

# Hunting for correctors of cystic fibrosis using existing data and systems biology thinking

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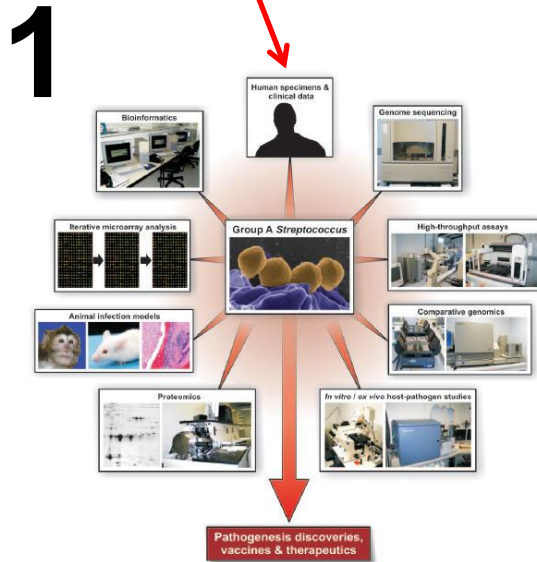
# Summary

- The themes of systems biology
- Cystic fibrosis and CFTR
- GSK-McGill collaboration
- Systems biology thinking as applied to the collaboration
- Impact and future plans

# The themes of systems biology

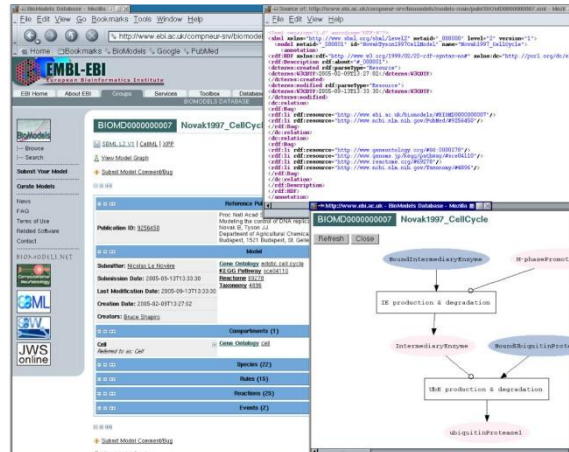
Systems biology is an approach by which biological questions are addressed through integrating experiments with computational modelling and theory, in re-enforcing cycles.

BBSRC <http://www.bbsrc.ac.uk/publications/corporate/systems-biology.aspx>



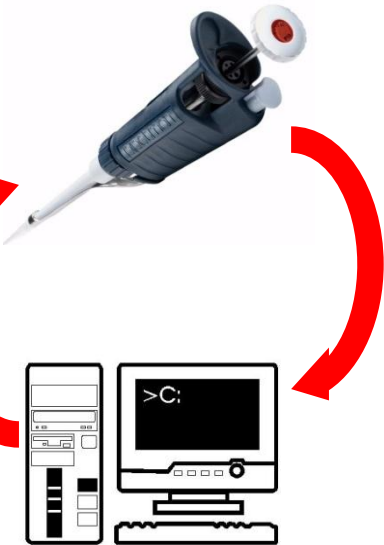
From: Musser and Leo (2005) Toward a Genome-Wide Systems Biology Analysis of Host-Pathogen Interactions in Group A *Streptococcus*. *American Journal of Pathology*, Vol. 167, pp1461-72

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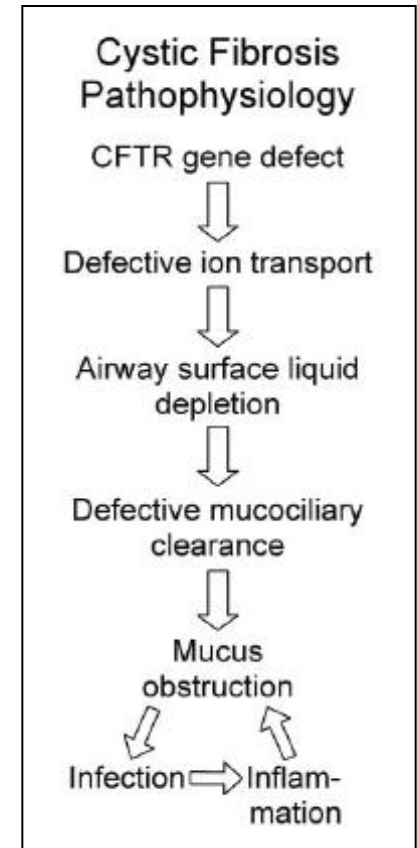
From: LeNovere (2006) Model Storage, Exchange and Integration. *BMC Neuroscience*, Vol 7, S11

**3**



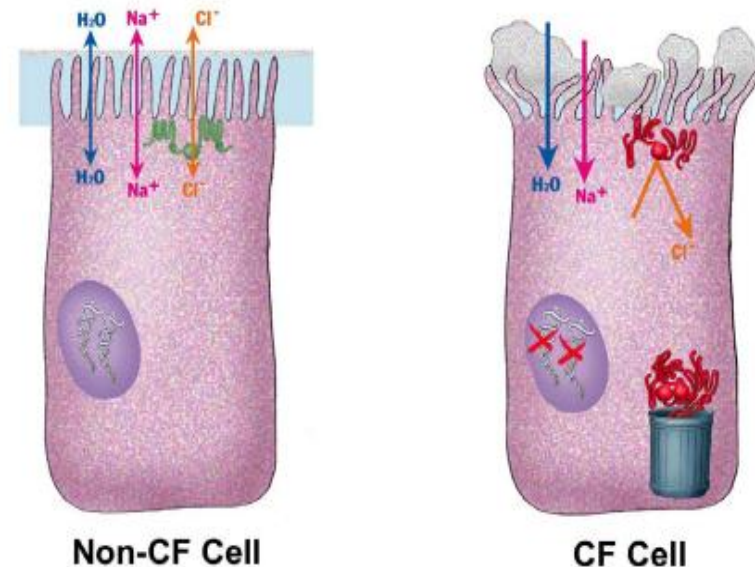
# Cystic Fibrosis (CF)

- Autosomal recessive disease
- Caused by mutations in *CFTR* gene
- Frequency ~1/2000 among Caucasians
- ~70,000 patients world-wide
- Affects pancreas, airways, exocrine glands (sweat, lacrimal, salivary), GI tract
- Airway disease is the main cause of morbidity and mortality
- Predicted median age of survival ~37 years



# Cystic fibrosis transmembrane conductance regulator (CFTR)

- CFTR is a cAMP regulated chloride channel, member of the ABC-transporter family
- Most patients with CF have a genetic mutation known as  $\Delta F508$ 
  - Causes misfolding of the CFTR protein which is then retained in the ER and degraded rather than trafficked to the plasma membrane
- Defects in the CFTR gene result in poor chloride flow across cell membranes
  - Abnormally thick, sticky mucus is produced, leading to chronic, life-threatening lung infections
- Believed that only 10-25% of ER-retained  $\Delta F508$ -CFTR needs to be rescued to provide therapeutic benefit



# CF treatment

Target	Potential treatments
Abnormal genes	Gene therapy Modifier genes
Abnormal CFTR protein	Protein rescue- “correction”
Altered ion transport, abnormal mucus secretion	Proper ion transport- CFTR “potentiation”
Infection & inflammation, tissue destruction	Anti-inflammatory agents Anti-infective agents
Organ destruction, respiratory failure	Transplantation

*Disease  
progression*



# GSK Respiratory CEDD-McGill University collaboration

## McGill

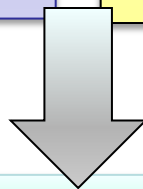
### *Biology platform and expertise*

- CFTR trafficking assay (correctors)
- Functional Ussing Chamber assay
- *In vivo* mouse efficacy model
- Clinical expertise, KOL in CF

## GSK

### *Integrated drug discovery platform*

- Medicinal Chemistry
- Computational biology and chemistry
- DMPK
- Safety



- Collaboration started Jan 2010
- Aggressive timelines for compound progression





# Literature searching

- We have developed methods to automatically extract genes associated with a PubMed query

## PubMed Query

("protein transport"[mh] OR "protein folding"[mh] OR "proteostasis deficiencies"[mh] OR "unfolded protein response"[mh]) AND ("cystic fibrosis"[mh] OR "Cystic Fibrosis Transmembrane Conductance Regulator"[mh] OR "cystic fibrosis transmembrane conductance regulator delta F508" [Substance Name])

112 genes

70 compounds, covering 4 targets

Trafficking assay (McGill)

Agarwal and Searls, *Brief. Bioinform.*  
2008 9(6):479-492

## 2) Use of (static) models: GeneGo

- Expert curated CF disease maps, funded by CF foundation

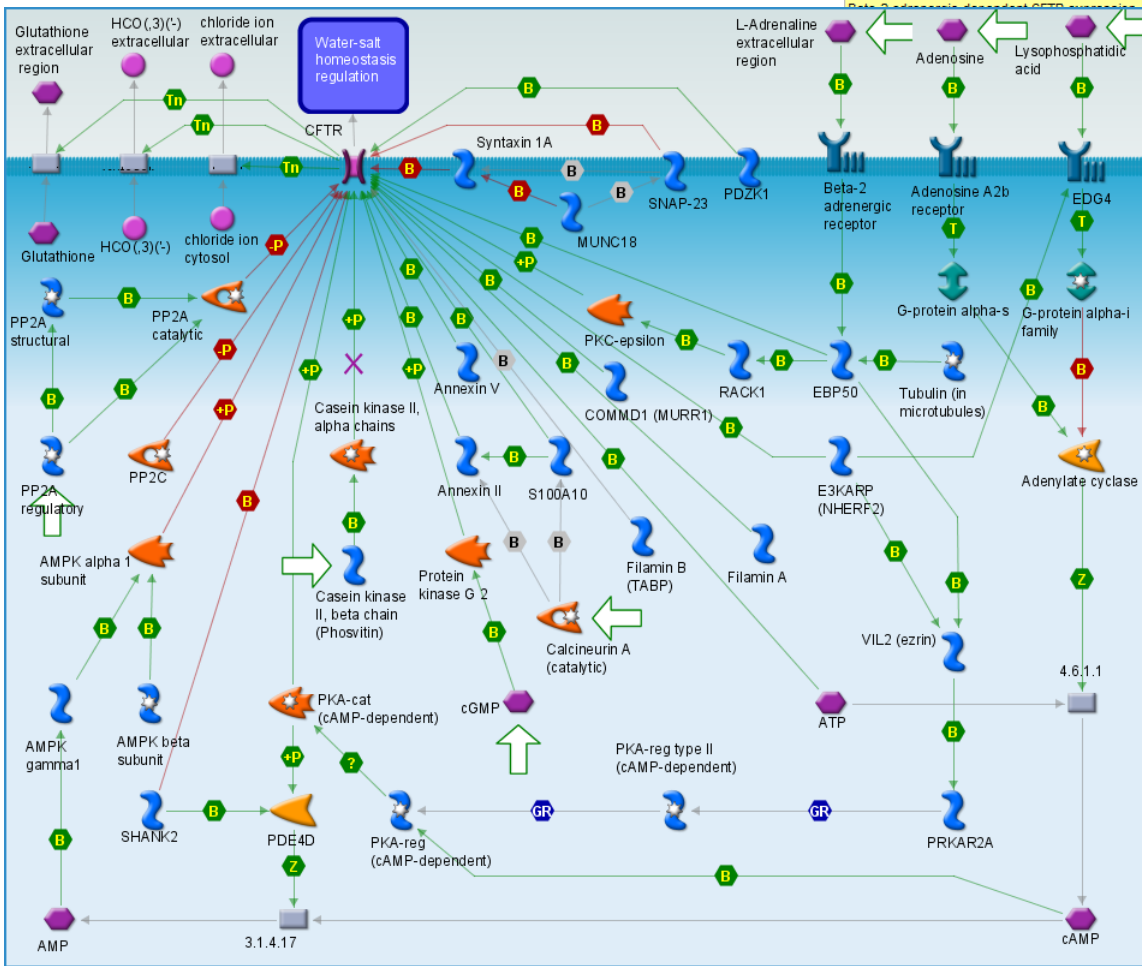
Map name	pValue	GSK targets	Pathway size
Cytokine production by Th17 cells in CF	1.97E-05	15	39
Cytokine production by Th17 cells in CF (Mouse model)	2.52E-05	17	49
ENaC regulation in airways (normal and CF)	2.80E-03	14	52
Bacterial infections in CF airways	8.35E-03	14	58
Immune response_Bacterial infections in normal airways	1.31E-02	12	49
Inhibitory action of Lipoxins on neutrophil migration	1.81E-02	13	57
	3.05E-02	5	15
	4.01E-02	5	16
pathways	9.35E-02	10	51
LTD4 signaling	1.50E-01	9	49
action in neutrophils	1.50E-01	9	49
	1.80E-01	10	58
neutrophil functions	2.29E-01	7	40
regulation of CFTR expression	2.46E-01	5	27
pathways	2.49E-01	6	34
way Epithelium	3.13E-01	7	44
g to plasma membrane in lung (normal and CF)	3.28E-01	4	23
	3.80E-01	7	47
ion	4.01E-01	3	18
ion in CF	5.72E-01	3	23
and Lysosome (norm and CF)	5.84E-01	2	15
	8.12E-01	3	33
ision (norm an	8.30E-01	1	13
	8.30E-01	1	13
	8.30E-01	1	13
	8.52E-01	1	14
	8.77E-01	2	27
	8.88E-01	1	16
rt from Golgi and ER to the apical membrane (normal and CF)	9.03E-01	3	40
	9.15E-01	1	18
icles formation (norm and CF)	9.26E-01	1	19
thione (normal and CF)	9.47E-01	3	46
ma (norm and CF)	9.66E-01	3	50
class I	9.76E-01	1	27
	1.00E+00	2	71

22 GSK targets on pathways linked to CFTR expression or activation

6 GSK targets on pathways linked to CFTR trafficking

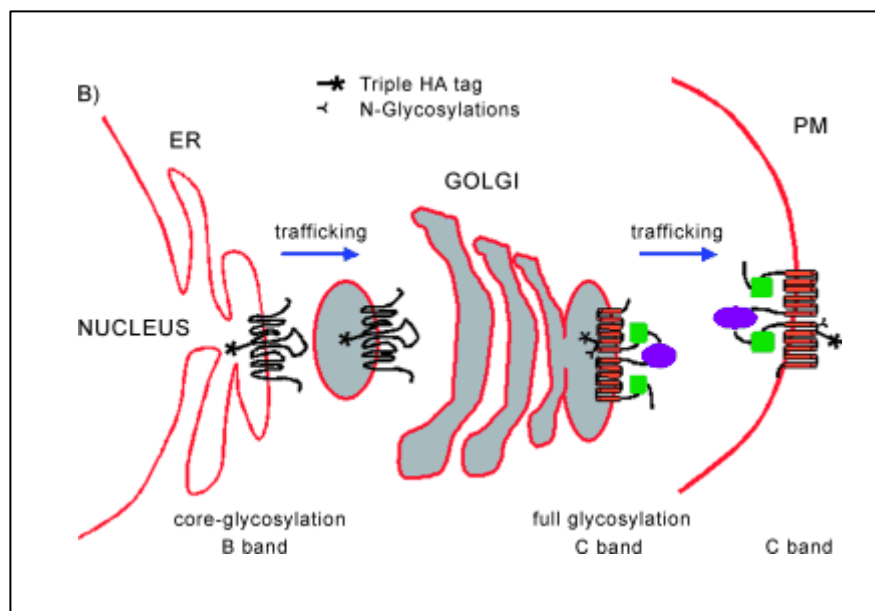
97 compounds, covering 14 targets

Trafficking assay (McGill)



### 3) Experimental validation: CFTR trafficking assay (McGill)

- Cell-based assay for monitoring the effects of chemical agents on the trafficking of mutated CFTR to the plasma membrane
- Triple HA-tag attached to extracellular loop allows detection of  $\Delta F508$ -CFTR at the cell surface by immunofluorescence staining

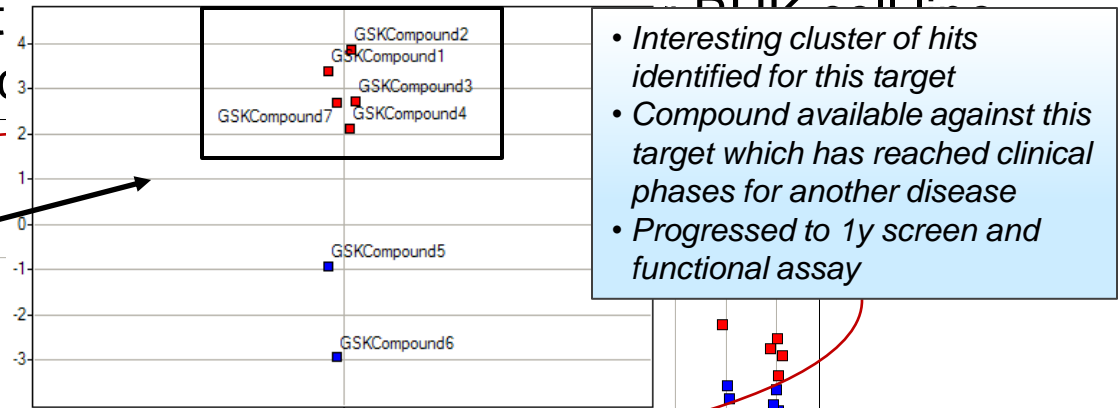
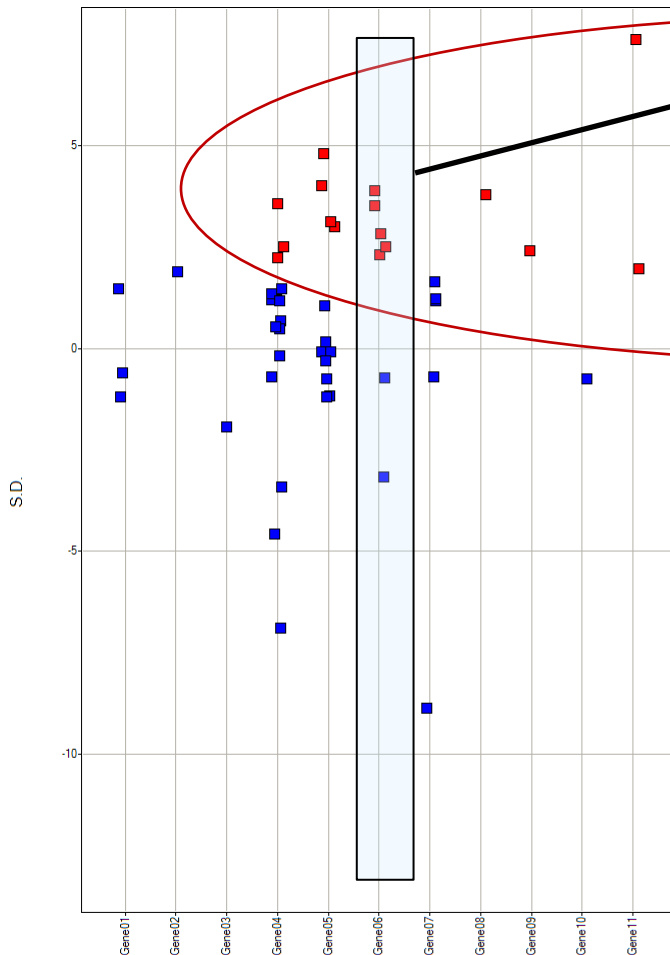


Trafficking assay

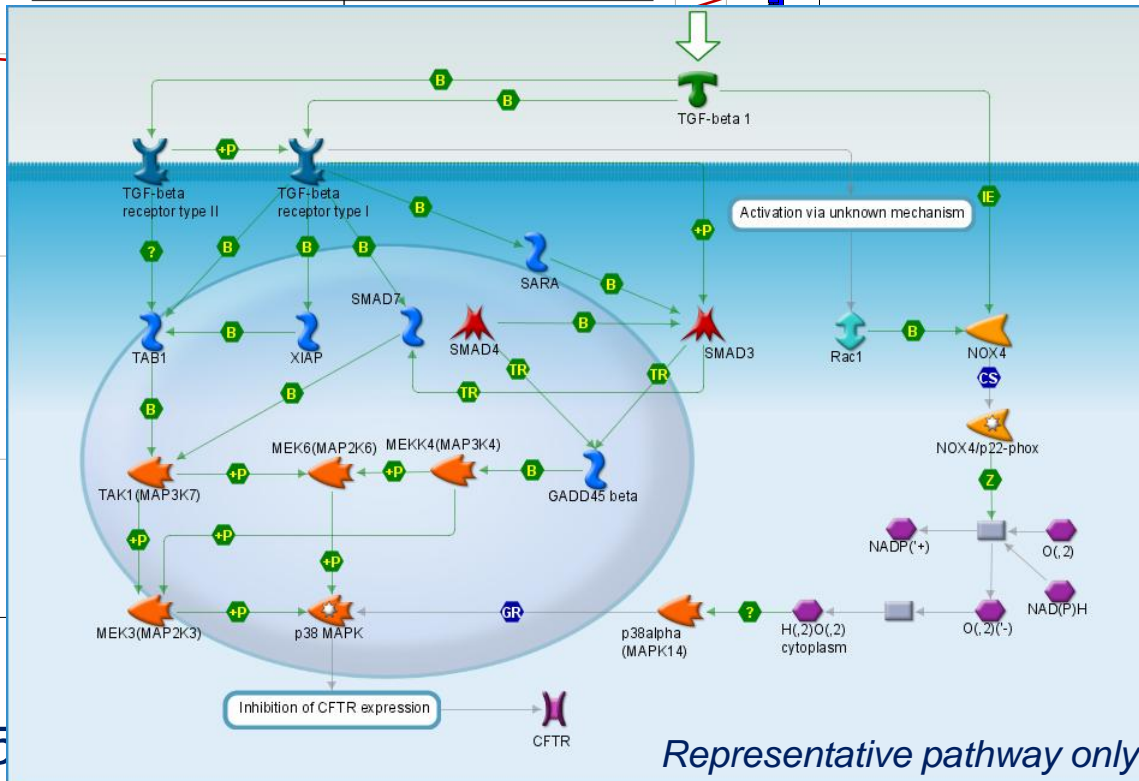
Carlile et al. 2007 Chembiochem. 8(9), 1012

# Experimental validation: CFTR trafficking assay results (McGill)

- Identification of correct expressing 3HA-tagged

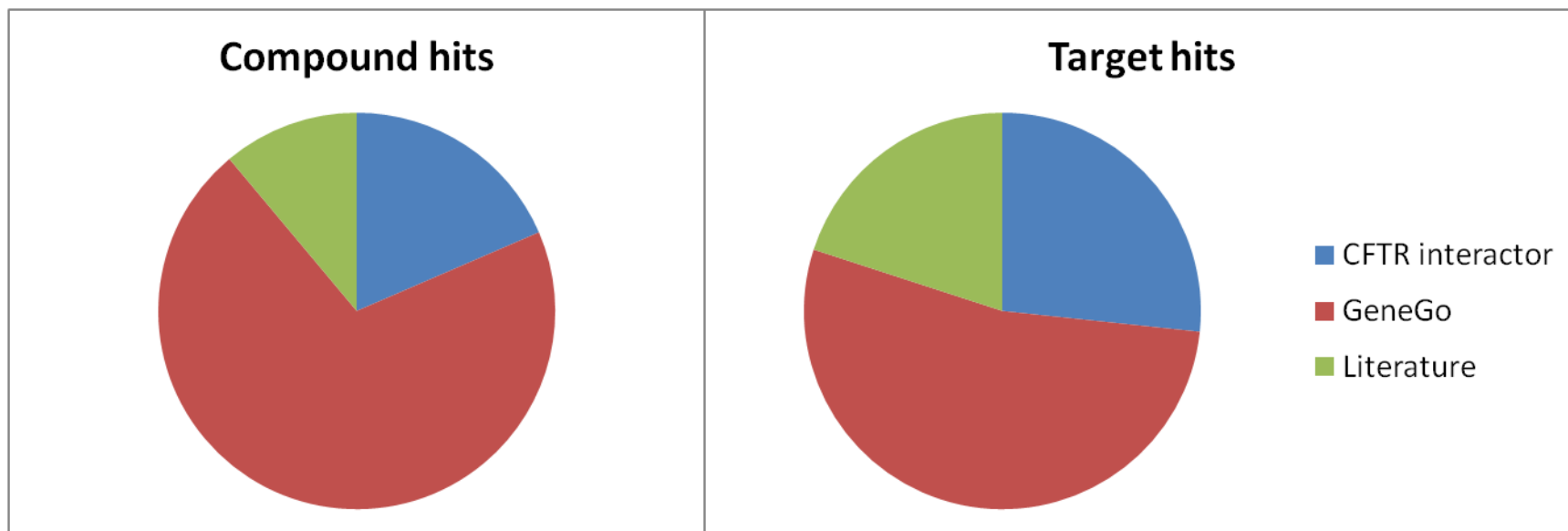


- Interesting cluster of hits identified for this target
- Compound available against this target which has reached clinical phases for another disease
- Progressed to 1y screen and functional assay



# Experimental validation: Summary of hits

- 180 compounds, covering 24 targets screened
- 27 initial hits (15%), covering 15 genes, identified for follow-up in functional assay



# Impact and future plans

- Successfully identified assets for screening through knowledge-based approaches
  - Achieved an initial 15% hit rate for follow up in functional assay
- GeneGo disease maps provided a valuable resource
  - Linking compound space to disease biology
  - Available to both GSK and our academic partner
- Collaborating with McGill over analysis of microarray data for corrector compounds
  - GeneGo maps will be used to help decipher compound mode-of-action

# Acknowledgements

## GSK

- Chris Larminie (Computational Biology)
- Samiul Hasan (Computational Biology)
- Pankaj Agarwal (Computational Biology)
- Veronique Birault (Respiratory CEDD)
- Helen Sneddon (Respiratory CEDD)
- Nicola Richmond (Computational Chemistry)

## McGill University

- David Thomas
- John Hanrahan
- Graeme Carlile
- Renaud Robert
- Suzana Anjos
- Fabiana Ciciriello