Novel potentiators for treating Cystic Fibrosis

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Poster #41

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Two potentiator series development and characterization

Potentiator series were identified using a YFP halide assay. Low Temperature rescued CFBe41o-cells were transduced with adenoviruses containing both F508del CFTR and YFP H148Q/I152L.

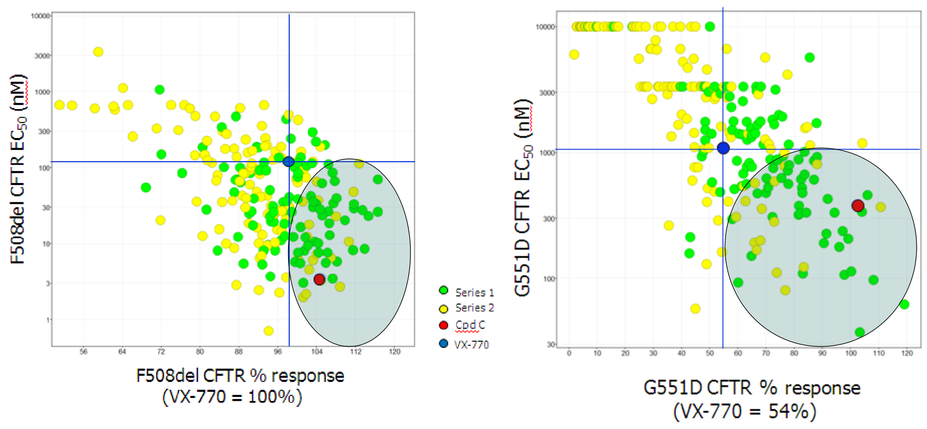
Compounds were added to the cells together with Forskolin (Fsk), and Iodide influx was followed by measuring quenching of YFP in time. Activities were calculated using VX-770 as control in the assay.

The potentiator series were developed by medicinal chemistry to improve the potency to open the F508del CFTR channel as well as the G551D CFTR.

Galapagos potentiators are very potent and improve CFTR channel opening

F508del CFTR **G551D CFTR**

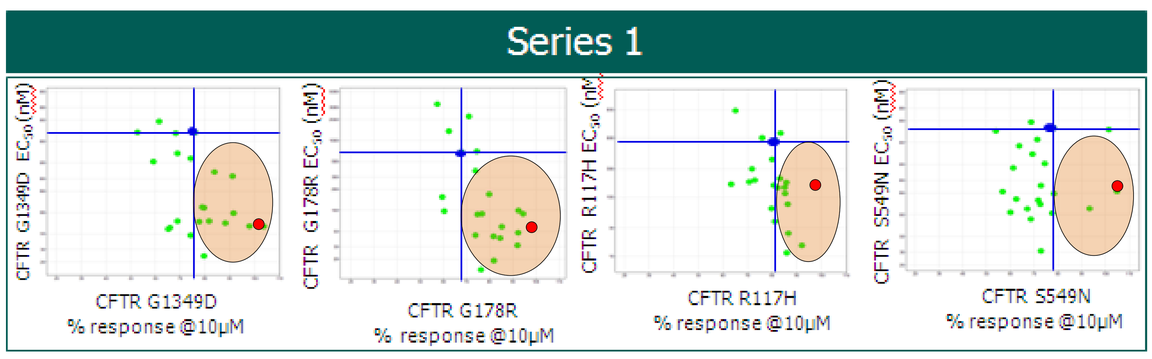
Series 1 YFP halide data	Cpd A	Cpd B	Cpd C	Cpd D	Cpd E
F508del EC_{50} (nM)	2.2	6.9	3.4	2.4	17.8
F508del % Act *vs* VX-770	96	92	104	92	102
G551D EC_{50} (nM)	80	111	379	122	367
G551D % Act *vs* VX-770	141	136	188	153	204

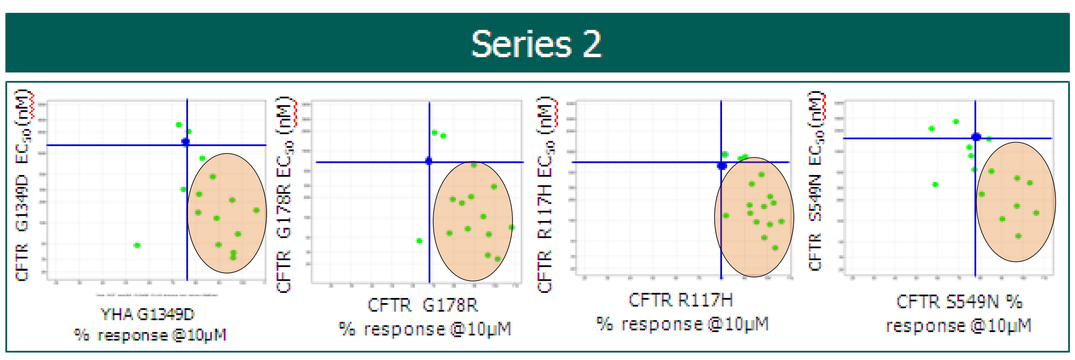


Series 2 YFP halide data	Cpd F	Cpd G	Cpd H	Cpd I	Cpd J
F508del EC_{50} (nM)	18.5	12.8	5.3	5.7	3
F508del % Act *vs* VX-770	106	106	92	99	101
G551D EC_{50} (nM)	113	97	62	106	37
G551D % Act *vs* VX-770	184	199	219	190	190

The most interesting compounds were profiled in additional YFP halide assays using class III and IV CFTR mutations. These assays were performed by transient transfection of CFTR mutant in HEK293 cells. All compounds tested were able to open the different CFTR mutants in an efficient manner. For several class III mutants, the maximal opening of the mutant channel exceeded that of VX-770, which was used as comparator in the assay. Furthermore, most compounds tested showed high potency against the mutants.

Galapagos potentiators have superior class III and class IV CFTR channel opening compared to VX-770

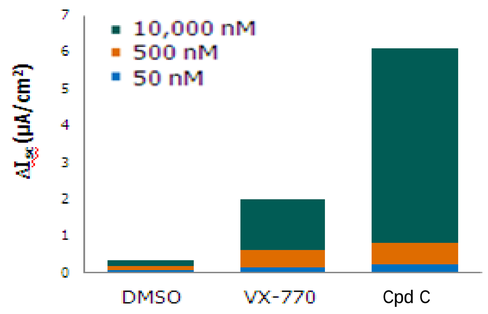




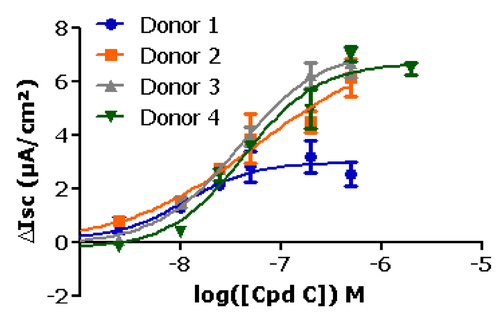
In Ussing chamber tests, multiple compounds of the series showed strong and dose-dependent activity, both on VX-809 rescued F508del homozygous and G551D/F508del heterozygous primary cells. The results on F508del homozygous primary cells were replicated in TransEpithelial Clamp Circuit (TECC) equipment which was used for determination of more accurate EC_{50} values. The YFP halide data appear to be predictive for the activity obtained on primary cells. The higher extent of channel opening observed in the YFP halide assay was confirmed on primary cells (multiple donors). In all CF-HBE donors evaluated, the increase in current after potentiation of VX-809 corrected F508del CFTR with Galapagos potentiators is higher than the comparator VX-770. Compound C shows similar potencies on all tested donors.

Higher extent of CFTR channel opening by Galapagos potentiators is confirmed on patient derived primary cells

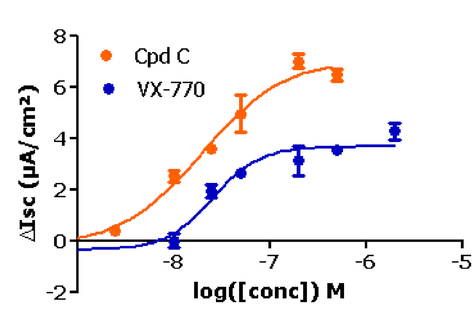
G551D/F508del primary cells Ussing chamber data



F508del/F508del primary cells DR data on 4 different donors (TECC data)



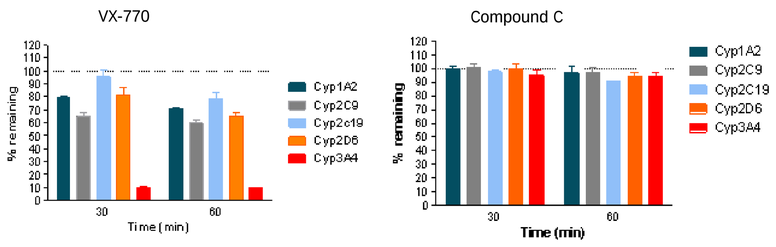
F508del/F508del primary cells DR Cpd C and VX-770 (TECC data)



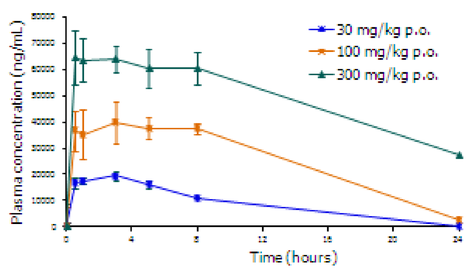
Compounds were not only profiled and optimized for their activity in opening CFTR channels, but also for their *in vitro* and *in vivo* ADME and DMPK properties. Good metabolic stability was general to the series, and good permeability was also found in multiple compounds, leading to excellent PK profiles. Compounds were also optimized for CYP liabilities; for the most advanced compounds no CYP interactions have been found, and route of clearance has been shown to be non- or multiple CYP mediated. Further broad selectivity panel testing has shown minimal interactions with kinases or other target classes.

Galapagos potentiators do not show any CYP liabilities as measured by *in vitro* ADME and are well exposed in animals

Galapagos potentiators are very slowly cleared by CYPs



Galapagos potentiators have excellent pharmacokinetic properties. Dose escalation PK study in rat (oral administration)



Plasma parameters		30 mg/kg p.o.	100 mg/kg p.o	300 mg/kg p.o
C_{max}	(ng/mL)	19,500	44,167	74,367
T_{max}	(h)	3	5	3
$AUC_{(0-24h)}$	(ng.h/mL)	178,997	488,195	1,134,998
C_{24h}	(ng/ml)	469	2,855	27,133
$T_{1/2}$	(h)	3.72	3.15	NC

Conclusions

Galapagos has developed two novel potentiator series with superior channel opening activity *versus* VX-770. The series show good metabolic stability and permeability, affording favorable PK profiles. The lack of CYP liabilities and lack of interactions with kinases or other target classes for these series also may afford a superior *in vitro* safety profile *versus* VX-770. These results encourage Galapagos to select a candidate for pre-clinical development from this series in 2013, on track to enter the clinic in 2014.

Poster available online at: www.glpg.com

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