

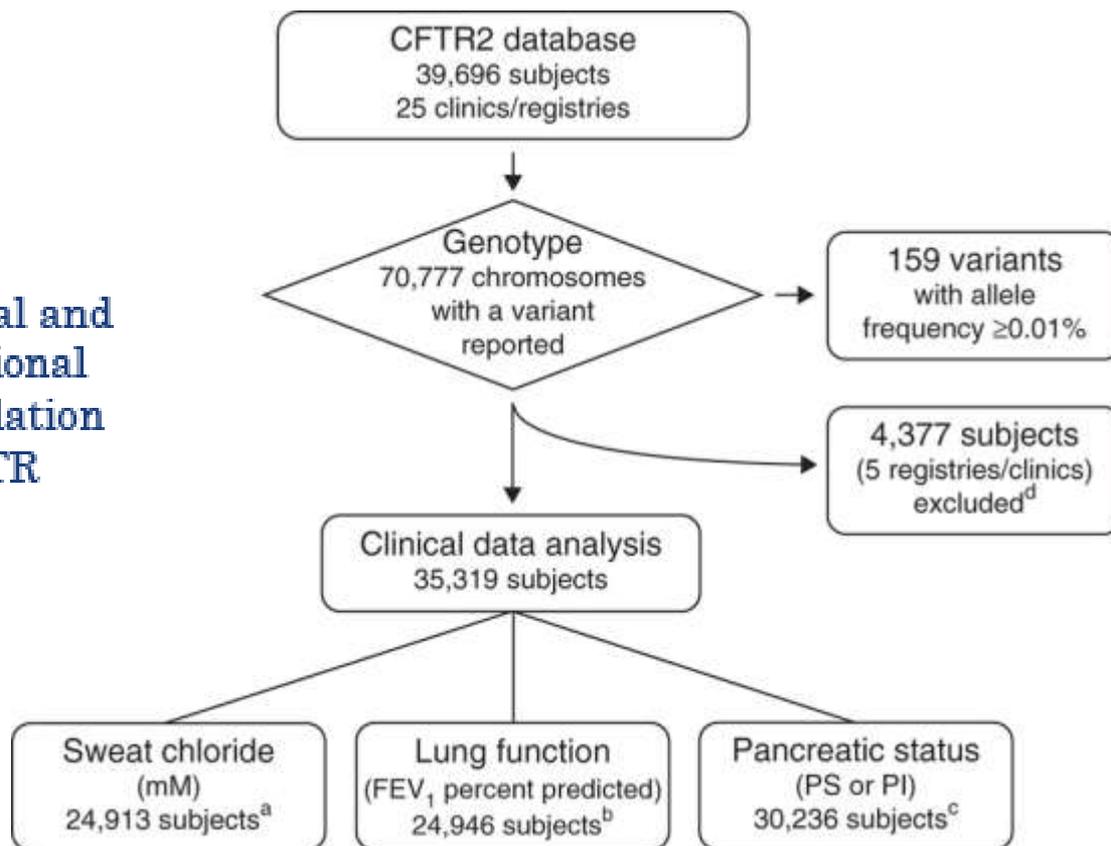
Cystic Fibrosis Translational Research: Analysis of Rare CFTR Alleles

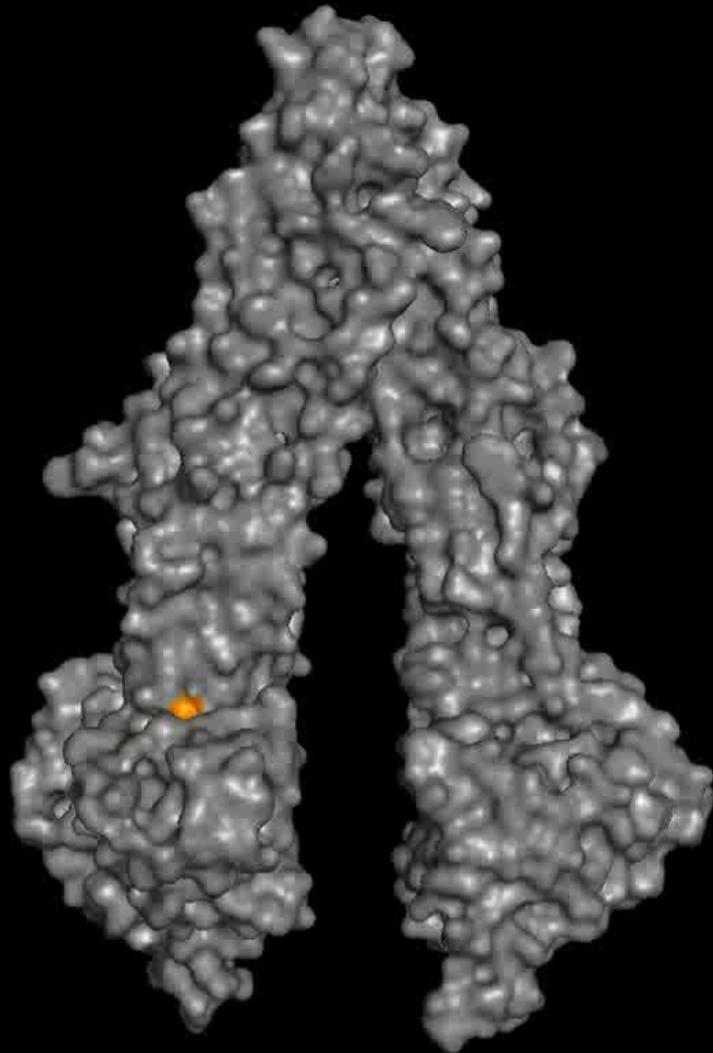
Eric J. Sorscher, MD
Emory University
Atlanta, Georgia

November 5, 2015



Clinical and
Functional
Translation
of CFTR





Folding Consortium Capabilities

CFTR dynamic folding mechanism

- *In vitro* NBD1 folding, subdomain interactions
- Cotranslational CFTR assembly
- MSD stabilization and CFTR biogenesis
- NBD stability
- CFTR domain interaction
- Corrector MOA
- CFTR2 pathogenesis



Assays and Reagents for drug development

- CFTR domain constructs
- ASL monitoring in primary HBE
- FRT flp-in™ and other cell lines
- HRP and HA reporter constructs
- NBD stability and yield
- Monitoring CFTR biogenesis with FAP
- Identifying CFTR subdomain targets for small molecule correction

Cellular defects and CF pathogenesis

- Cellular contributors to CFTR2 folding/maturation
- Peripheral stability
- Quality control machinery
- Emerging strategies for CFTR2 rescue
- Ubiquitination and augmenting correctable CFTR
- SUMOylation and CFTR correction

Domain	Folding defect		Legacy Name
N-terminal (1-79)	No defect		
	Mild (3)	1	E56K
		2	P67L
		3	R74W
	Severe		
	Nonsense Mutations (3)	4	Q39X
		5	E60X
		6	R75X

TMD1(80-389)			
No defect (4)	7	R334W	
	8	T338I	
	9	R347H	
	10	R352Q	
Mild (11)	11	D110H	
	12	R117C	
	13	R117H	
	14	G178R	
	15	H192G	
	16	V232D	
	17	F311del	
	18	I336K	
	19	S341P	
	20	R347P	
	21	Q359K/ T360K	
Severe (7)	22	G85E	
	23	G91R	
	24	E92K	
	25	H199Y	
	26	P205S	
	27	L206W	
	28	L227R	
Nonsense Mutations (5)	29	E92X	
	30	Q98X	
	31	Y122X	
	32	Q220X	
	33	G330X	

NBD1(390-673)			
No defect (2)	34	S549N	
	35	G551D	
Mild (3)	36	S549R	
	37	D579G	
	38	D614G	
Severe (14)	39	A455E	
	40	L467P	
	41	L470P	
	42	G480C	
	43	S492F	
	44	I506T	
	45	I507del	
	46	F508del	
	47	V520F	
	48	L558S	
	49	A559T	
	50	R560K	
	51	R560T	
	52	Y569D	
Nonsense Mutations (10)	53	W401X	
	54	S466X	
	55	S489X	
	56	Q493X	
	57	Q525X	
	58	G542X	
	59	R553X	
	60	Q552X	
	61	E585X	
	62	R709X	

R domain (674-829)			
No defect (2)	63	K710X	
	64	L732X	
	65	R764X	
	66	E822X	
Severe - nonsense mutations (4)	67	L927P	
	68	G970R	
	69	S977F	
	70	F1052V	
	71	G1069R	
	72	D1152H	
	73	S945L	
	74	R1070Q	
	75	R1070W	
	76	L1065P	
No folding defect (6)	77	R1066C	
	78	R1066H	
	79	L1077P	
	80	M1101K	
Mild (3)	81	E831X	
	82	W846X	
	83	R851X	
Severe (5)	84	Q890X	
	85	E1104X	
	86	W1089X	
	87	Y1092X	
	88	R1158X	
Nonsense mutations (10)	89	R1162X	
	90	S1196X	

NBD2 (1201-)			
No folding defect (4)	91	I1234V	
	92	G1244E	
	93	S1251N	
	94	D1270N	
Mild			
Severe (1)	95	N1303K	
Nonsense mutations (3)	96	W1204X	
	97	W1282X	
	98	Q1313X	

FRT cells expressing CFTR2 mutants

Cell lines

- Isogenic expression of CFTR
- Clonal cells selected
- mRNA level matched within 50% variation of wild type CFTR expressing cells
- Expression level does not diminish at higher passage numbers (tested up to p31)

Assay

- Isc Measurement: $1\sim 5 \times 10^4$ cells seeded/filter (day 0) and assayed on day 5
- Cells incubated with drug for 48 hours: VX-809(3 μ M), VX-661(3 μ M), G418(250 μ g/mL)
- Drug added on both apical and basolateral sides on day 3
- Serum lot influences the magnitude of CFTR Isc:
 - the same lot used for all CFTR2 assays allowing comparison among different CFTR2 lines

FRT cell lines generated to date encoding CFTR2 mutations

	Legacy Name	domain	mutation class	Patient # (CFTR2)	p. 470
1	wt				M
2	P67L	NT	IV	238	M
3	G85E	TMD1	II	580	V
4				-	M
5	E92K	TMD1	II	33	M
6	R117H	TMD1		2042	M
7	Y122X	TMD1	I	75	M
8	R334W	TMD1	IV	404	V
9	R347P	TMD1	IV	512	V
10					M
11	A455E	NBD1	V	495	M
12	S492F	NBD1	II	24	M

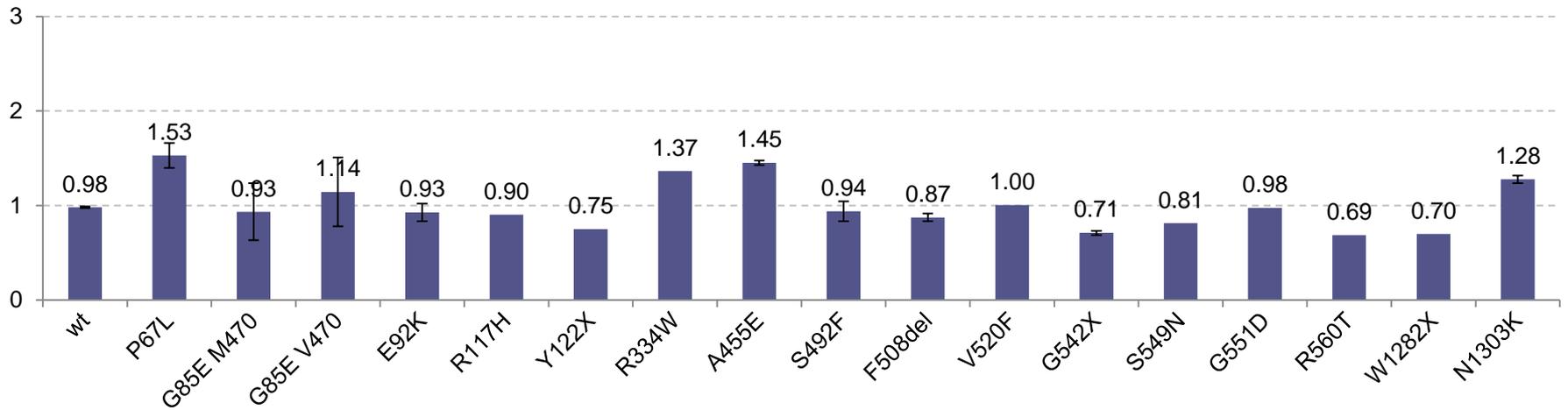
	Legacy Name	domain	mutation class	Patient # (CFTR2)	p. 470
13	F508del	NBD1	II	64,868	M
14	V520F	NBD1	III	155	M
15	G542X	NBD1	I	3,474	M
16	S549N	NBD1	III	184	M
17	S549R	NBD1	III	61	M
18	G551D	NBD1	III	2,915	M
19	R560T	NBD1	III	340	M
20	L1077P	TMD2	III	93	M
21	M1101K	TMD2	II	176	V
22	W1282X	NBD2	I	1,552	M
23	N1303K	NBD2	II	2,130	M

FRT lines completed for mechanistic studies

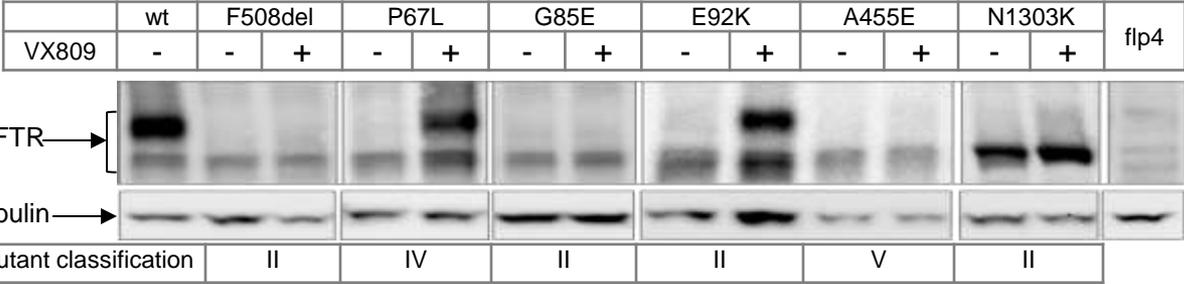
	Legacy Name	p.470
1	F508G	M
2	F508/V510D	M
3	F508G/V510D	M
4	Δ F/V510D	M
5	Δ F/I539T	M
6	Δ F/R555K	M
7	Δ F/R1070W	M
8	wt -HRP	M
9	Δ F/ -HRP	M
10	Δ F/I539T -HRP	M

	Legacy Name	p.470
11	Δ F/R555K -HRP	M
12	Δ F/R1070W -HRP	M
13	Δ F/R555K+R1070W -HRP	M
14	Luciferase	M
15	Empty	M
16	Y122X-HRP	M
17	G542X-HRP	M
18	W1282X-HRP	M

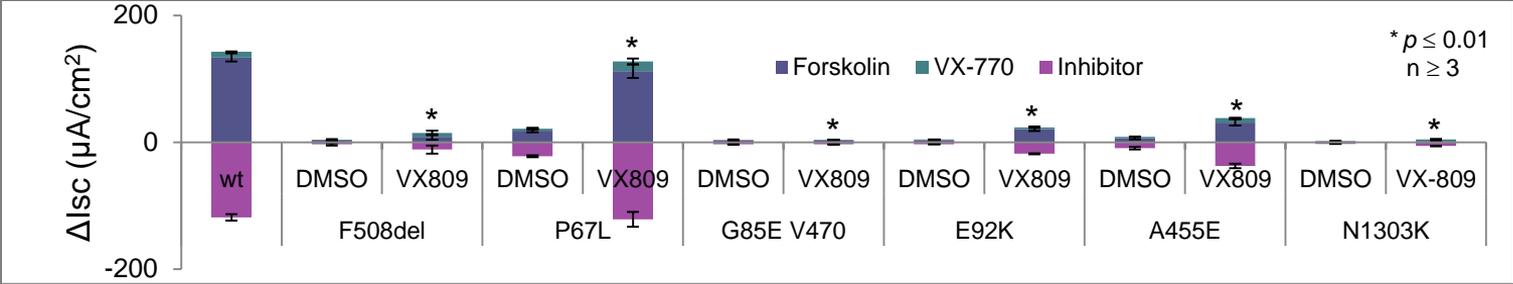
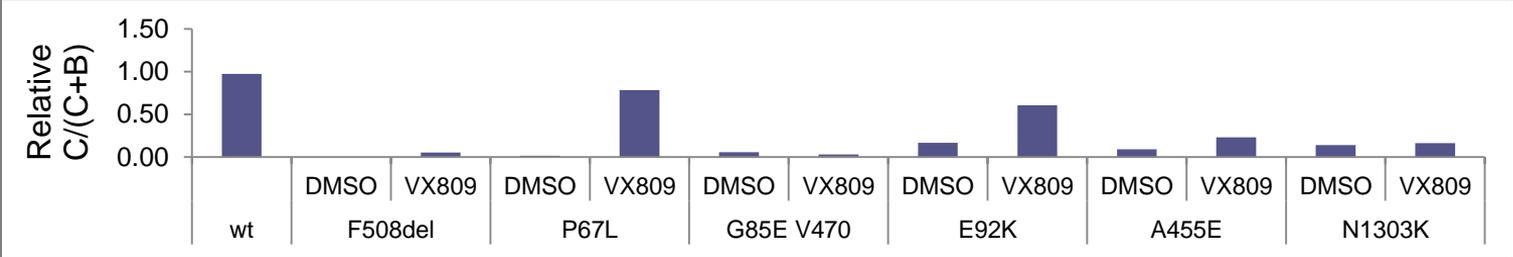
Relative level of mRNA in FRT – CFTR2 cell lines



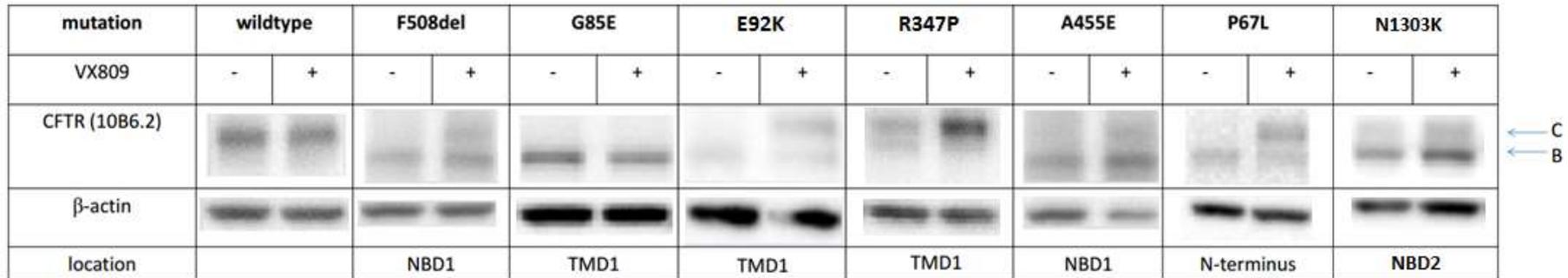
Diversity of corrector effect



FRT cells, 3 μM VX809



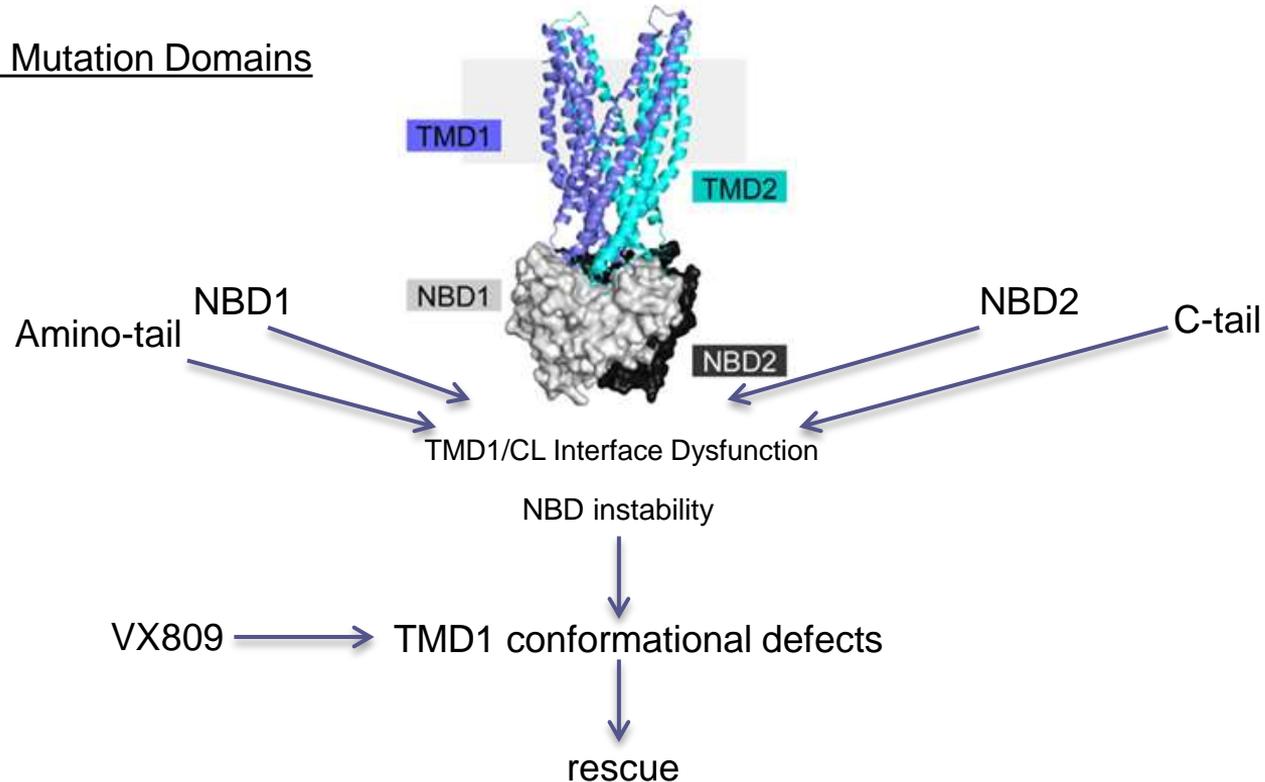
Diversity of Corrector Effect (cont.)



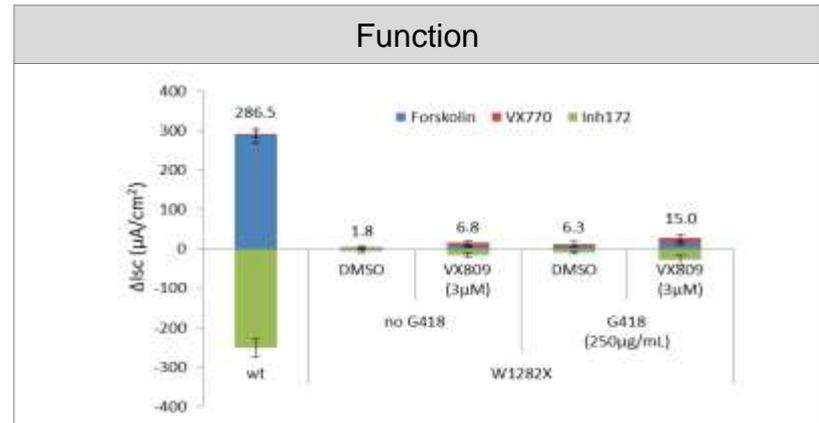
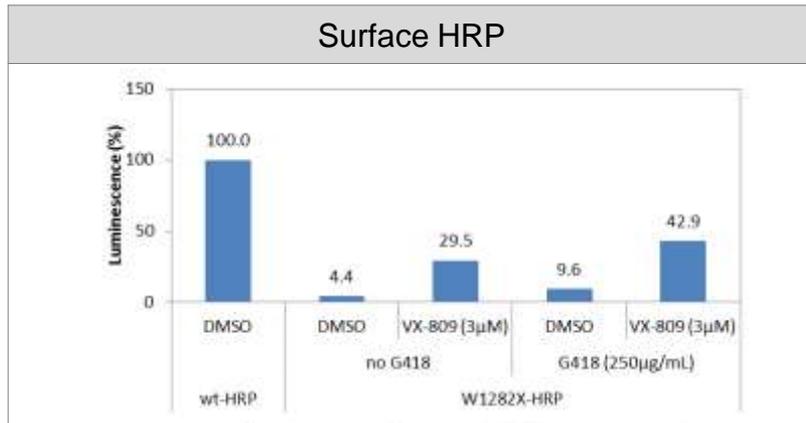
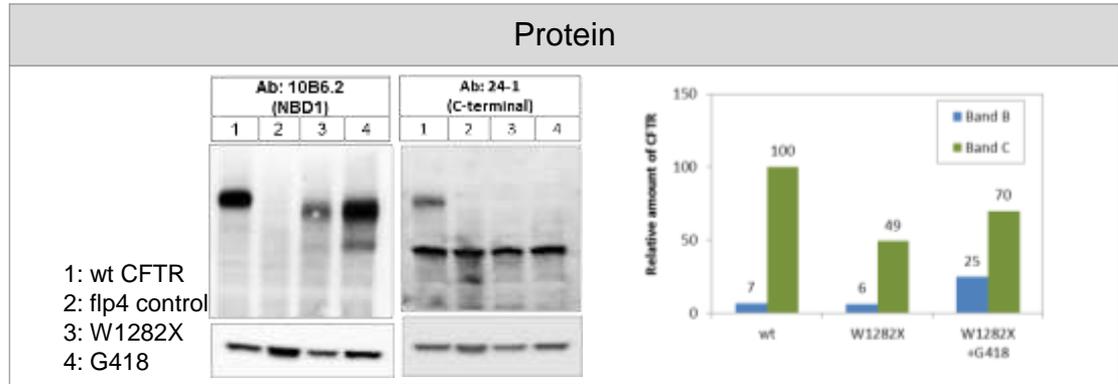
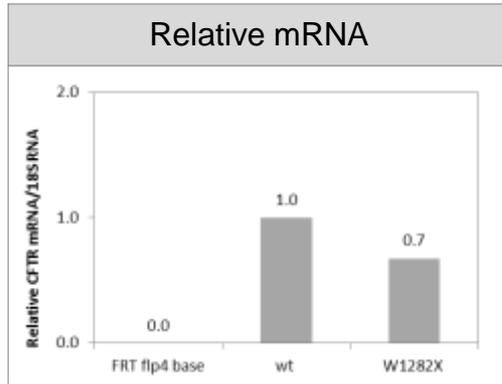
3 uM VX809, HEK 293
C. Sabusap

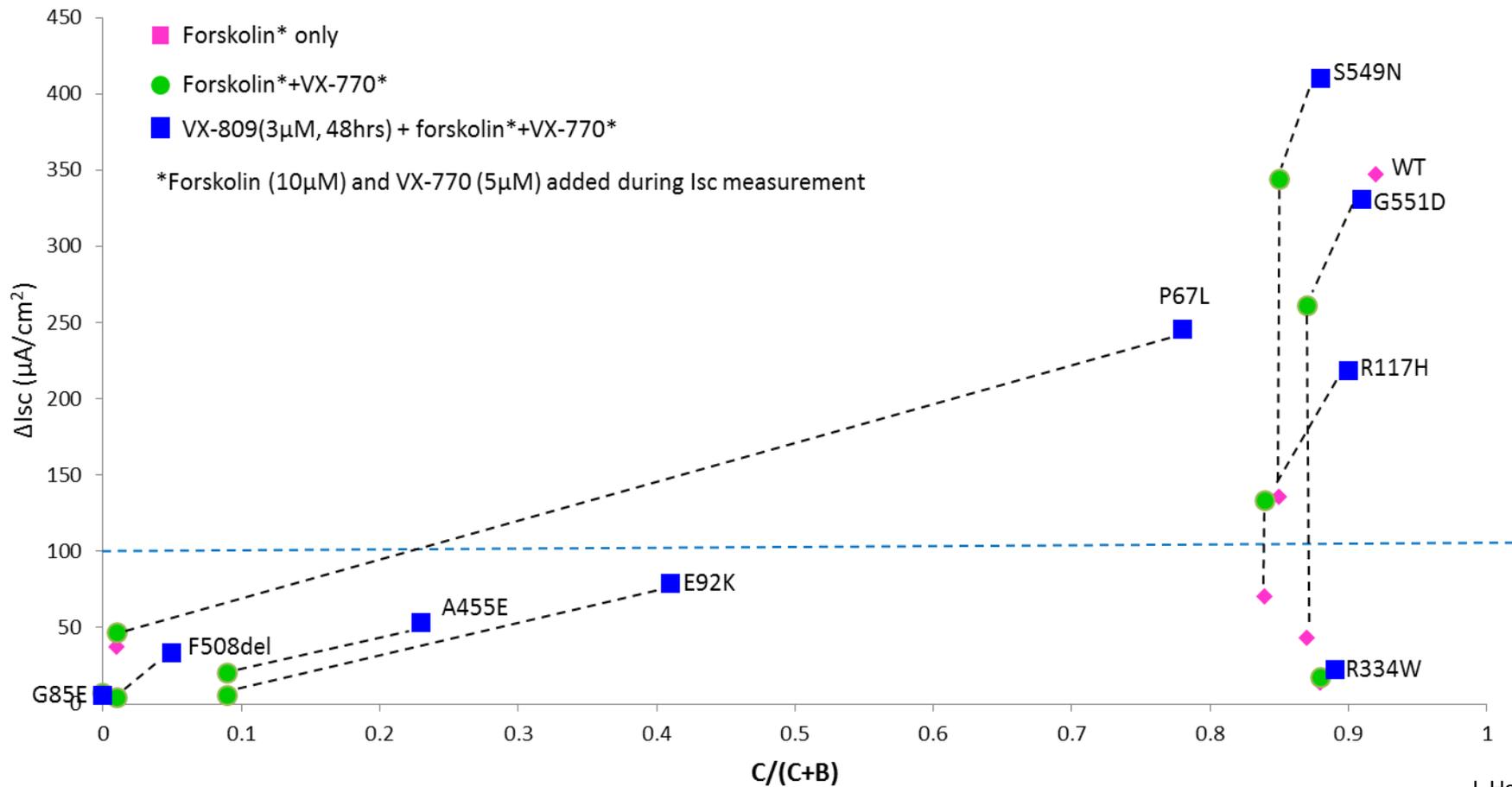
Misfolded TMD-1 as a common checkpoint for correction

Clinical CFTR Mutation Domains



FRT flp4 W1282X





					C/C+B		Fsk stimulated Isc (over baseline)		VX-770 stimulated Isc (over forskolin)	
							$\mu\text{A}/\text{cm}^2, n \geq 3$			
Mutation	Location	Folding Defect	Mean Sweat [Cl-]	Mean Lung Function (FEV ₁ %pred)	DMSO	VX-809 (3 μM)	DMSO	VX-809 (3 μM)	DMSO	VX-809 (3 μM)
Wild-type					0.92		347.2		3.08	
F508del	NBD1	Severe Folding	102	73.7	0	0.05	5.6	22	1.7	11.6
P67L	N-terminal	Mild folding	61	72.5	0.01	0.78	37.18	215.66	9.65	30.05
G85E (M)	TMD1	Severe Folding	100	71.7	0.03	0.06				
G85E (V)	TMD1	Severe Folding	100	71.7	0.01	0	3.96	6.04	-0.08	-0.21
E92K	TMD1	Severe Folding	77	44.1	0.09	0.41	4.7	65.5	0.8	13.7
R117H	TMD1	Mild folding	60		0.84	0.9	69.8	123.73	63.18	94.56
R334W(V)	TMD1	No Folding Defect	100	78.5	0.88	0.89	13.61	17.79	3.31	4.35
S549N	NBD1	No Folding Defect	101	72.7	0.85	0.88	135.2	156.2	208.9	253.6
G551D	NBD1	No Folding Defect	101		0.87	0.91	43.3	53.3	218	277.3
A455E	NBD1	Severe Folding	83	75.7	0.09	0.23	19.3	51.5	1	2.1
S492F	NBD1	Severe Folding	72	67.9			9.2	38.9	0.04	1.9
R347P	TMD1	Mild folding	100	73.6						
V520F	NBD1	Severe Folding	108	78.2			1.65	4.79	0.01	0.4
R560T	NBD1	Severe Folding	103	76.2			0.55	0.56	0.04	0.03
M1101K	TMD2	Severe Folding	102	65						
R1066C	TMD2	Severe Folding	106	74.4						
N1303K	NBD2	Severe Folding	103	72.5			1.9	3.8	3.3	7
L1077P	TMD2	Severe Folding	101	69.4						

Mechanistic Analysis & Theratyping of Rare CFTR2 Alleles

P67L OVERVIEW

MUTATION

GENETIC/PREVALENCE IN CFTR2

P67L [c.200C>T]

alleles: 77/70777

patients: 76/39696

complex genotype(s): 97%M470, 3% V470

CLINICAL SEVERITY & AVAILABLE TARGETED THERAPIES

<i>folding/function</i>		<i>Clinical characteristics</i>			
<u>Maturation</u> : 36%	<u>Cl cond</u> : 7.8%	<u>FEV1</u> : 73-102%	<u>PI</u> : 26%	<u>pA</u> : 42%	<u>Sweat Cl</u> : 60

Kalydeco status as of 2/2014: not aproved

MOLECULAR PATHOLOGY IN MODELS

Maturation/Folding: minor effect

Conductance: minor effect

Gating: minor effect

Consistency with disease severity: consistent

THERATYPE: AGENT RESPONSIVENESS

<i>models</i>	<i>patients</i>
CORRECTORS <u>Step 1:</u> TBD <u>Step 2 (809):</u> effective	CORRECTORS <u>Step 1:</u> TBD <u>Step 2 (809):</u> unknown
POTENTIATORS <u>ivacaftor:</u> effective <u>combinations:</u> effective <u>non-CFTR targets:</u> TBD	POTENTIATORS <u>ivacaftor:</u> unknown <u>combinations:</u> unknown <u>non-CFTR targets:</u> unknown

CONSORTIUM DATA

- Significant rescue by VX-809, ~78% (C/C+B): FRT cells
 - Fsk stimulated Isc (over baseline) with VX-809: 61.5% of wt
 - VX-770 stimulated Isc (over baseline) with VX-809: 8.39% of wt
 - Activation of gating and mild rescue of chloride transport by VX-809 in primary cells (F508del/P67L)
- Summaries of cell surface stability, ubiquitin modification, subdomain folding, proteomic analysis, molecular rescue strategies, NBD stability and yield, and SUMOylation have also been compiled.

Additional data/comments:

Ren et al, MBoC, 2013

Significant rescue by VX-80-9, ~83% (C/C+B)

Van Goor et al, JCF, 2013

CFTR maturation in FRT cells (normal CFTR), 28.4%

Chloride transport: 5.6 fold increase over baseline with ivacaftor

Citations:

- Yousef et al, Improved clinical and radiographic outcomes after treatment with ivacaftor in a Young Adult with Cystic Fibrosis with the P67L CFTR Mutation. CHEST, 2015
- Giffillan et al, P67L: a cystic fibrosis allele with mild effects found at high frequency in the Scottish population. J Med Genet., 1988
- Ren et al, VX-809 corrects folding defects in cystic fibrosis transmembrane conductance regulator protein through action on membrane-spanning domain 1. Mol Bio Cell, 2013
- Van Goor et al, Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. JCF, 2014

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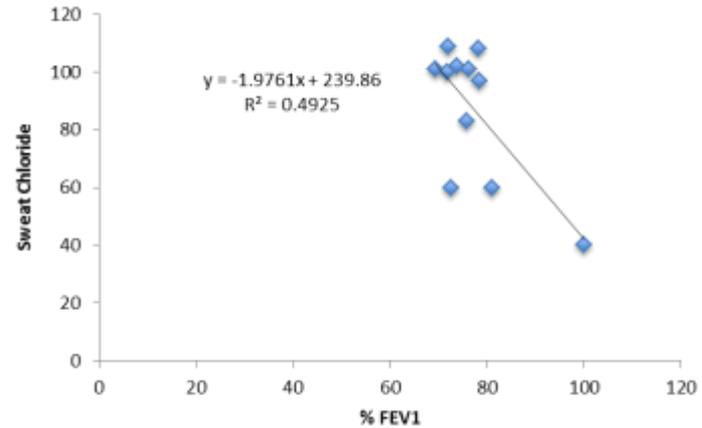
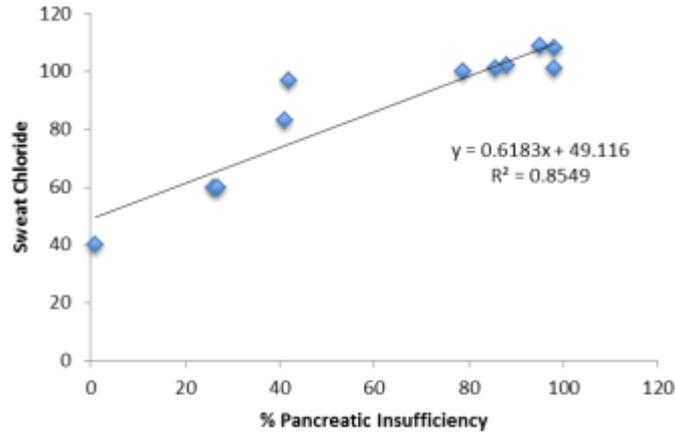
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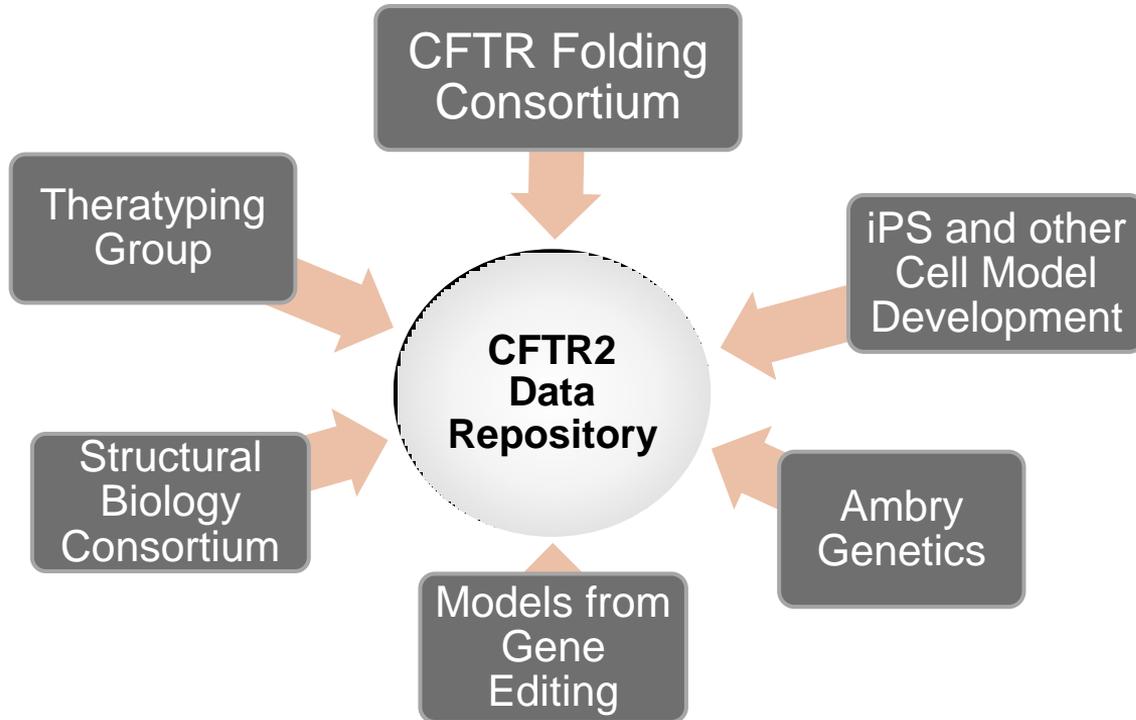
Pilot heat map profiling analysis

Mutation	WILDTYPE	P67L	G85E	R117H	R334W	A455E	F508del	V520F	S549R	R560T	L1077P
# of Alleles		239	610	1852	425	496	98735	156	70	343	96
Allele Frequency		0.00169	0.00432	0.0131	0.00301	0.00351	0.69856	0.0011	0.0005	0.00243	0.00068
Patients		238	580	1817	404	495	64,868	155	61	340	93
Domain		N-terminal	TMD1	TMD1	TMD1	NBD1	NBD1	NBD1	NBD1	NBD1	TMD2
Biogenesis Defect		Minor	Major	No Effect	No Effect	Moderate	Severe	Major	Minor	Major	Moderate
%FEV1	100	72.5	71.70	81.00	78.5	75.7	73.7	78.2	72.00	76.2	69.40
%PI	0.01	0.26	0.79	0.27	0.42	0.41	0.88	0.98	0.95	0.98	0.86
%Patients	0.01	0.42	0.58	0.24	0.42	0.49	0.56	0.41	0.54	0.68	0.48
%Sweat Cl	40	60	100	60	97	83	102	108	109	101	101
Pulse chase, VX809	10	12	1	10	10	1	3	1	10	1	5
WB,Band B , C18	100	541	412	457	442	470	615	477	806	336.9	637
WB, Band C, C18	100	177	*	72	47.5	104	63	*	92.4	*	66.1
WB,Band B , PIAS4	100	14250	10814	2895	2677	1948	7536	11494	8723	14105	4746
WB, Band C, PIAS4	100	410	*	784	533	259	62	*	2389	*	141.8
WB,Band B , Combo	100	14163	11569	2462	2543	7117	8149	12066	9298	19568	7503
WB, Band C,Combo	100	11059	*	1362	823	702	118	*	6115	*	1398
CS, C18	100	13.4	53	274	499	110	43.6	6.9	98	89	8
CS,PIAS4	100	13.5	11.2	235	389	28.2	4.2	11.2	82	30	1.3
CS,C18+PIAS4	100	182	56	505	659	111	56.9	11.9	273	51	16.4
Forsk,DMSO		37.18	3.96	69.8	13.61	19.3	5.6	1.65	9.2	0.55	*
Forsk, 3 uM VX809		215.66	6.04	123.73	17.79	51.5	22	4.79	38.9	0.56	*
770, DMSO		9.65	-0.08	63.18	3.31	1	1.7	0.01	0.04	0.04	*
770,3 uM VX809	347.2	30.05	-0.21	94.56	4.35	2.1	11.6	0.4	1.9	0.03	*
DMSO,C/C+B	100.00	1.09	3.26	91.30	95.65	17.39	*	*	92.39	*	*
VX809, C/C+B	100	84.7	6.52	97.8	96.73	44.5	5.43	*	95.65	*	*
MATURATION	100	12.5	0.39	*	*	1.5	0.55	0.138	28.7	0.03	0.5
THERATYPE	1	1	3	*	*	2	2	3	1	3	2
M470	55	97	0	75	20	100	100	80	100	100	100
V470	45	3	100	25	80	0	0	20	0	0	0

Relationship between sweat chloride, pancreatic insufficiency, and FEV1



Sources for CFTR2 Data Repository



Summary

- A multidisciplinary and comprehensive effort is in progress to characterize rare CFTR2 alleles.
- The project highlights shortcomings of traditional disease categories organized by molecular pathogenesis to specify molecular mechanism.
- The approach will aid in binning CFTR2 alleles according to theratype.
- New tools and reagents generated through the initiative are available to the CF academic and pharmaceutical research communities.



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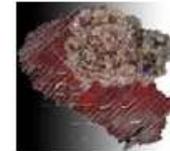
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CFTR Folding Consortium

Sponsored By

cystic fibrosis foundation
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With Thanks.