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

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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 US[1] and 1 in ca. 2500 to 1 in ca. 6000 in European countries[2].

Worldwide, **circa 95,000 persons suffer from CF**
ca. 54,000 in Europe, ca. 32,000 in USA, ca. 4,300 in Canada, ca. 4,300 in Australia + New Zealand.

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.

Over 2000 mutations have been described in the CFTR gene. Of these, 360 are CF-causing[3].

1) Stephenson et al., *J. Cystic Fibrosis* **2023**, 22, 443-449; 2) P. M. Farrell, *J. Cystic Fibrosis* **2008**, 7, 450-453
3) The Clinical and Functional Translation of CFTR (CFTR2). Available at: https://cftr2.org.

Cystic Fibrosis: CFTR

CFTR protein consists of 1480 amino acids.

CFTR protein is a member of the ATP-binding cassette (ABC) transporters family.

ABC transporters are multi-domain membrane proteins that mediate diverse ATP-driven transport processes[1].

There are 48 distinct human ABC transporters, which belong to various subfamilies.

Domain arrangement of ABC transporters

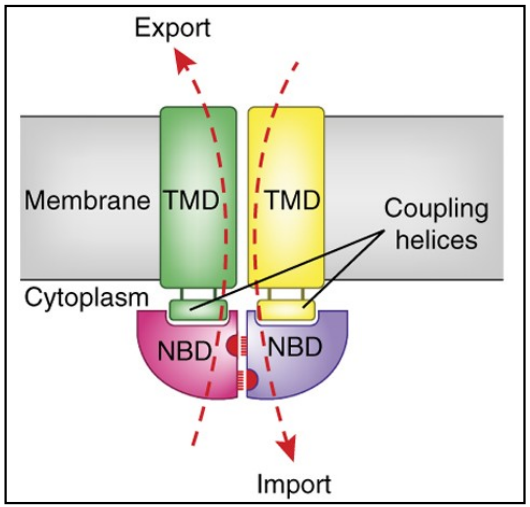


Image adapted from Ref. 1

Some human ABC transporters have functions other than substrate translocation:

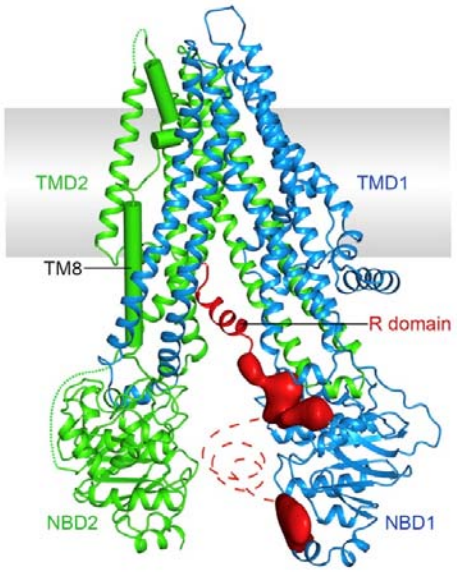
CFTR is an ion channel transporting Chloride and Bicarbonate anions in multiple organs.

CFTR is widely expressed in epithelial cells, regulating salt and fluid homeostasis in a variety of tissues.

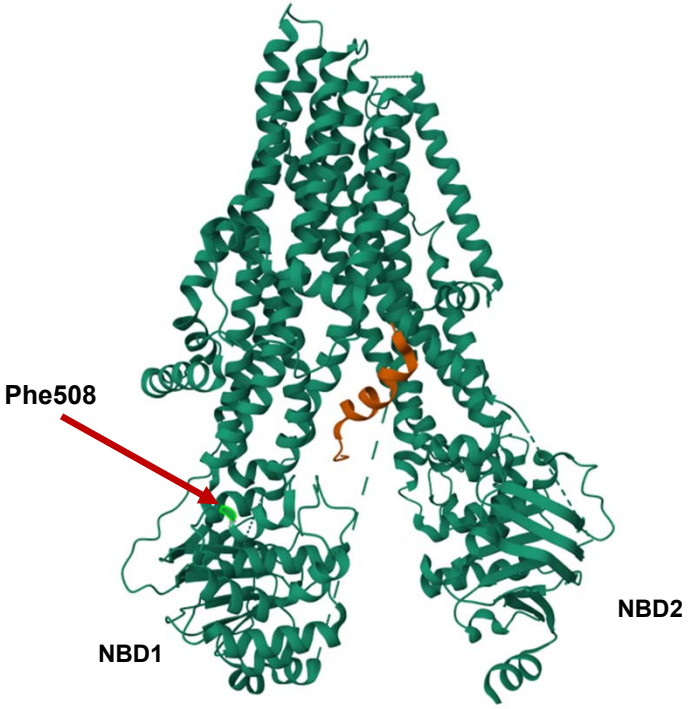
1) K.P. Locher *Nat. Struct. Mol. Biol*. **2016**, 23, 487 - 493

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 USA[1].



Molecular structure of human CFTR determined in the dephosphorylated, ATP-free form
Liu et al., *Cell*, 2017, 169, 85–89



PDB structure: 5UAK

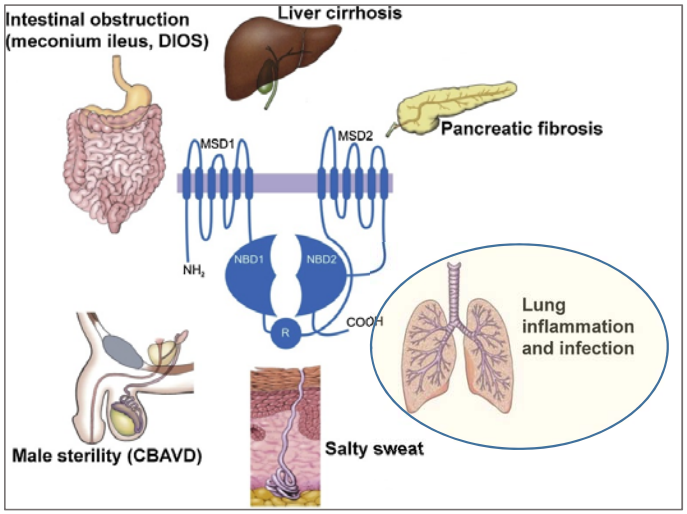
1) Data from: *European Cystic Fibrosis Society*, Patient Registry, Annual Data Report 2021, and *Cystic Fibrosis Foundation*, 2021 Patient Registry

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.

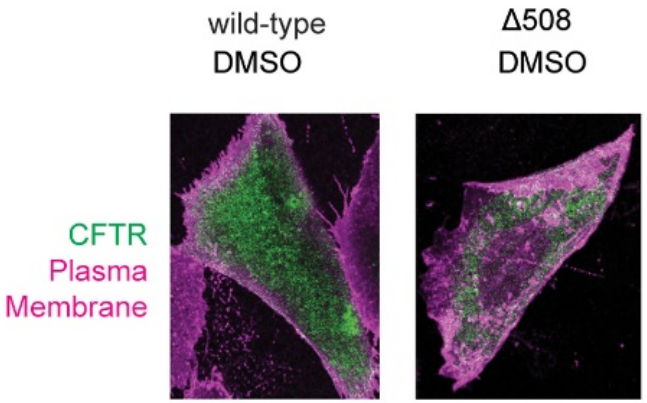


Adapted from Ikpa et al., *Int. J. Biochem. Cell Biol*. 2014, 52, 192-200

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

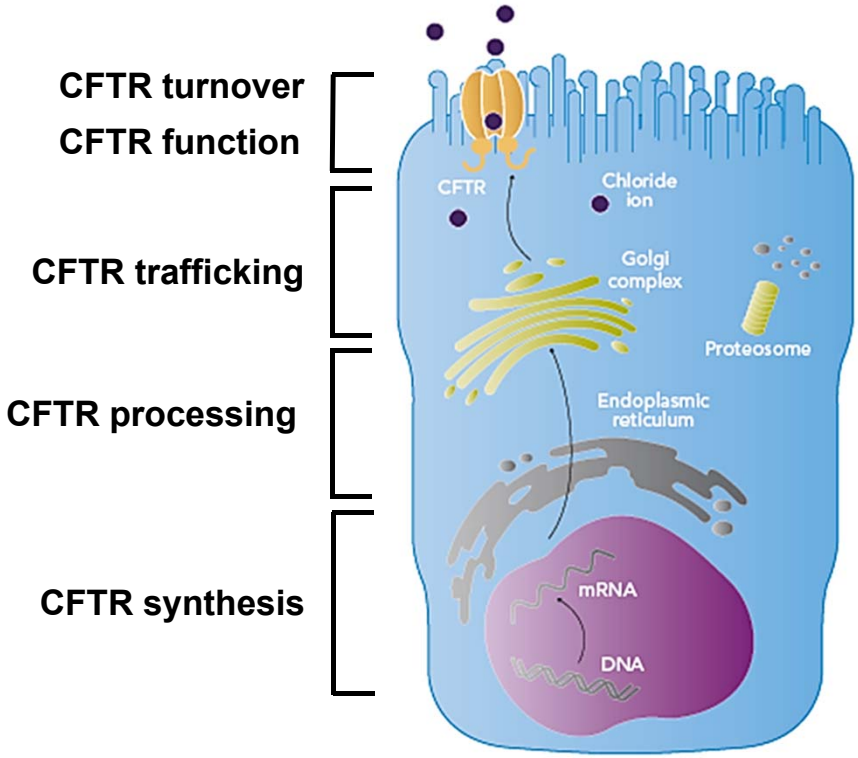
Mutations in the CFTR gene affect different processes

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.

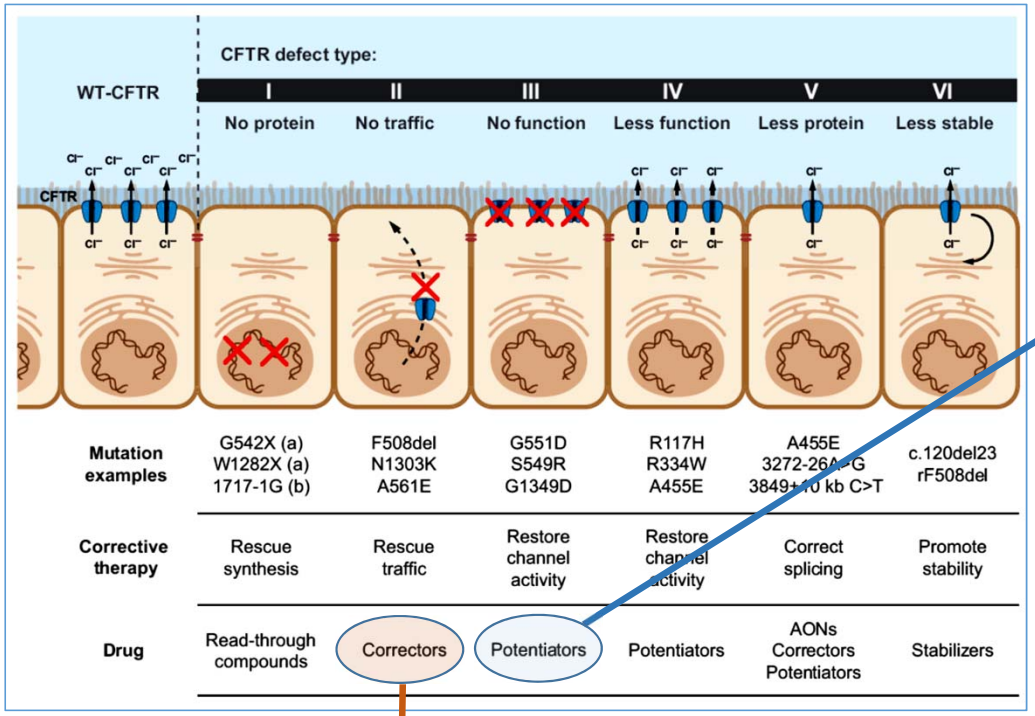
Image adapted from: Fiedorczuk et al., *Science* 2022, 378, 284 - 290



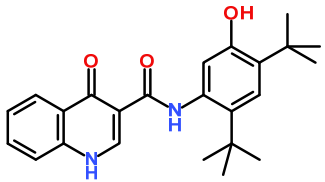
Credit: *Vertex Pharmaceuticals Inc.*

CF disease-modifying therapy:
an example of personalized medicine

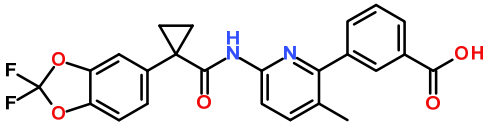
CFTR mutations are classified in 6 classes based on their phenotypic consequences.



Iva
(2012)

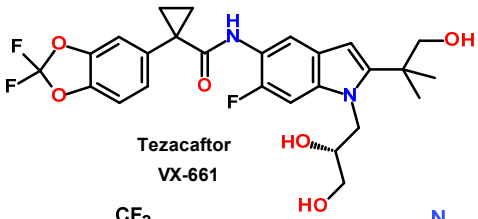


Ivacaftor
VX-770



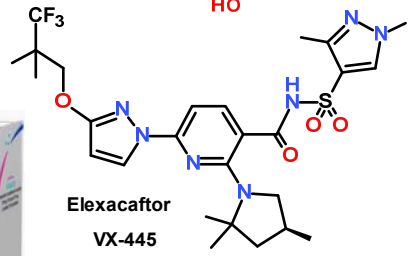
Lumacaftor
VX-809

Iva + Luma
(2015)



Tezacaftor
VX-661

Iva + Teza
(2018)



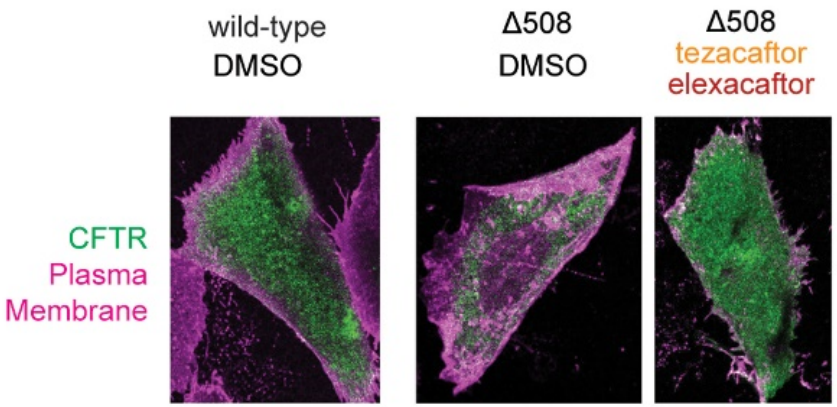
Elexacaftor
VX-445

Iva + Teza + Elexa
(2019)

Adapted from M.D. Amaral, *Journal of Internal Medicine* **2015**, 277, 155-166

F508del-CFTR protein can be rescued with correctors

Treatment of F508del-CFTR CHO cells with the combination of correctors tezacaftor plus elexacaftor allowed mutant CFTR to 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.

Image adapted from: Fiedorczuk et al., *Science* 2022, 378, 284 - 290

Search for new modulators of mutant CFTR

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. Galietta (now at TIGEM)

Nicoletta Pedemonte

Istituto Italiano di Tecnologia (IIT)

Tiziano Bandiera

Project funded by FFC

Fondazione Ricerca Fibrosi Cistica - Onlus
italian cystic fibrosis research foundation

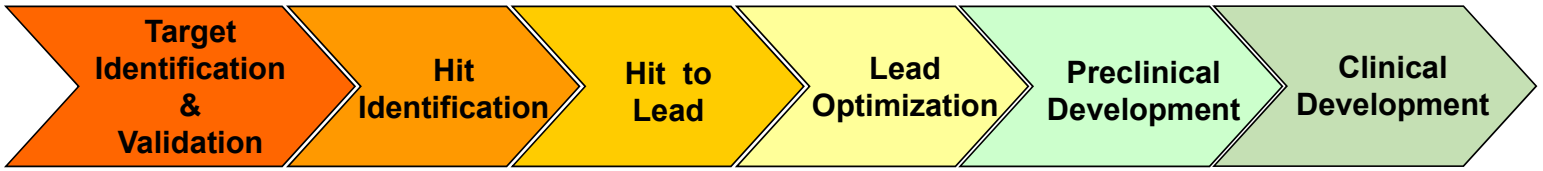
http://www.fibrosicisticaricerca.it/

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)



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).

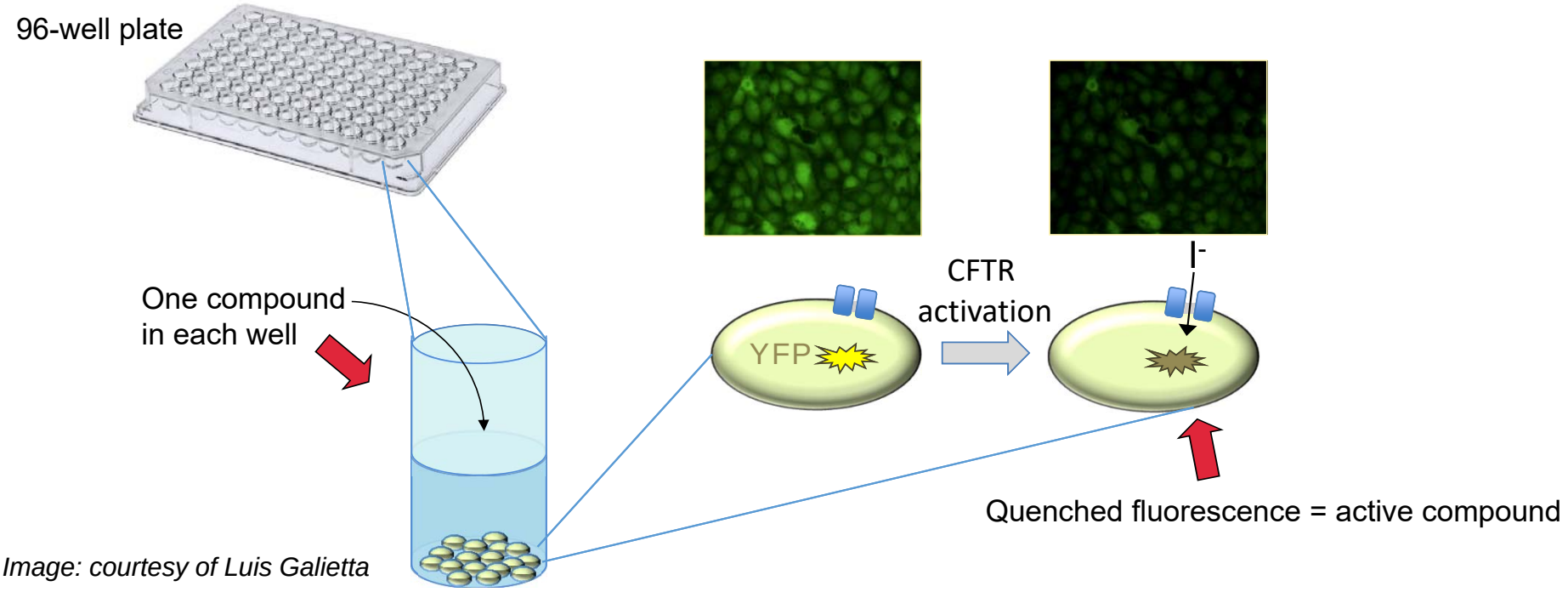
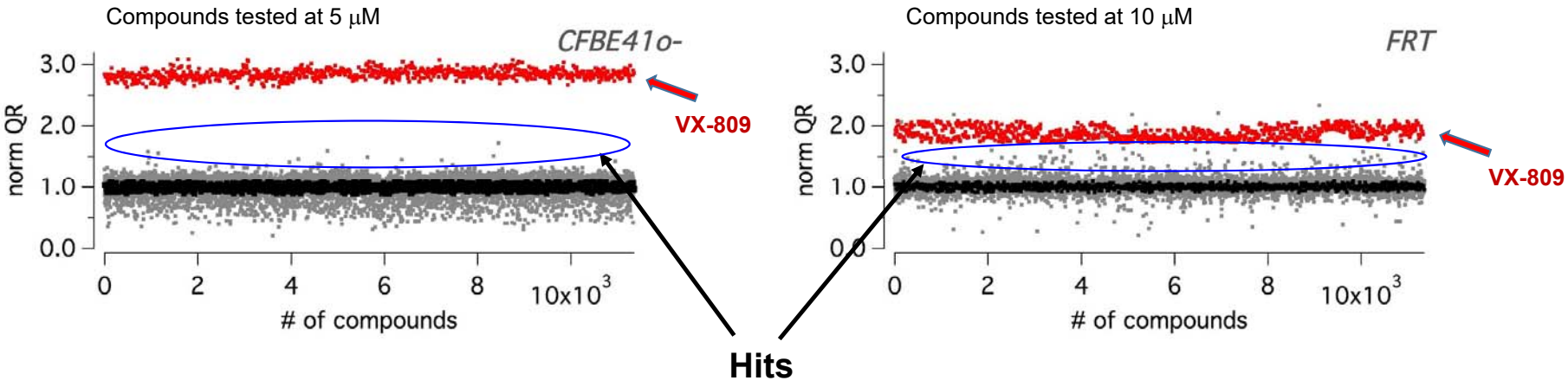


Image: courtesy of Luis Galietta

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

Phenotypic HTS: results

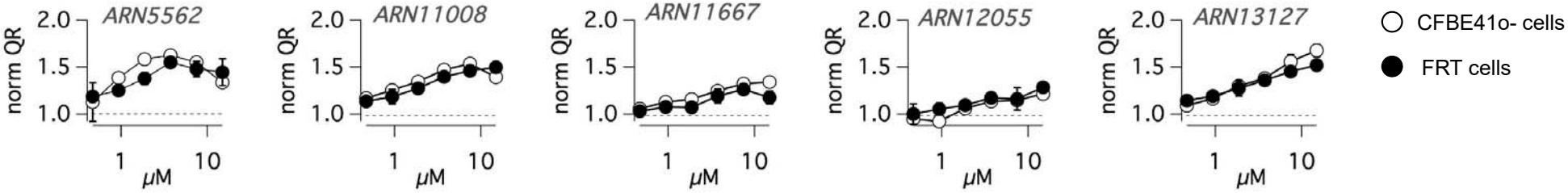
The hit rate was higher in FRT than in CFBE41o- cells.



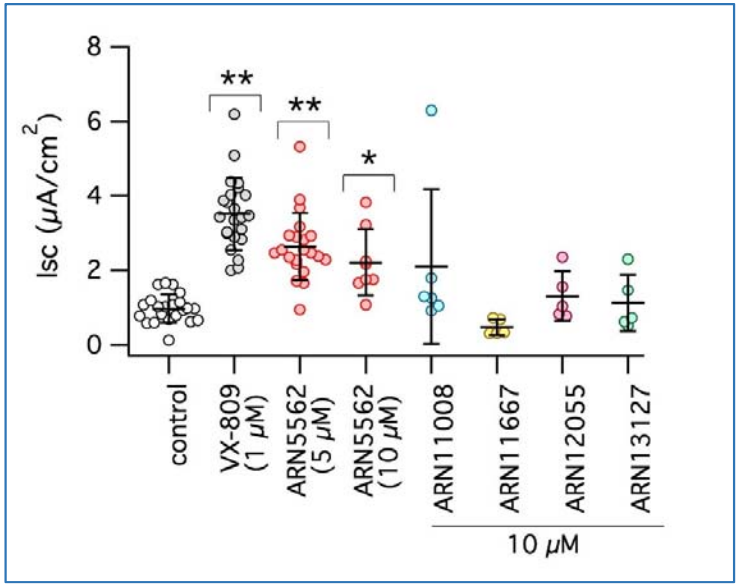
Pedemonte, Bertozzi... Bandiera* & Galietta*, *Science Advances* 2020; **6**: eaay9669

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.



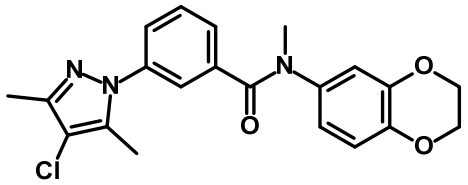
Short-circuit current recording in Ussing chamber.

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

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

Pedemonte, Bertozzi... Bandiera* & Galietta*, *Science Advances* 2020; **6**: eaay9669

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**

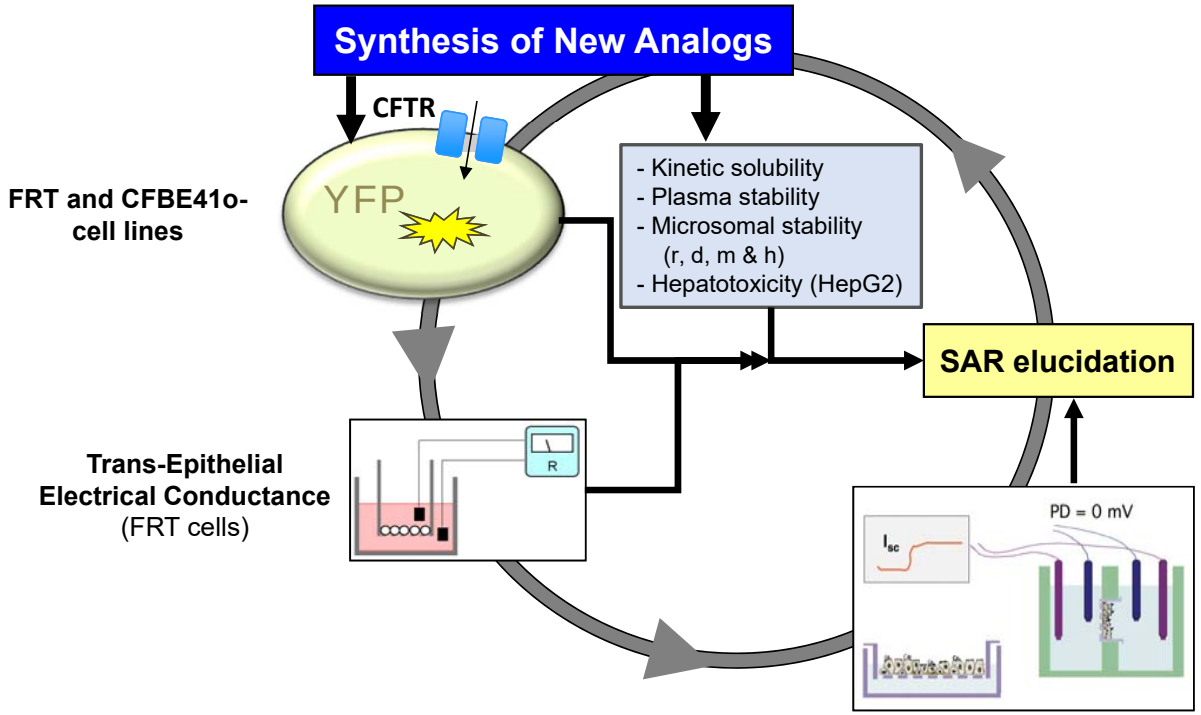
Drug-like properties

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

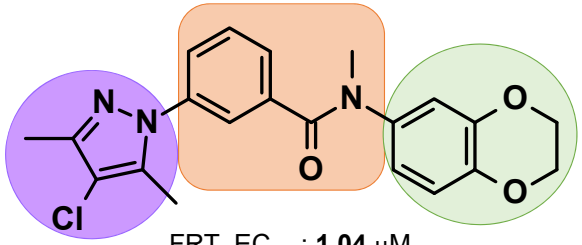
All new compounds were tested on both FRT and CFBE41o- cells.

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

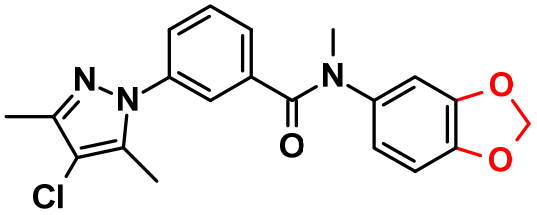


Hit-to-Lead: evolution of hit ARN5562

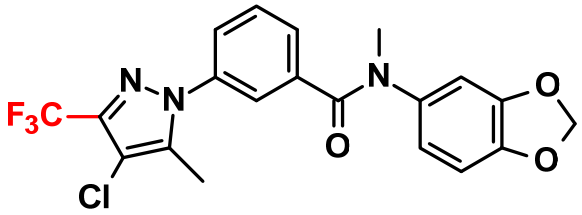
Certain modifications in each of the three parts of the hit were beneficial for activity.



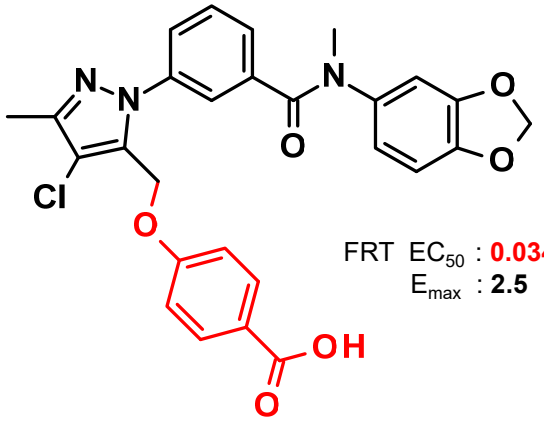
FRT EC_{50} : **1.04** µM
E_{max} : **1.56**



FRT EC_{50} : **0.76** µM
E_{max} : **2.01**



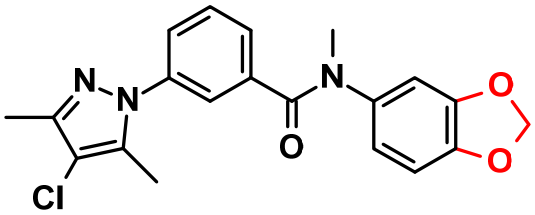
FRT EC_{50} : **0.34** µM
E_{max} : **2.2**

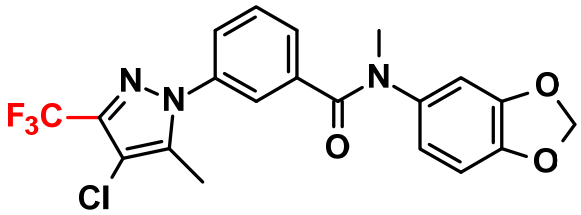


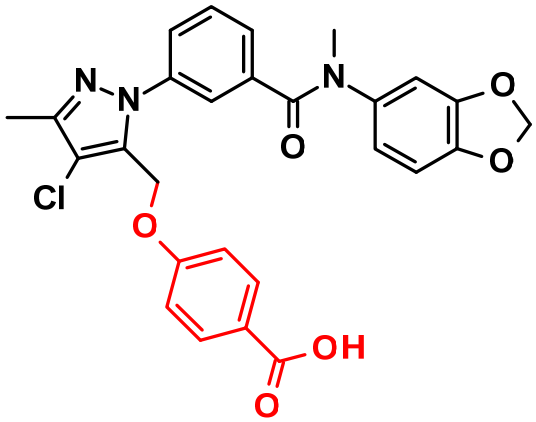
FRT EC_{50} : **0.034** µM
E_{max} : **2.5**

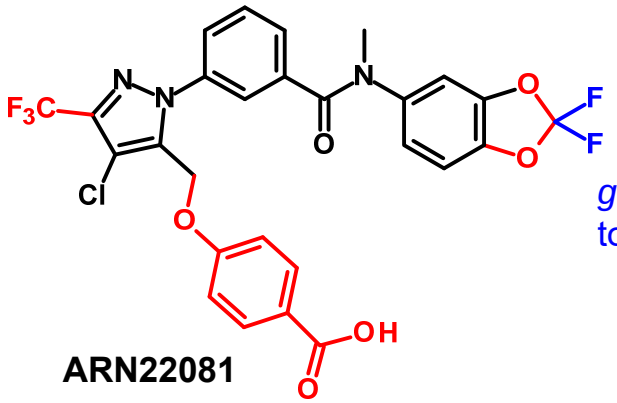
Hit-to-Lead: nomination of a Lead

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







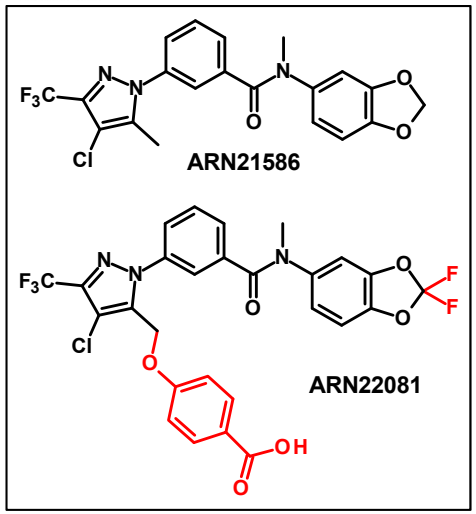


gem-di-F introduced to block metabolism

ARN22081

Lead Compound

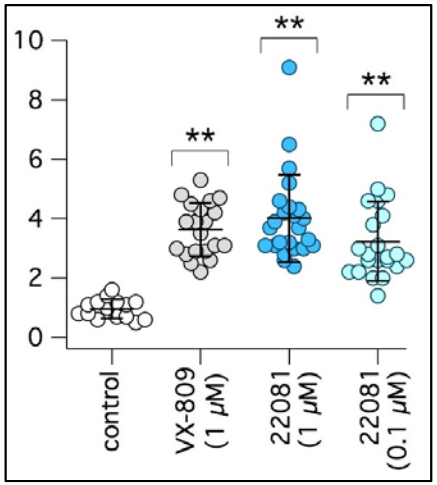
ARN22081 was the lead compound for this series



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**

F508del/F508del HBE cells

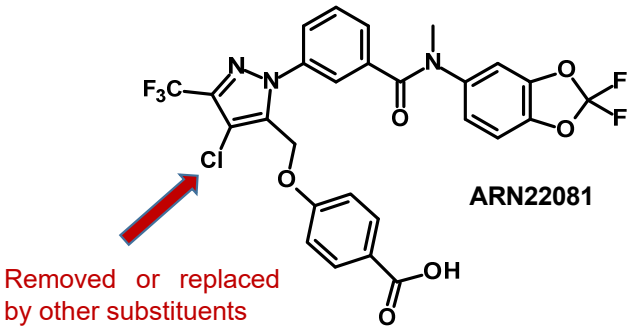


Short-circuit current recording in Ussing chamber.

Drug-like properties

ID	Kinetic Solubility	Metabolic stability (NADPH)				Hepatotoxicity	Caco-2 permeability	
	PBS pH 7.4	Rat LM	Dog LM	Monkey LM	Human LM	HepG2	A-B	B-A
	(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**

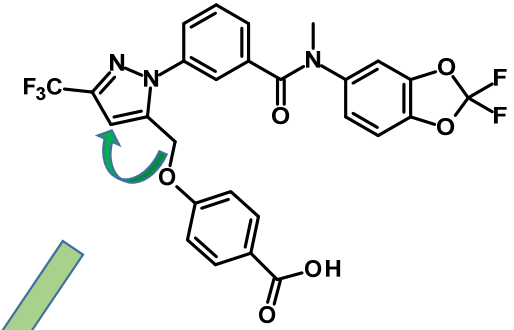
From ARN22081 to an optimized lead



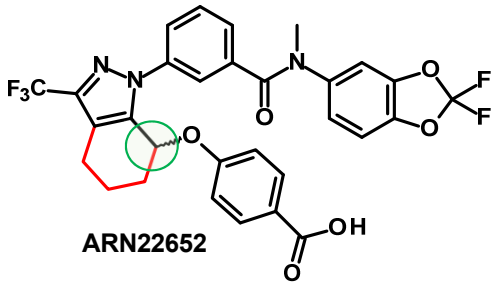
ARN22081

Removed or replaced by other substituents

CFBE41o- EC_{50} : **0.0014** μM
E_{max} : **2.81**



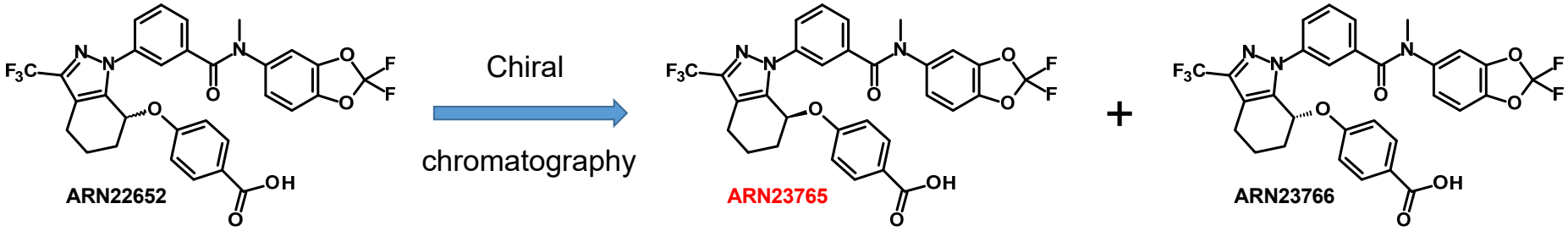
CFBE41o- EC_{50} : **0.064** μM
E_{max} : **2.52**



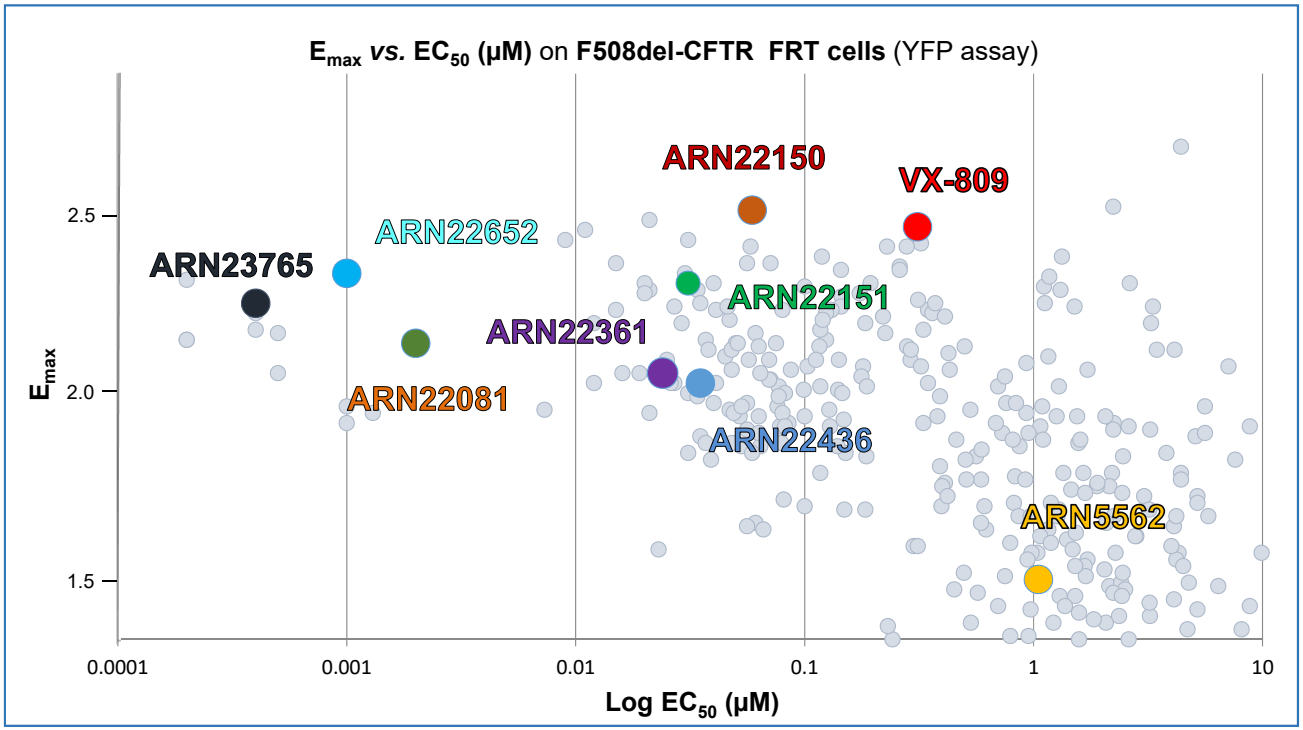
ARN22652

CFBE41o- EC_{50} : **0.006** μM
E_{max} : **2.68**

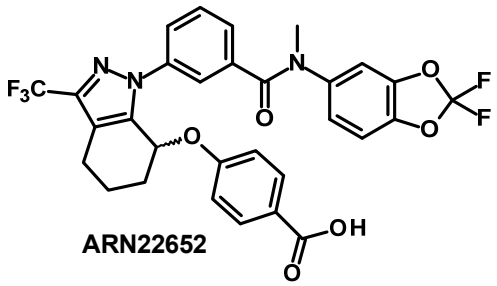
ARN23765 is the eutomer of ARN22652

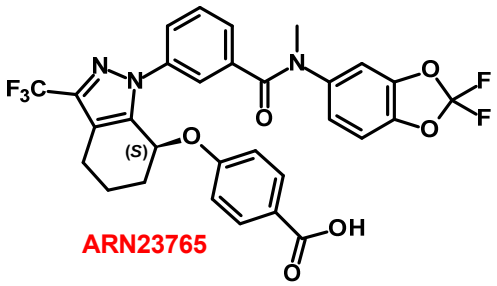


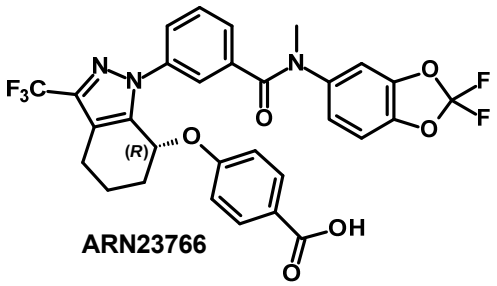
~ 400 compounds synthesized



ARN23765 is a very potent F508del-CFTR corrector



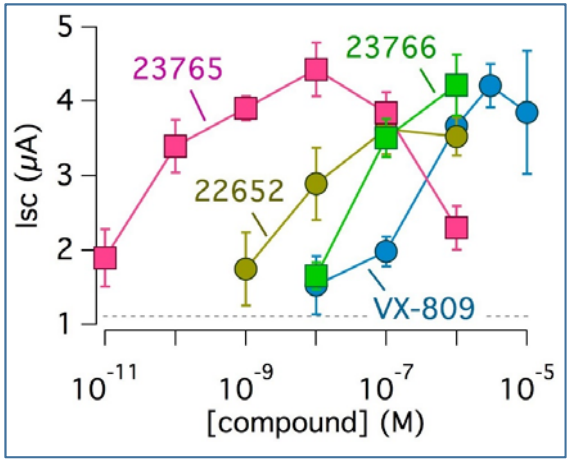




Activity data in cells

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**

F508del/F508del HBE cells



Short-circuit current recording in Ussing chamber.

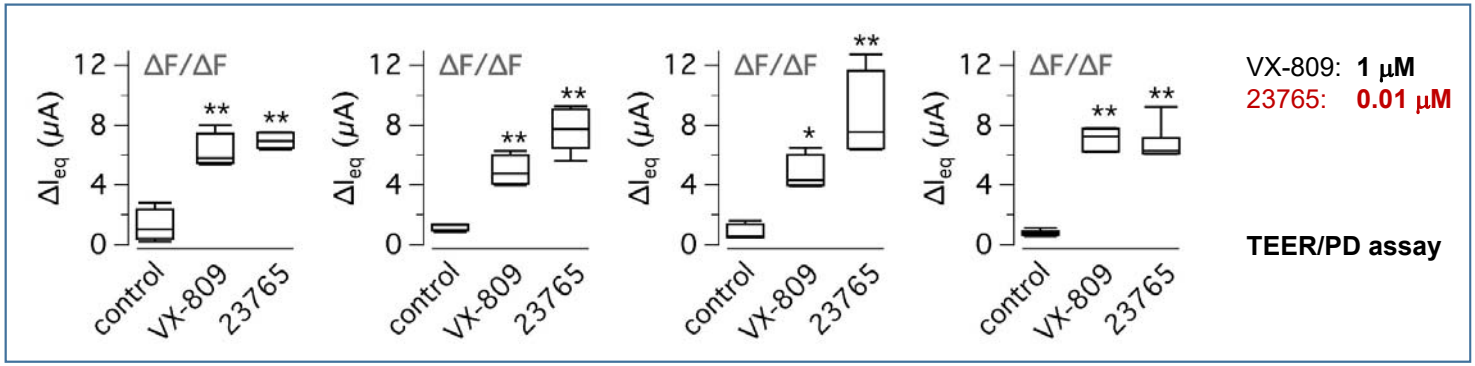
ARN23765 EC_{50} : **0.038** nM

VX-809 EC_{50} : **~200** nM

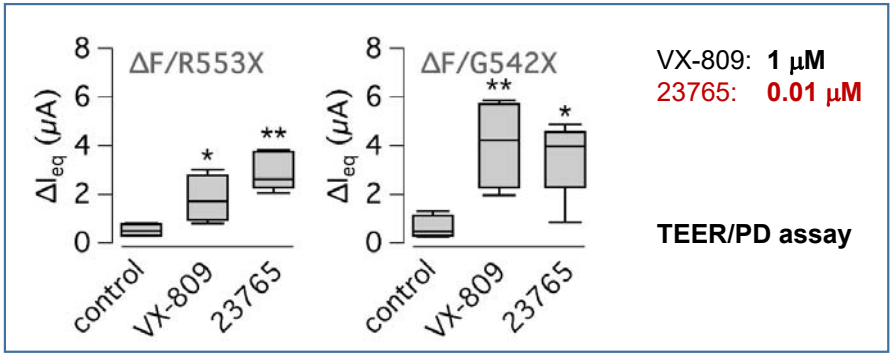
(*R*) and (*S*) configuration of enantiomers determined from X-ray structures.

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.



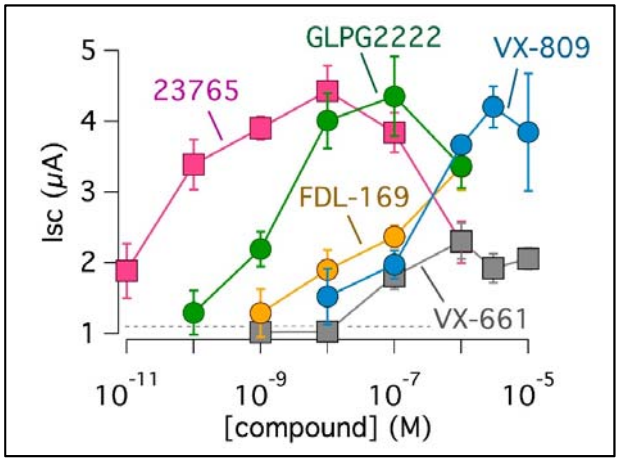
*P < 0.05 and **P < 0.01 (ANOVA with Dunnett's post hoc test; n = 5 to 6)

Pedemonte, Bertozzi... Bandiera* & Galietta*, *Science Advances* 2020; **6**: eaay9669

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 EC_{50} : **0.038** nM
VX-809 EC_{50} : **~200** nM

>5,000 fold more potent

Pedemonte, Bertozzi... Bandiera* & Galietta*, *Science Advances* 2020; **6**: eaay9669

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

ID	Kinetic Solubility	Hepatotoxicity	Caco-2 permeability	
	PBS pH 7.4	HepG2	A-B	B-A
	(μM)	(% Survival)	(10^{-6} cm/s)	(10^{-6} cm/s)
ARN22081	**>250**	**>80**	**4.0**	**0.5**
ARN23765	**228 ± 3**	**>80**	**9.9**	**0.7**
VX-809	**>250**	**>80**	**n.t.**	**n.t.**
VX-661	**151**	**>80**	**n.t.**	**n.t.**

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

ARN23765 did not show any alert when tested at 10 μM in a panel of 44 targets[1] 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: *in vivo* data

ARN23765 showed ca. 20% oral bioavailability in Sprague-Dawley 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**

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

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

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

Search for new mutant CFTR modulators

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

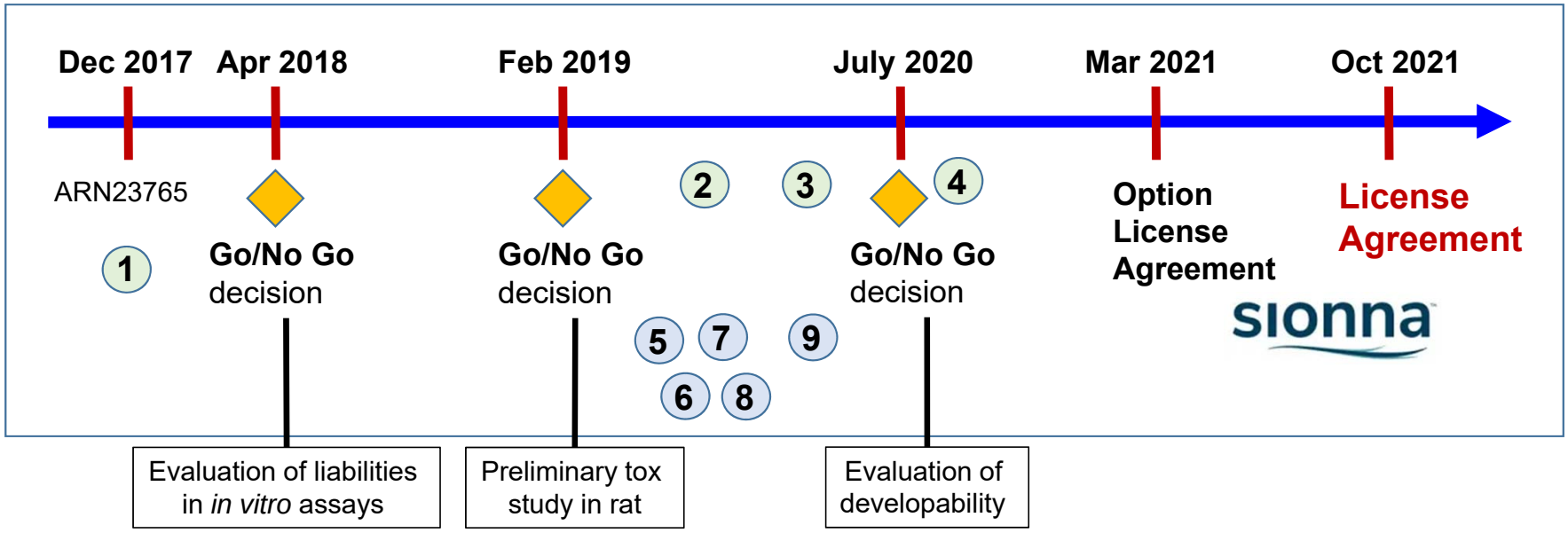
WO2022/175889 claiming a new synthetic process for ARN23765

ARN23765 licensed to a US biopharma company

Timeline for ARN23765 de-risking and search for a preclinical development partner

TFCF project presented to

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



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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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