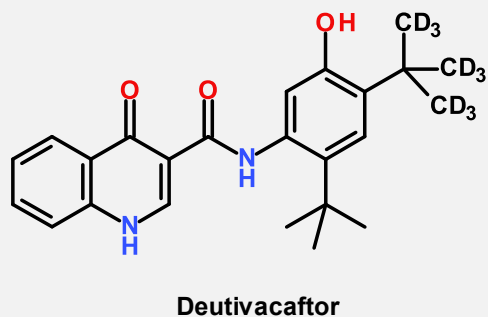
an example of precision medicine



Morning dose: 2 fixed-dose combinations tablets (each containing elelacaftor 100 mg; teza 50 mg; iva 75 mg)

Evening dose: 1 tablet containing 150 mg of ivacaftor.

Iva + Teza + Elelacaftor
(2019)



Single dose

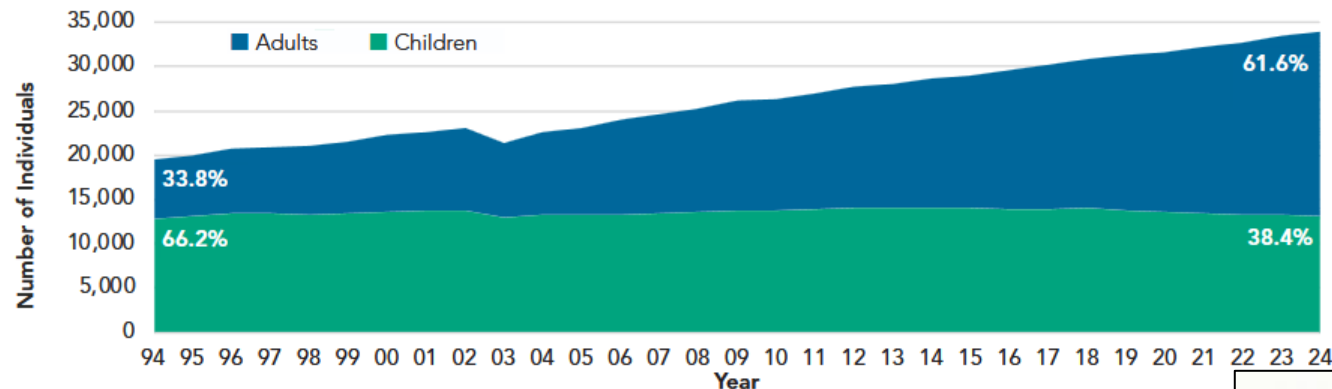
Adults (>40 kg): 2 tablets (each containing vanza 10 mg; teza 50 mg; deutiva 125 mg)

Deutiva + Teza + Vanza
(2024)

CF disease-modifying therapy: Trends in pwCF USA, Europe

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Number of Children and Adults With CF, 1994–2024



Source: 2024 Patient Registry Annual Data Report

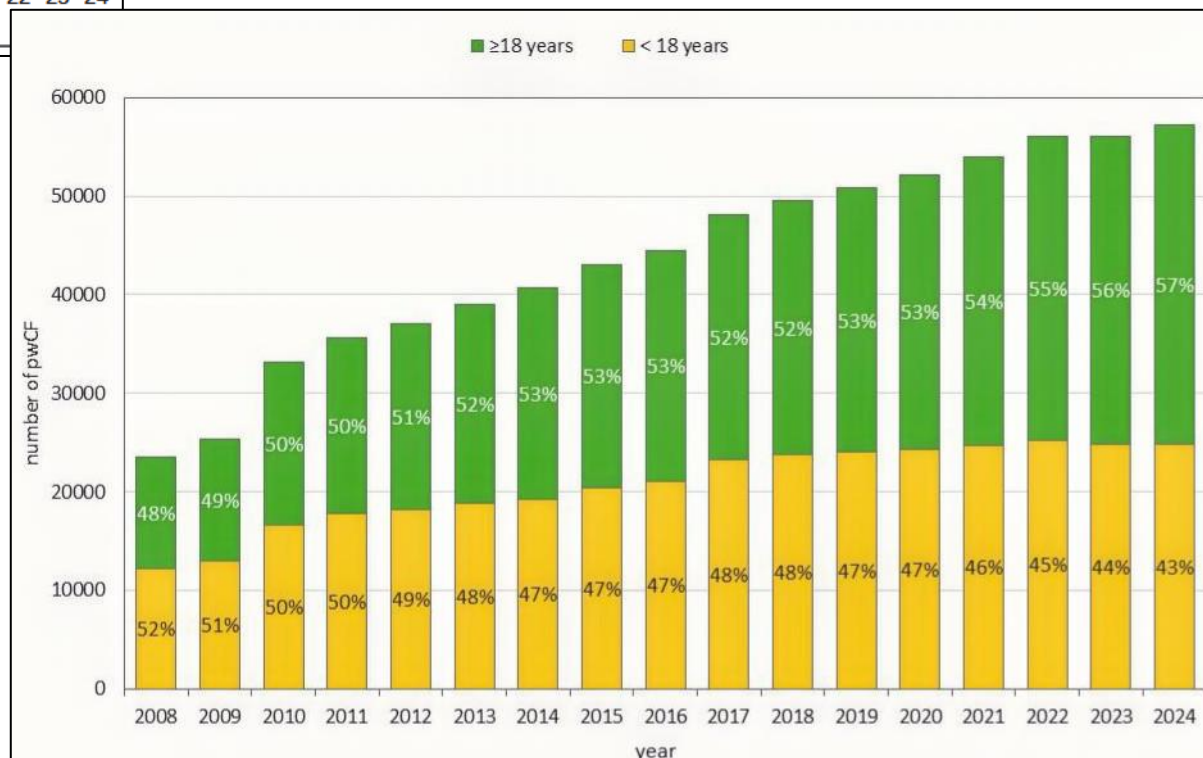


In the US, the number of children (<18 years old) with CF has remained constant over the last 30 years, but their percentage in the people with CF almost halved.



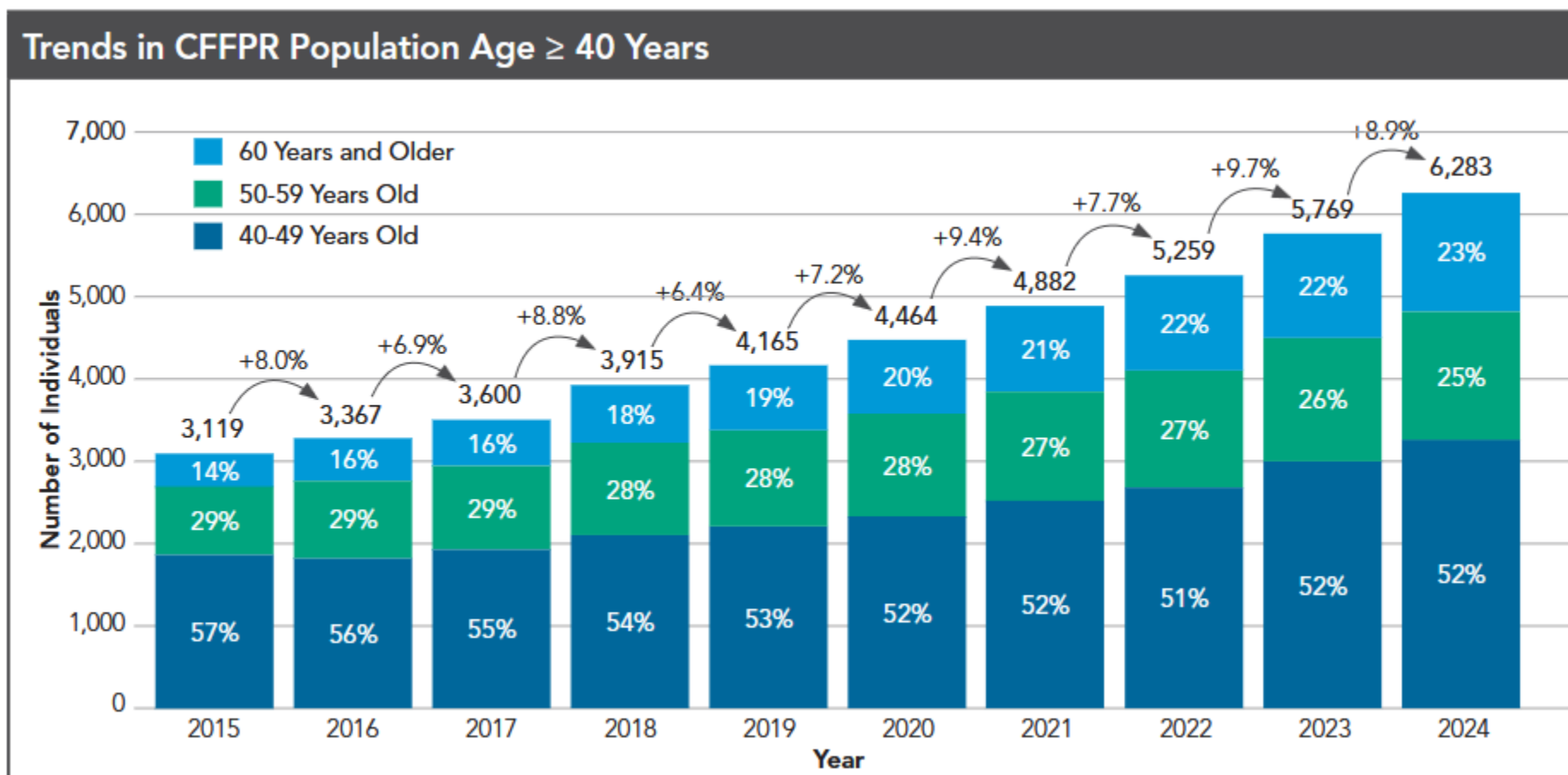
In Europe, the percentages of pwCF <18 years old and pwCF >18 years old swapped over the last 16 years.

Source: 2024 ECFS Patient Registry Annual Data Report



CF disease-modifying therapy: Trends in US CF population

In the US, the number of people with CF and >40-year-old has doubled in the last ten years.



Source: 2024 Patient Registry Annual Data Report



Search for new modulators of mutant CFTR

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The Task Force for Cystic Fibrosis (TFCF) Project

A collaborative drug discovery project aimed at the identification of new drugs for the treatment of CF.



Istituto Giannina Gaslini (IGG)

Luis J. V. Galletta (now at TIGEM)

Nicoletta Pedemonte



Istituto Italiano di Tecnologia (IIT)

Tiziano Bandiera

Project funded by FFC



<http://www.fibrosicisticaricerca.it/>

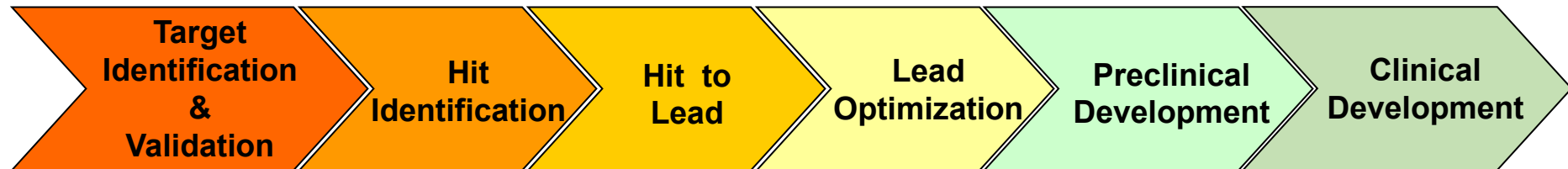
Search for new modulators of mutant CFTR

Approach: High Throughput Screening of the IIT compound collection

Evolution of Hits up to an optimized Lead

Goal: Nominate a Preclinical Development Candidate (PDC)

Resources: 3 FTEs at IGG, 5 FTEs at IIT



TFCF Project

Hits found by **phenotypic HTS** of IIT compound collection

A collection of ca. 11,300 compounds was screened on two cell lines:

- CFBE41o- and
- FRT

Both cell lines stably expressed F508del-CFTR and the halide-sensitive yellow fluorescent protein (HS-YFP).

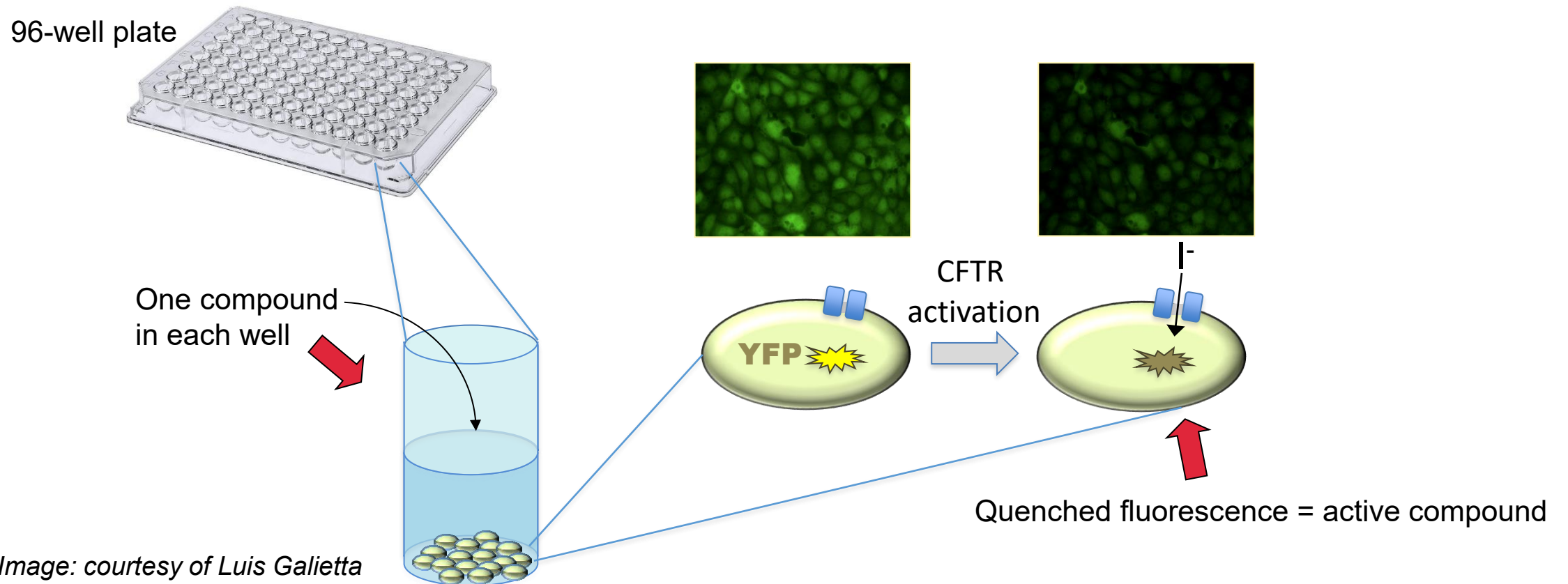
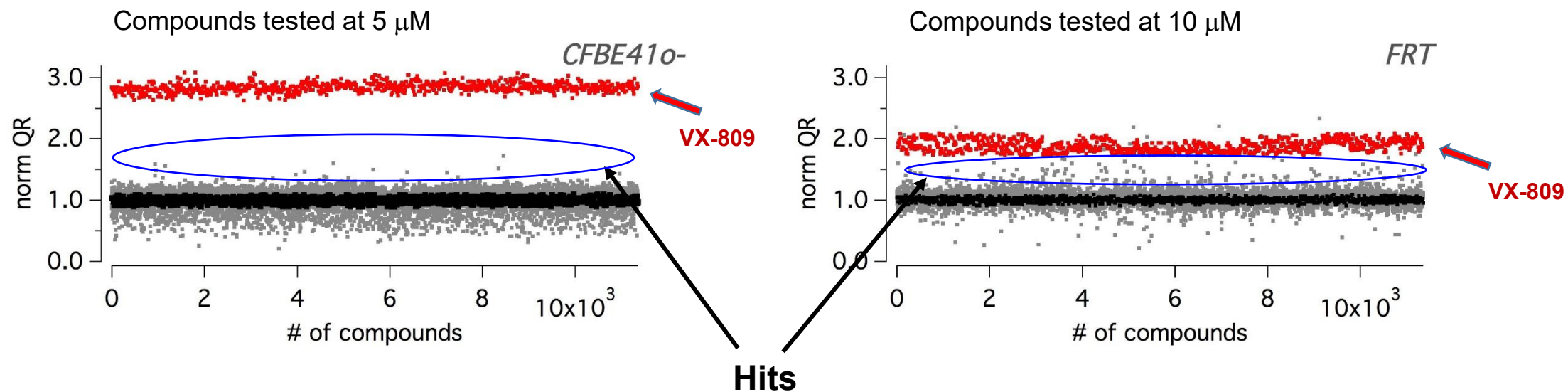


Image: courtesy of Luis Galletta

CFBE41o-: Cystic Fibrosis Bronchial Epithelial cells; **FRT**: Fisher Rat Thyroid cells

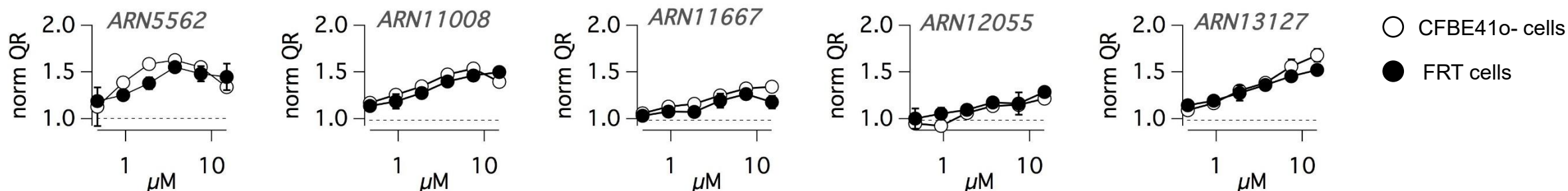
Phenotypic HTS: results

The hit rate was higher in FRT than in CFBE41o- cells.



Hit confirmation

5 hits active on both FRT and CFBE41o- were tested in dose-response in the two cell lines.

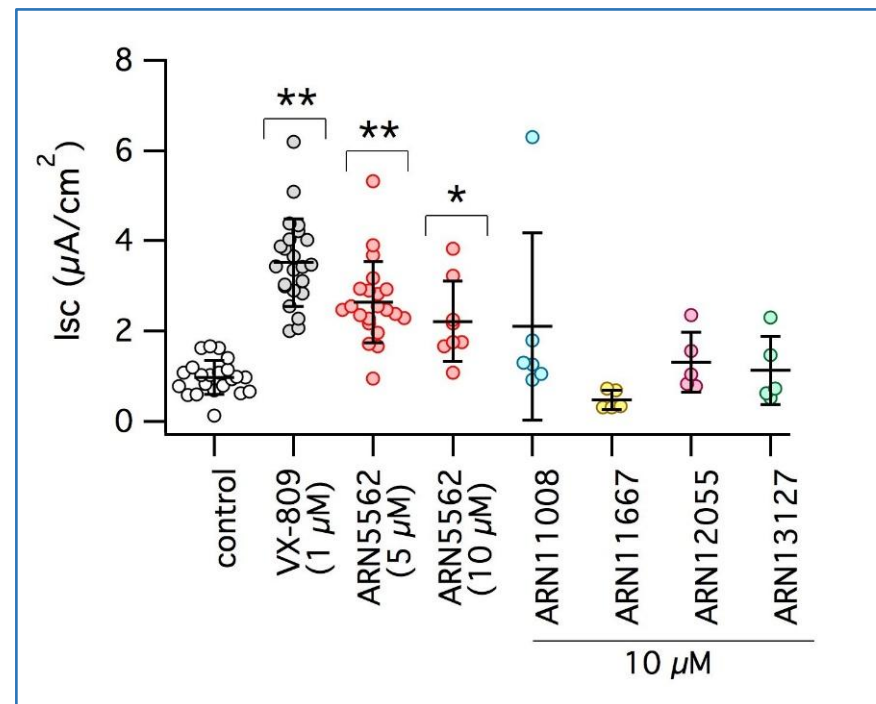


To further confirm activity, the hits were tested on F508del/F508del primary human bronchial epithelial (HBE) cells from lungs of CF patients who underwent transplantation.

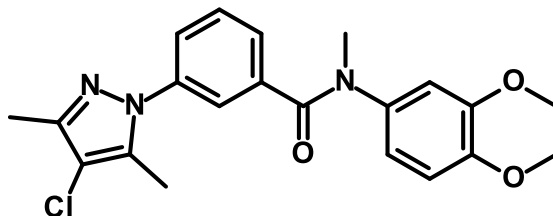
Short-circuit current recording in Ussing chamber.

I_{sc} : amplitude of the current blocked by the CFTR channel inhibitor, Inh-172.

* $P < 0.05$ and ** $P < 0.01$ versus control



ARN5562 selected as one of the hits for follow up



Activity data in cells

CFBE41o- cells		FRT cells	
EC ₅₀ (μM)	E _{max}	EC ₅₀ (μM)	E _{max}
1.45	1.47	1.04	1.56

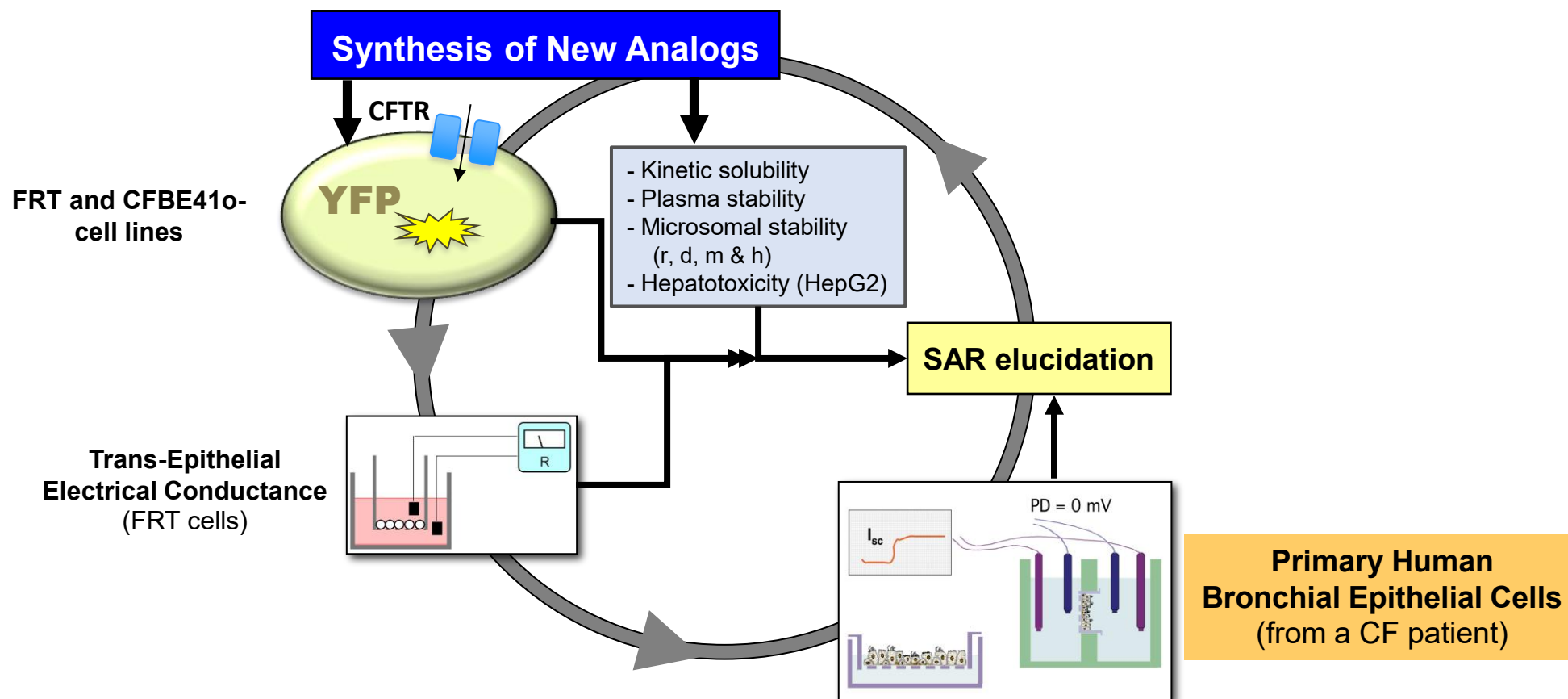
Drug-like properties

Kinetic Solubility	Metabolic stability (NADPH)				Hepatotoxicity
PBS pH 7.4	Rat LM	Dog LM	Monkey LM	Human LM	HepG2
(μM)	t _{1/2} (min)	t _{1/2} (min)	t _{1/2} (min)	t _{1/2} (min)	(% Survival)
81 ± 10	<5	8 ± 2	<5	<5	74

Hit-to-Lead: flow of activities in the project

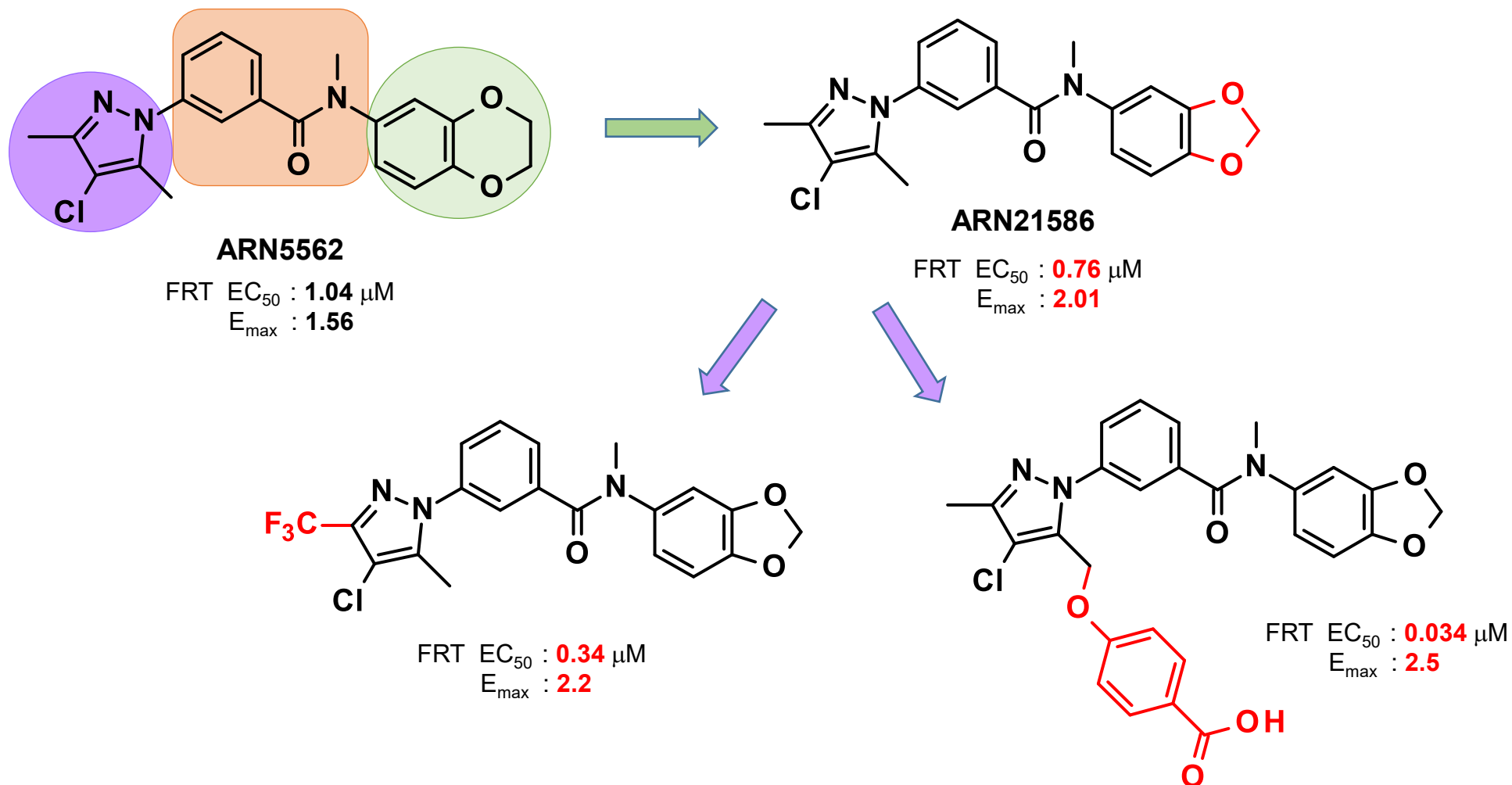
All new compounds were tested on both FRT and CFBE41o- cells.

The most interesting ones were further characterized in secondary biological assays and for their drug-like properties.



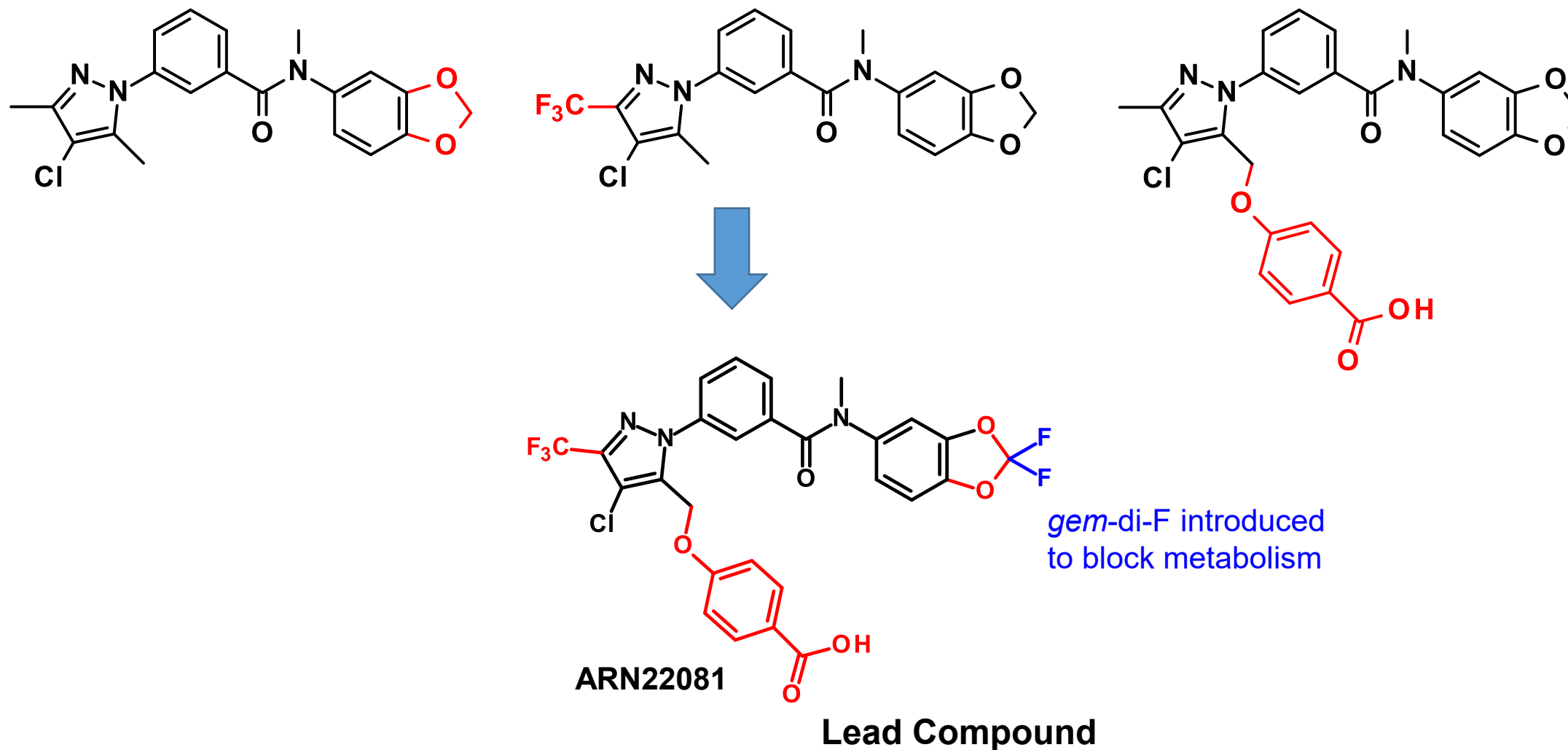
Hit-to-Lead: evolution of hit ARN5562

Certain modifications in each of the three parts of the hit were beneficial for activity.

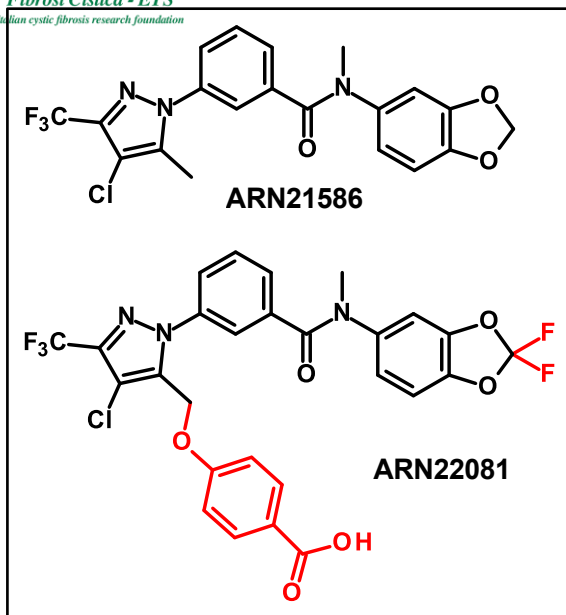


Hit-to-Lead: nomination of a Lead

Combination of modifications beneficial for activity resulted in the Lead Compound.



ARN22081 was the Lead compound for this series

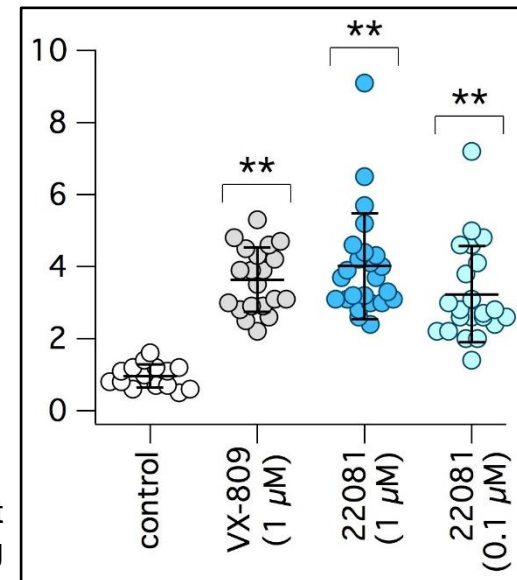


Activity data in cells

ID	CFBE41o- cells		FRT cells	
	EC ₅₀ (μM)	E _{max}	EC ₅₀ (μM)	E _{max}
ARN5562	1.45	1.47	1.04	1.56
ARN21586	0.36	2.7	0.34	2.3
ARN22081	0.0014	2.81	0.0019	2.19
VX-809	0.205	2.5	0.319	2.53

Short-circuit current
recording in Ussing
chamber.

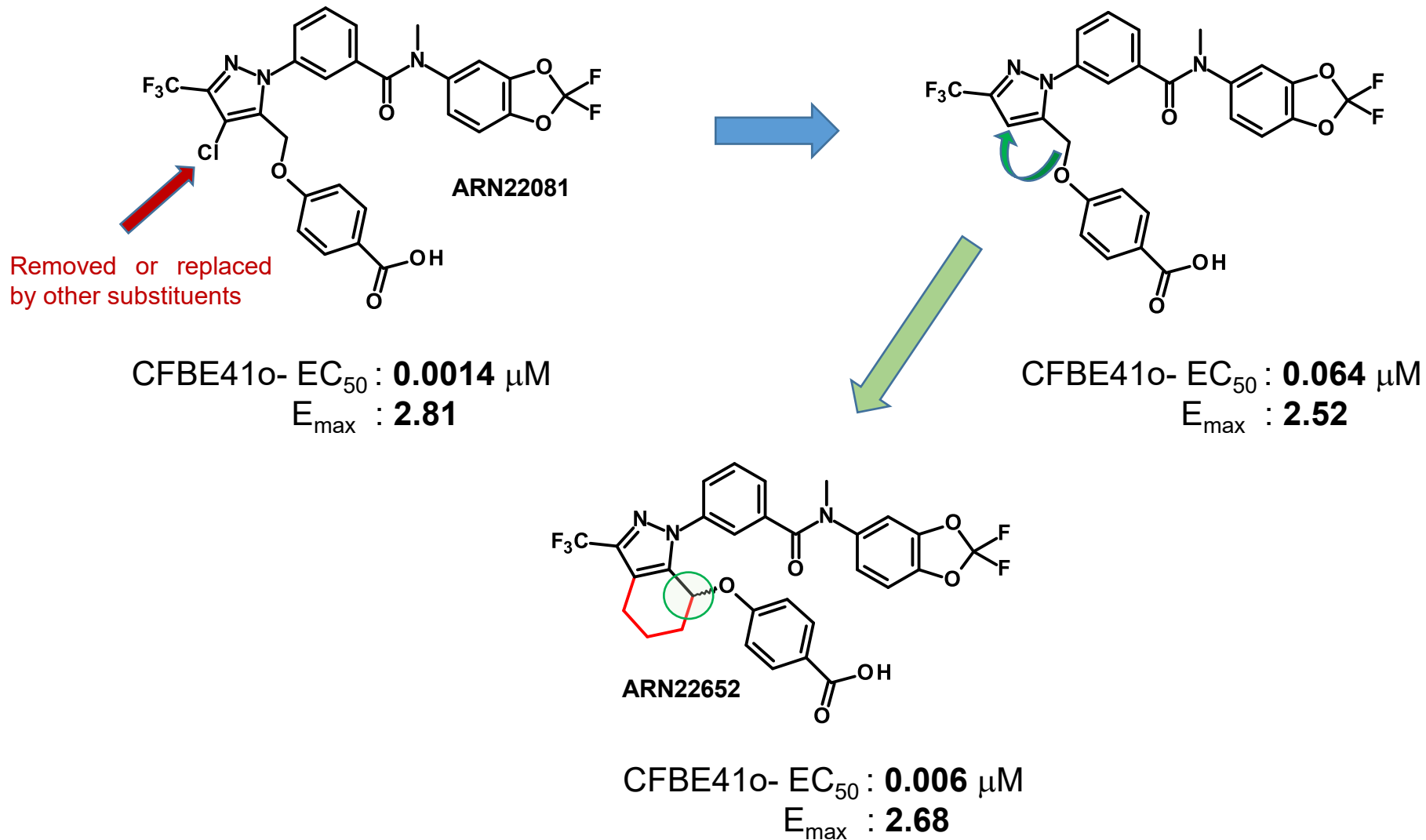
F508del/F508del HBE cells



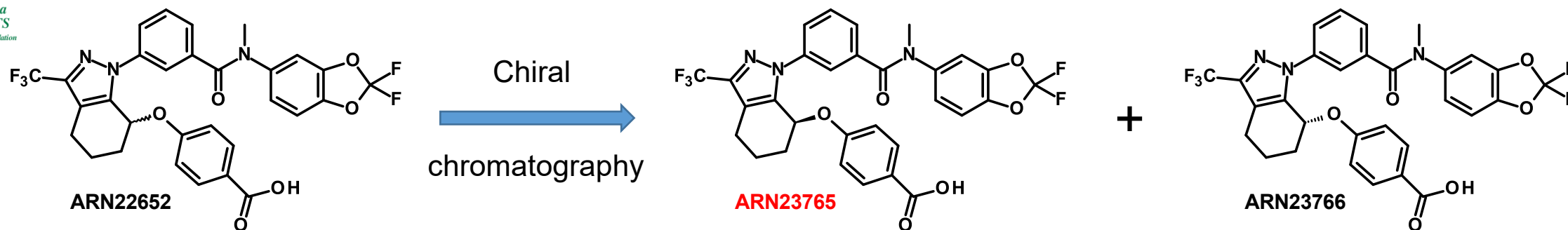
Drug-like properties

ID	Kinetic Solubility	Metabolic stability (NADPH)				Hepatotoxicity	Caco-2 permeability	
		Rat LM	Dog LM	Monkey LM	Human LM		A-B	B-A
	PBS pH 7.4					HepG2		
	(μM)	t _{1/2} (min)	t _{1/2} (min)	t _{1/2} (min)	t _{1/2} (min)	(% Survival)	(10 ⁻⁶ cm/s)	(10 ⁻⁶ cm/s)
ARN5562	81 ± 10	<5	8 ± 2	<5	<5	74	n.t.	n.t.
ARN21586	8 ± 2	<5	17 ± 2	<5	10 ± 1	64	n.t.	n.t.
ARN22081	>250	19 ± 3	>60 (77%)	10	50 ± 2	>80	4.0	0.5

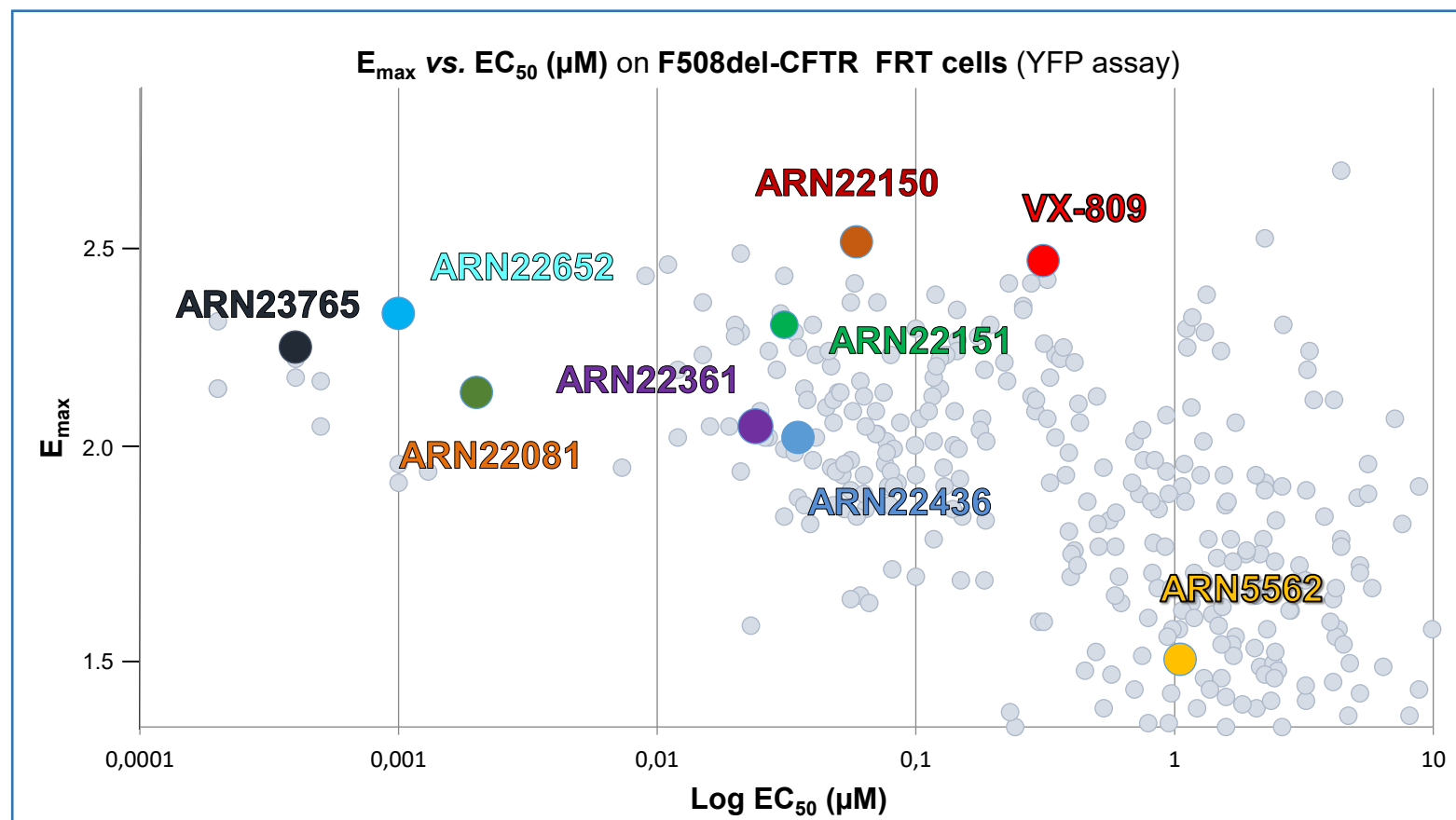
From ARN22081 to an optimized Lead



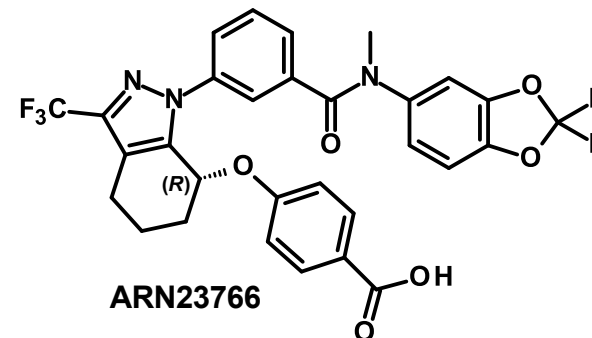
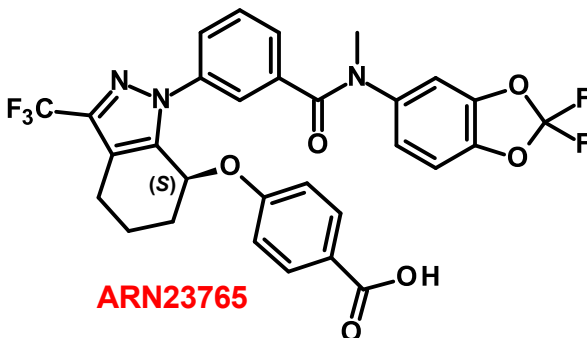
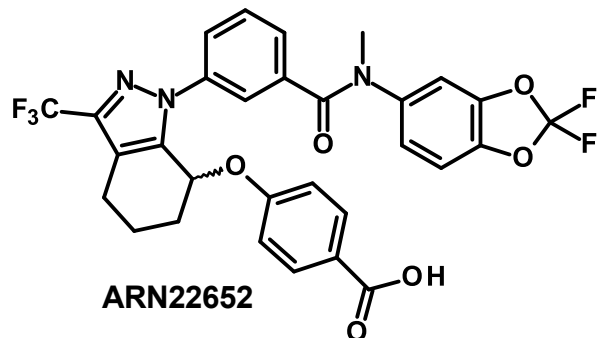
ARN23765 is the eutomer of ARN22652



~ 400 compounds
synthesized



ARN23765 is a very potent F508del-CFTR corrector

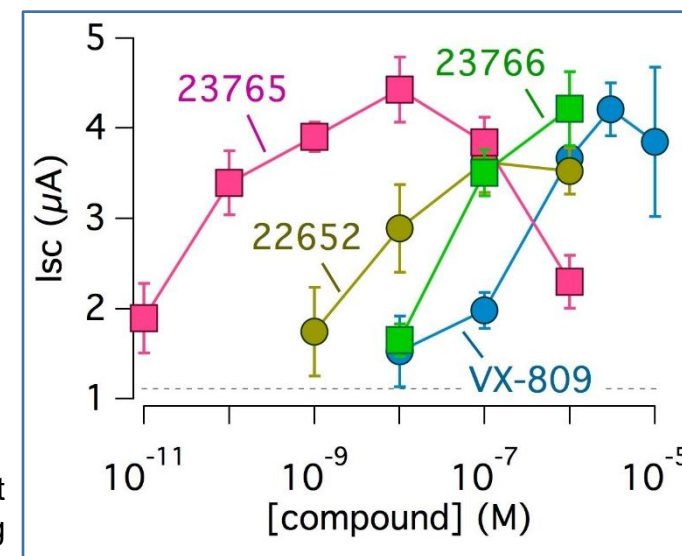


Activity data in cells

ID	CFBE41o- cells		FRT cells	
	EC ₅₀ (μM)	E _{max}	EC ₅₀ (μM)	E _{max}
ARN22652	0.006	2.68	0.001	2.4
ARN23765	0.0004	2.49	0.0004	2.28
ARN23766	0.069	2.74	0.063	2.18
VX-809	0.205	2.5	0.319	2.53

Short-circuit current
recording in Ussing
chamber.

F508del/F508del HBE cells

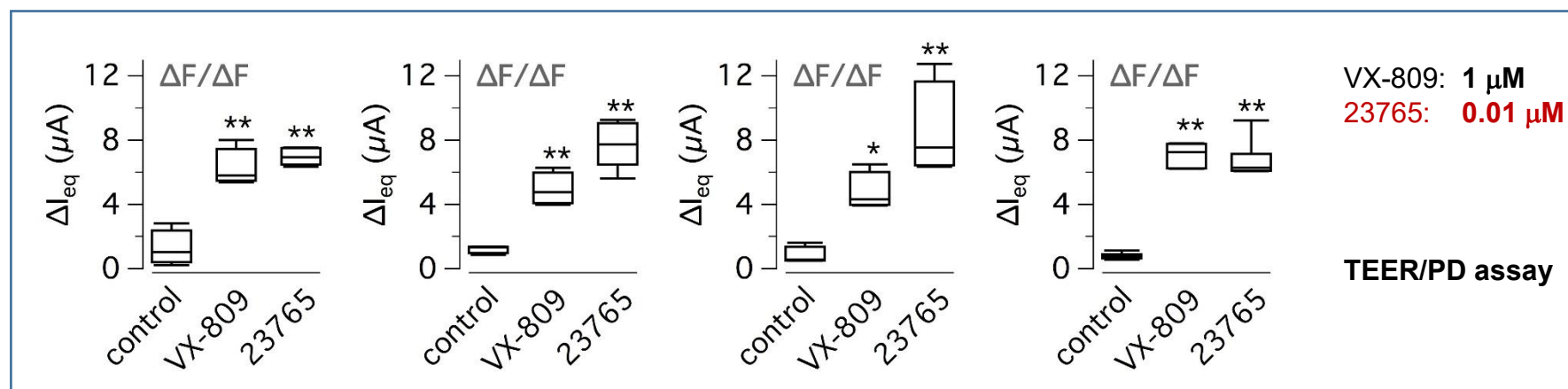


ARN23765 EC₅₀: **0.038 nM**
VX-809 EC₅₀: **~200 nM**

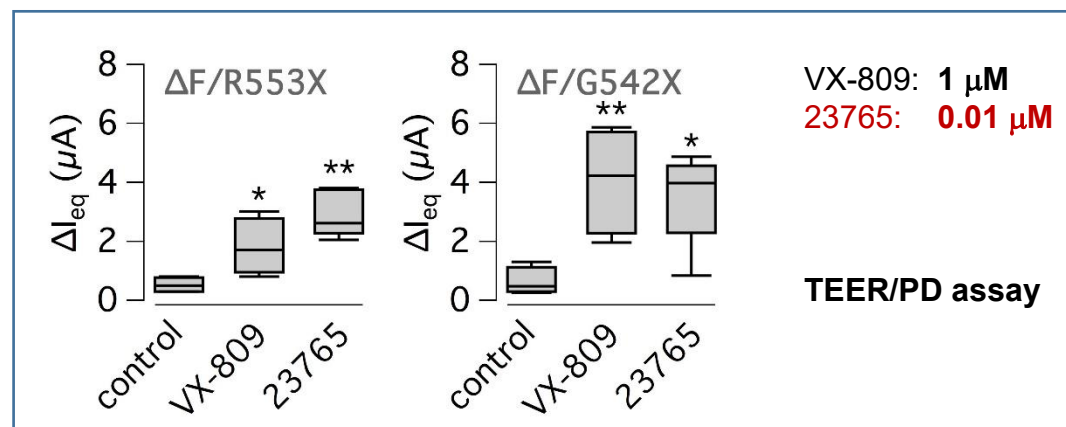
(R) and (S) configuration of enantiomers determined from X-ray structures.

ARN23765 partially rescues the activity of mutant CFTR

CFTR activity in primary bronchial epithelial cells from different CF patients homozygous for the F508del mutation;



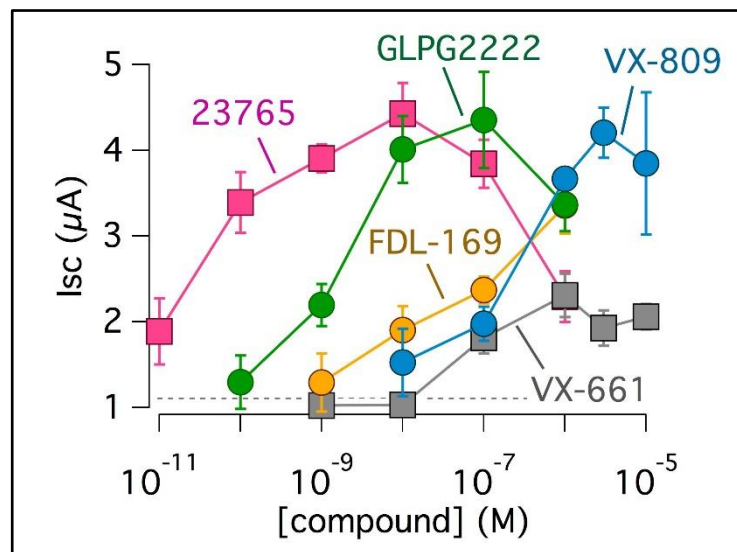
CFTR activity in primary bronchial epithelial cells from CF compound heterozygous patients.



ARN23765 has higher potency than CF drugs

ARN23765 has higher potency than:

- the drugs VX-809 (lumacaftor) and VX-661 (tezacaftor),
- the correctors GLPG-2222 and FDL-169, former drug candidate from Galapagos/AbbVie and Flatley Labs.



Dose-response study in primary human bronchial epithelial cells from CF patient homozygous for the F508del CFTR mutation (Ussing chamber).

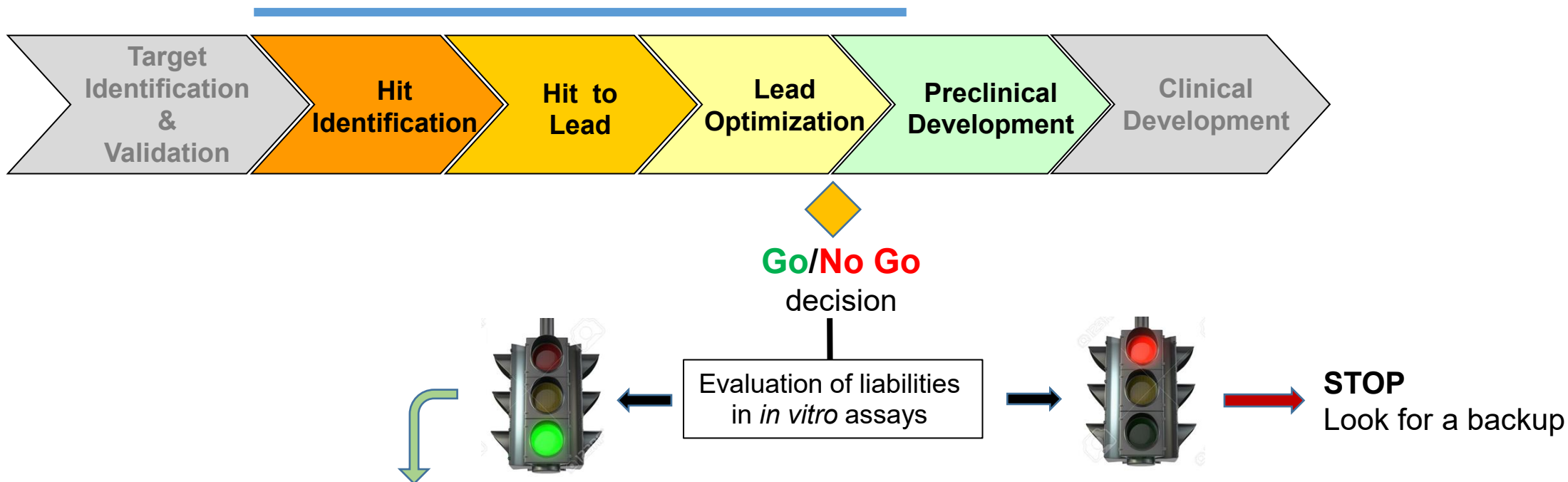
ARN23765 EC₅₀: **0.038 nM**
 VX-809 EC₅₀: ~**200 nM** **>5,000 fold more potent**

Search for new modulators of mutant CFTR

Approach: HTS of the IIT compound collection
Evolution of Hits up to an optimized Lead

Goal: Nominate a Preclinical Development Candidate (PDC)

TFCF Project



Preclinical Development Candidate: ARN23765



De-risking of ARN23765: *in vitro* drug-like properties

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ARN23765 represents an improvement over ARN22081 in terms of drug likeness.

ARN23765 did not inhibit hERG tail currents by greater than 10% at 10 μ M when tested in an electrophysiological assay on HEK cells.

ARN23765 did not show any alert when tested at 10 μ M in a panel of 44 targets¹ relevant for potential toxicities, including receptors, ion channels, monoamine transporters, and enzymes.

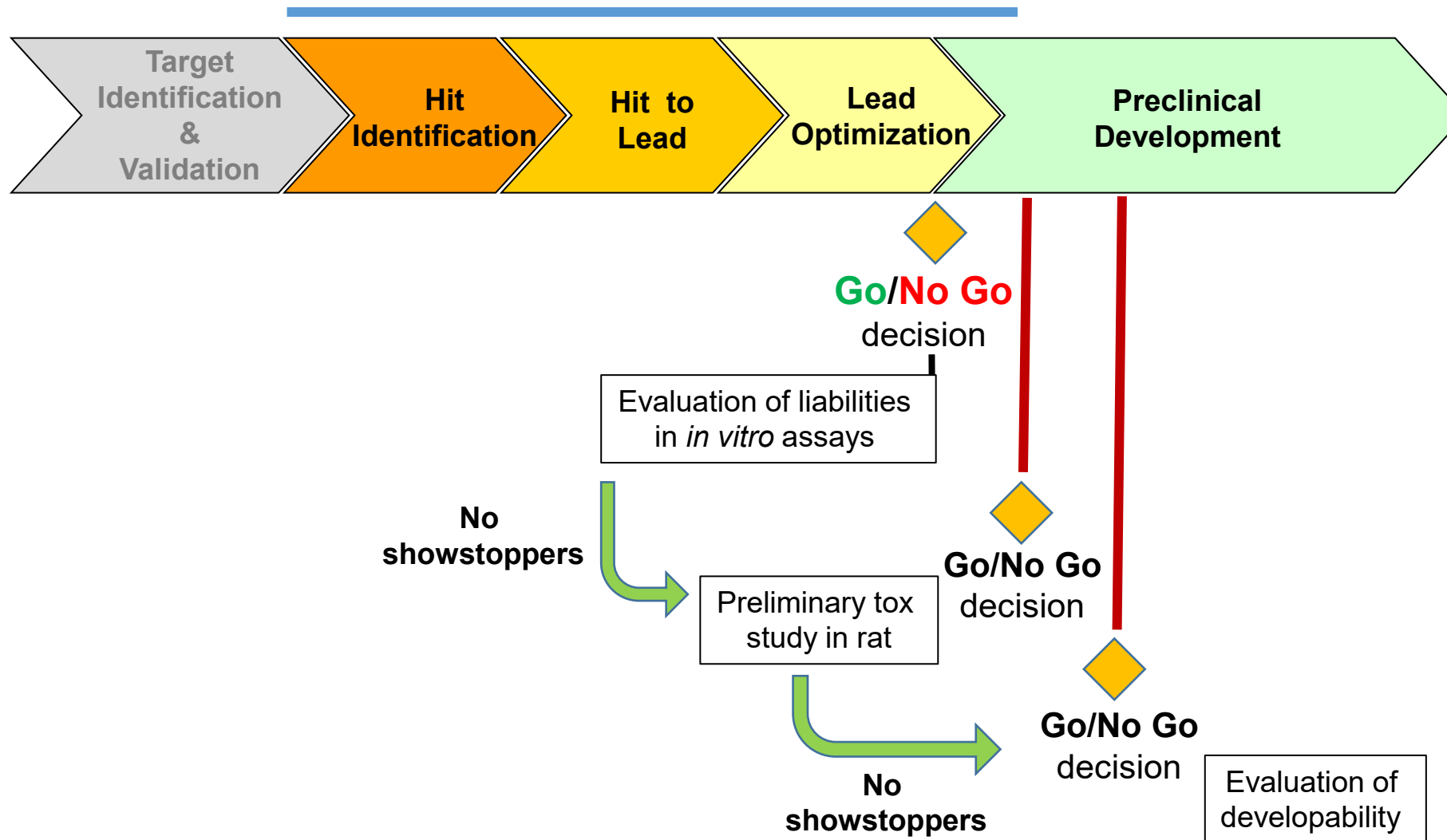
ARN23765 did not show any significant mechanism-based or time-dependent inhibition of CyP1A, CyP2C9, CyP2C19, CyP2D6, and CyP3A.

ARN23765 was negative in the Ames fluctuation assay (*Salmonella typhimurium*) and in the Micronucleus assay (*CHO cells*).

1) Eurofins SafetyScreen 44 Panel

De-risking of ARN23765

TFCF Project



De-risking of ARN23765: *in vivo* data

No showstopper found in a 7-day Dose Range Finding toxicity study in SD male rats.

ARN23765 distributed well to lungs and pancreas following oral administration to Sprague-Dawley rats.

Tissue	Tissue/Plasma at 1h	Tissue/Plasma at 4h
Lung	1.33	1.16
Pancreas	1.45	1.43
Brain	0.04	0.056

Under the same experimental conditions, the lung-to-plasma ratio of VX-809 (lumacaftor) is reported to be **0.18**.

ARN23765 was dosed orally in Sprague-Dawley rats at 300 mg/kg. Plasma and tissue samples were collected after 1 and 4 hours.

ARN23765 was tolerated up to 300 mg/kg/day in a 14-day Dose Range Finding toxicity study in rat (both sexes).



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Search for new modulators of mutant CFTR

Approach: HTS of the IIT compound collection
Evolution of Hits up to an optimized Lead

Goal: Nominate a Preclinical Development Candidate (PDC)

Preclinical Development Candidate: ARN23765

Intellectual Property: 4 PCT applications filed claiming compounds and their use

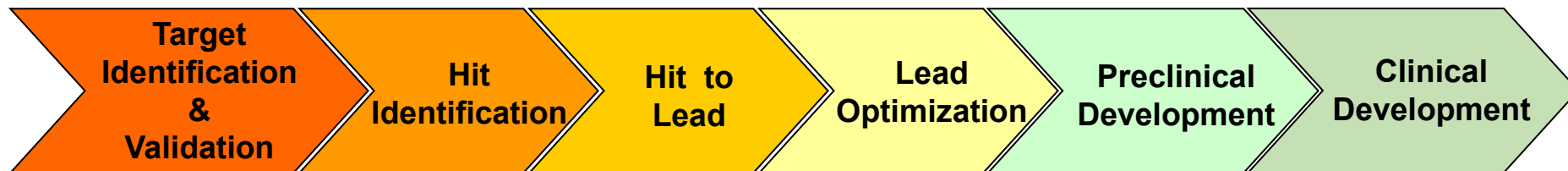
2 granted US patents claiming ARN23765 and analogs

US No. 10,745,407 and US No. 10,968,225

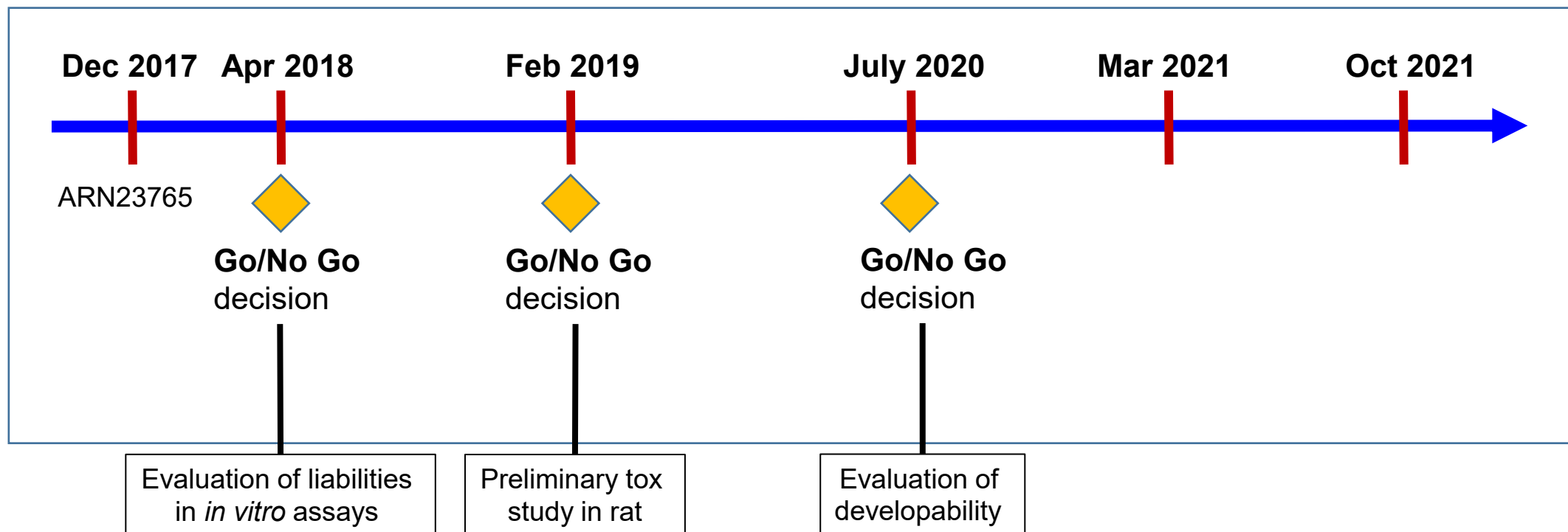
European Patent No. EP3596074A1 claiming ARN23765 and analogs

WO2022/175889 claiming a new synthetic process for ARN23765

De-risking of ARN23765: timeline



TFCF Project

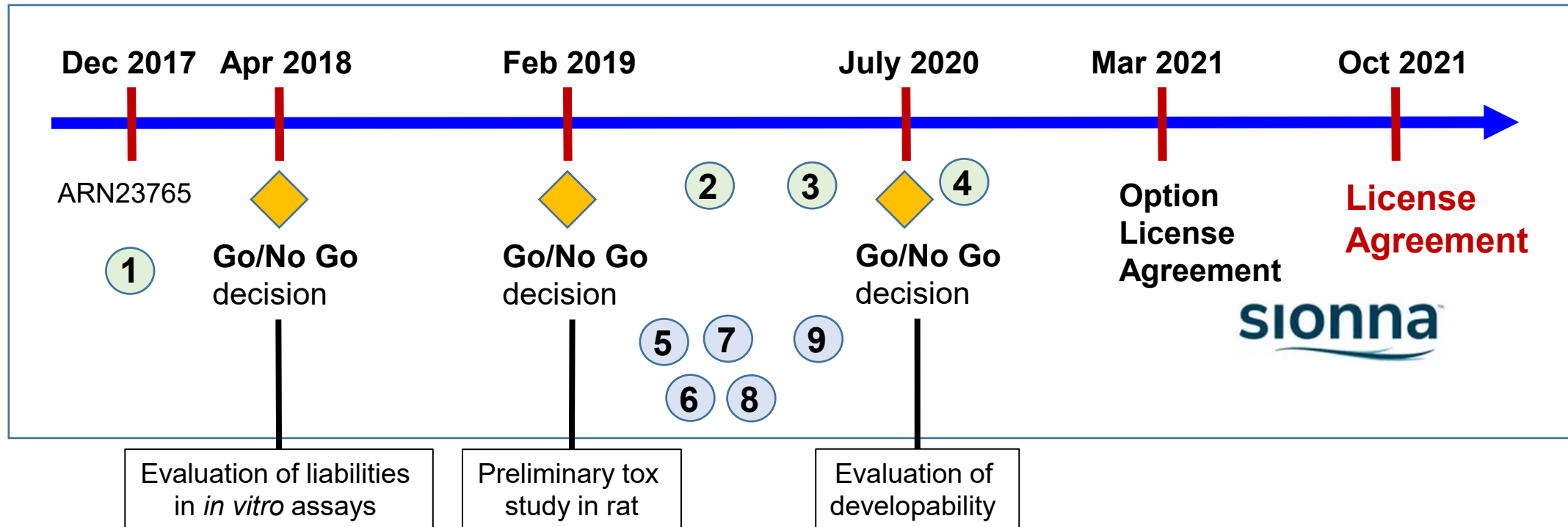


ARN23765 licensed to a US biopharma company

Search for a preclinical development partner

TFCF project presented to

- **4 Companies**
- **5 Investors**



Conclusions

Phenotypic screening of the IIT compound collection identified a few hits active as correctors in 2 cell lines expressing F508del-CFTR.

One of the hits was evolved up to the identification of a candidate for Preclinical Development, i.e., ARN23765.

ARN23765 shows picomolar EC_{50} when tested in primary human bronchial epithelial cells from a CF patient homozygous for the F508del mutation in CFTR.

ARN23765 shows higher potency than the corrector drugs VX-809 and VX-661.

Initial de-risking of ARN23765, *in vitro* and *in vivo*, did not highlight any showstopper for the progression of the compound to full Preclinical Development.

ARN23765 was licensed to Sionna Therapeutics in 2021.



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Rosalia Bertorelli

Ilaria Penna

Natasha Margaroli

Debora Russo

Raffaele Spanò

Maria Summa

Sine Mandrup Bertozzi

Andrea Armirotti



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Nicoletta Pedemonte

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sulla fibrosi cistica - onlus*
italian cystic fibrosis research foundation