



# Screening of a Chemokine Receptor – a Ligand Biased Approach

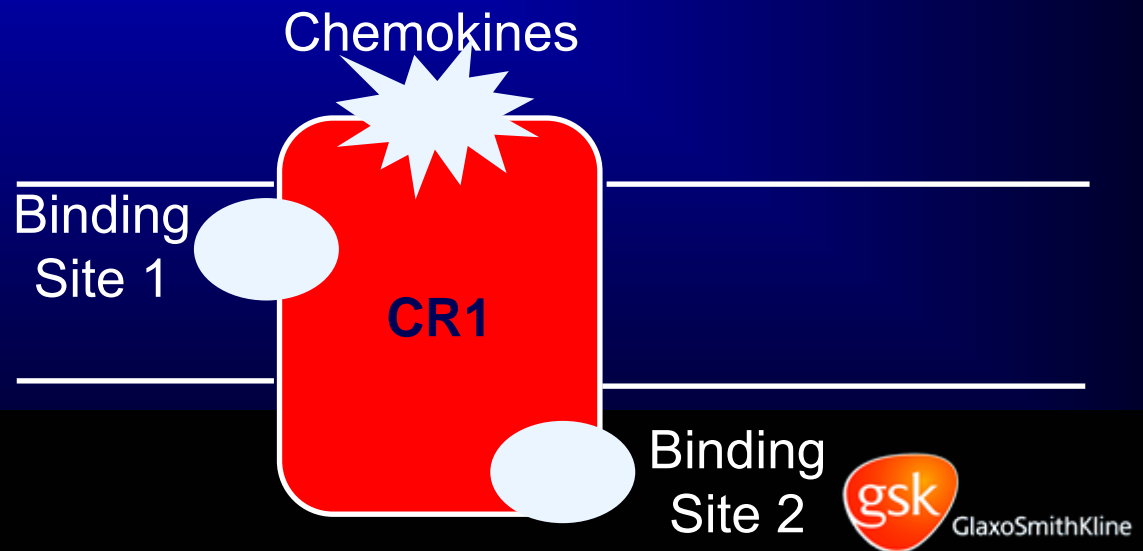
Angela Dunne  
Screening & Compound Profiling  
GSK, Harlow, UK

# Overview

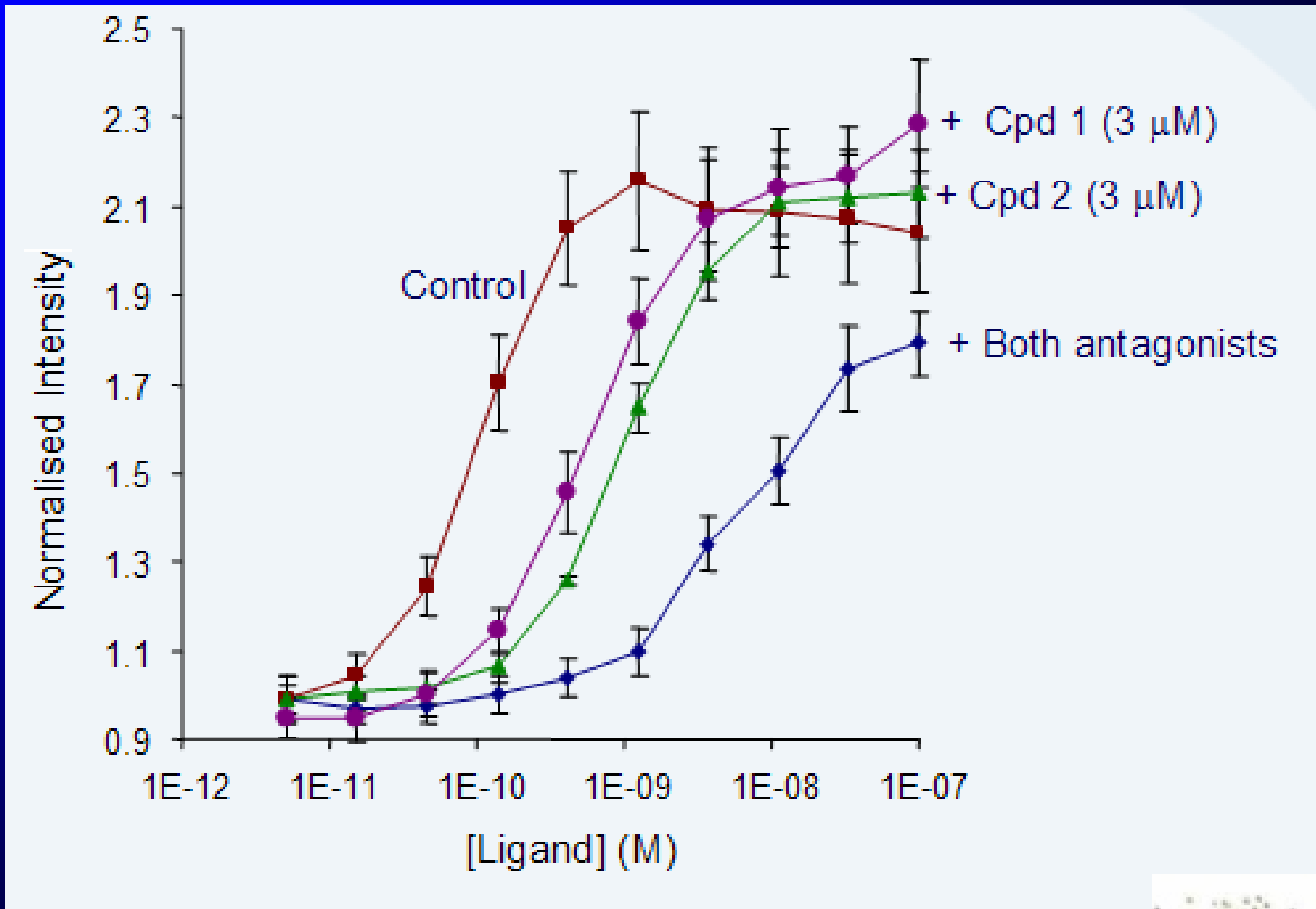
- Screening a Chemokine Receptor (CR1)
- Classic GPCR Approach
- Chemotaxis Assays
  - How can we utilise ‘secondary assays?’
- G protein dependent and G protein independent HIT ID for CR1
  - DiscoverX PathHunter  $\beta$ -arrestin HTS campaign
  - Ligand Biased signalling in a phenotypic chemotaxis assay

# Chemokine Receptor 1 (CR1)

- CR1 target for Asthma
  - Gi-coupled 7TM
  - Expressed on Th2 cells
    - Eosinophil recruitment
    - Airway inflammation
- CR1 Antagonism to inhibit Th2 cell chemotaxis
  - Reduce Th2 cell numbers and cytokine levels at sites of allergic inflammation



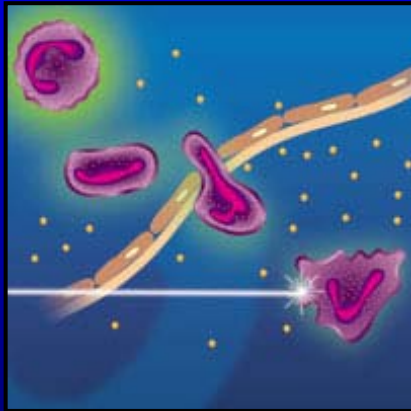
# Interaction Studies Allow Classification of Antagonist Binding Site Selectivity



# CR1 Drug Discovery Program History

- Identification of 2 binding sites facilitates Med Chem
- HTS Campaigns
- Focussed Screens
- Cross Screening
- Label Free Detection trial

No suitable molecules  
identified  
(FLIPR (Gqi) assay)



Phenotype: Inhibition of T cell chemotaxis



# Classic Screening Cascade

*Target is cloned and expressed in a recombinant system*



Assay developed dependent on signalling cascade



**HTS Hit Identification** - Full diversity screen (HTS) run to identify small molecule agonists or antagonists (SS. XC50)



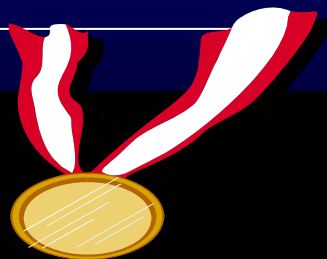
SAR Lead Identification - Hits confirmed & pharmacologically validated at GPCR, *in vitro* selectivity & native tissue assays (XC50)



Molecule optimised for potency, efficacy, developability etc



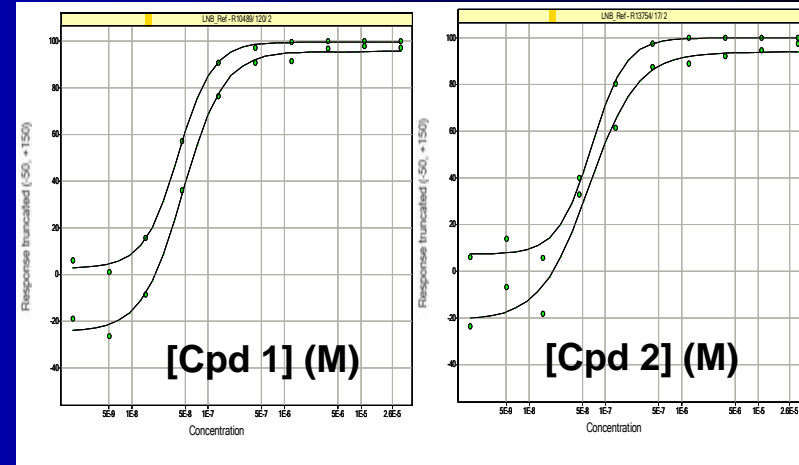
**Lead declared**



# What is the best strategy?

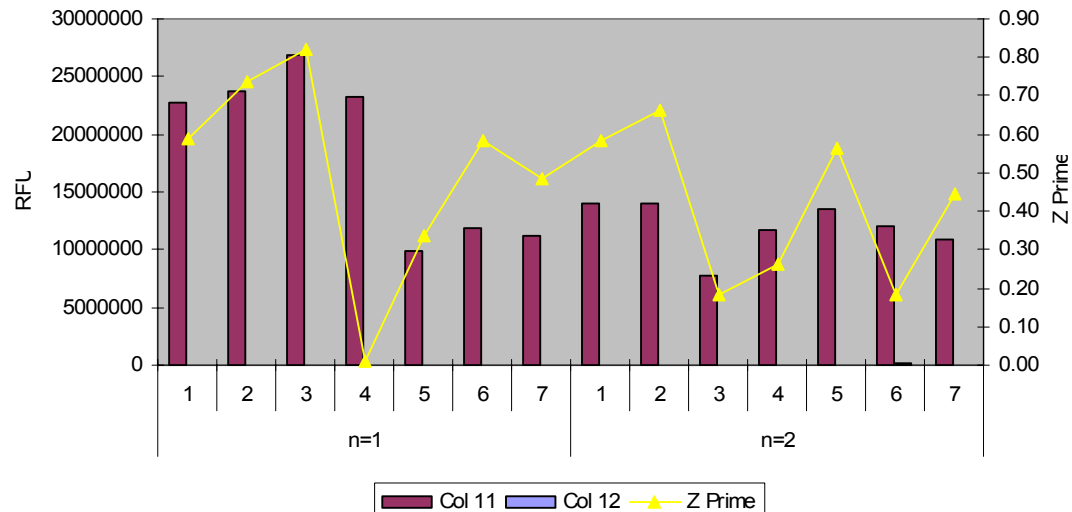
- What molecules to screen?
  - Focussed sets
  - Diversity
- What are the assay requirements?
  - Robust assay
  - Validated against tool compounds
  - Suitable for high throughput

## Phenotypic Chemotaxis assay in Native Tissue



- What assay?
  - Binding
  - Signalling
  - Functional
  - Phenotypic

## CR1 Ligand induced T Cell Chemotaxis Assay Performance



# Chemotaxis Assay for HIT ID....Work in Progress

- Throughput

- Current assay is 96 well
- 384 well assay in development

	96 well (n=6)	384 well (n=3)
Cpd 1	6.6 +/- 0.79	6.7 +/- 0.21
Cpd 2	6.7 +/- 0.44	6.9 +/- 0.16

- Cell type

- HUT78 T cell line
- Th2 cells derived from human blood

- Surrogate chemotaxis assays

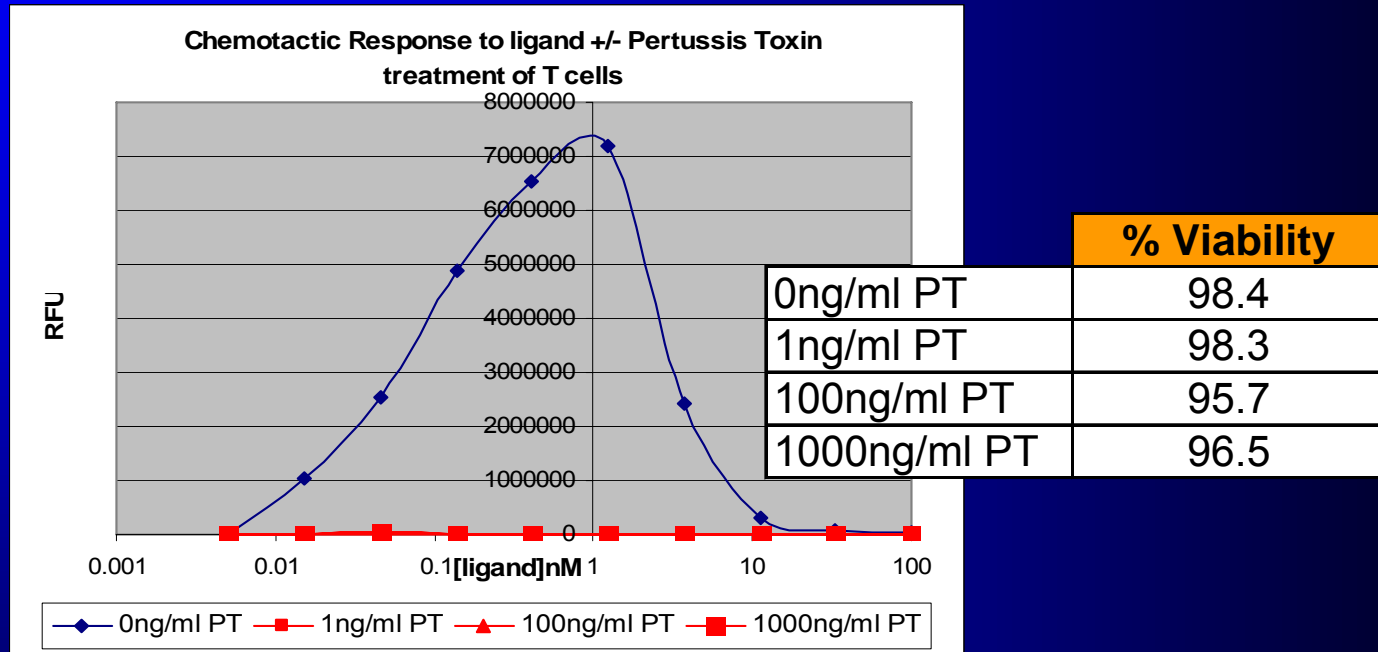
- Actin polymerisation
- Shape Change
- Cell Adhesion

- Alternative Signalling/Functional Assays?

Chemotaxis assay  
currently secondary  
assay in screening  
cascade

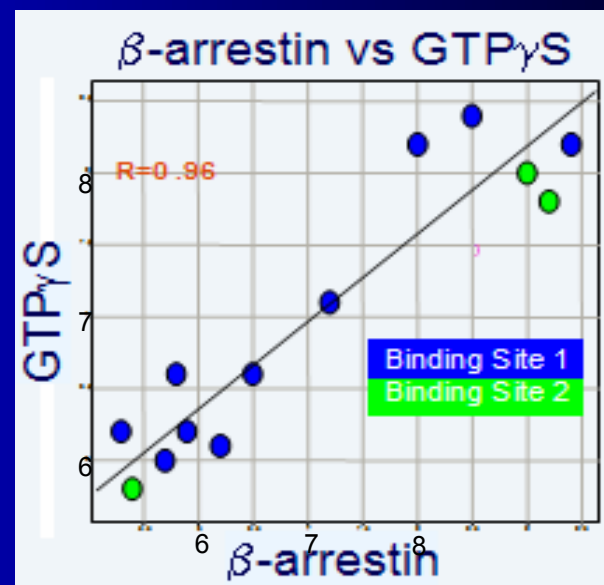
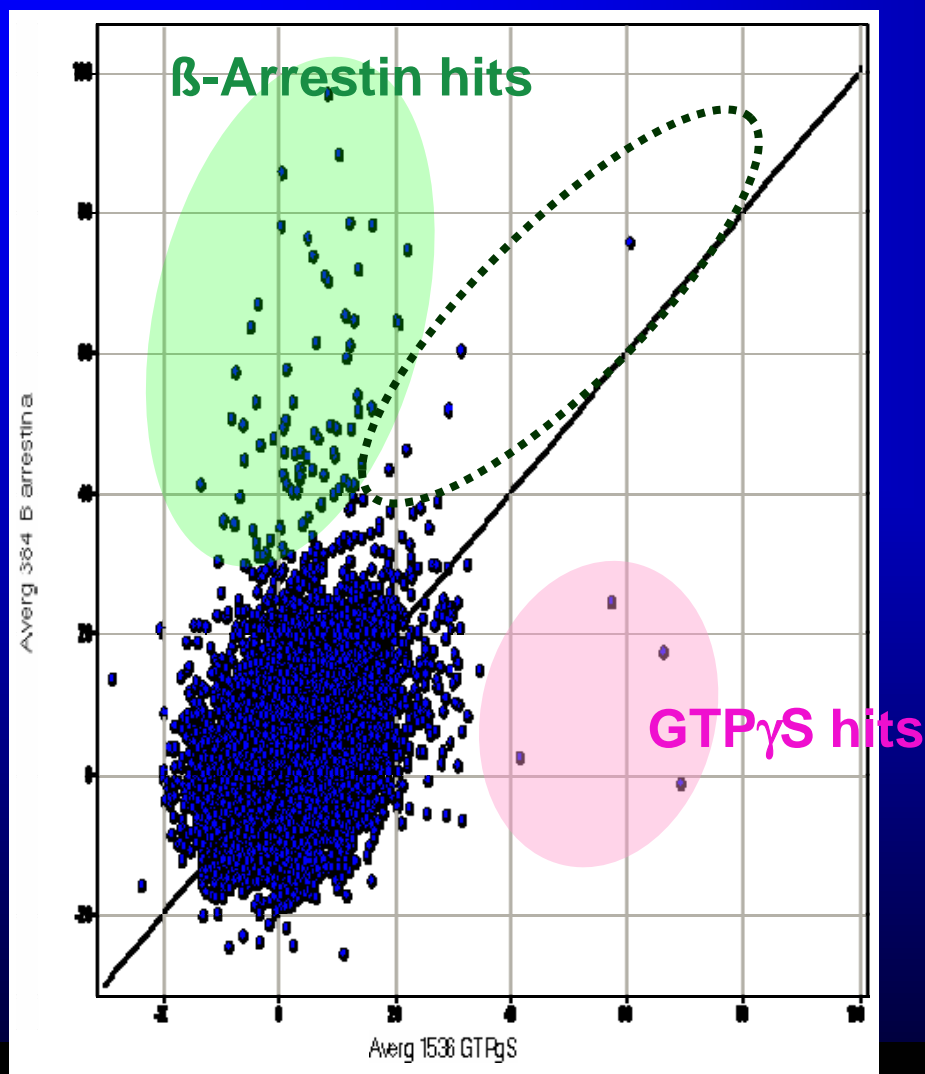
# CR1 Signalling and Functional Assays

- CR1-ligand mediated T cell chemotaxis is Gi protein dependent
  - Pertussis Toxin treatment of cells blocks chemotactic response



- $\beta$ -arrestin plays a significant role in downstream effector response for chemotaxis
- Can we identify novel CR1 antagonists via G protein dependent and independent assay formats?

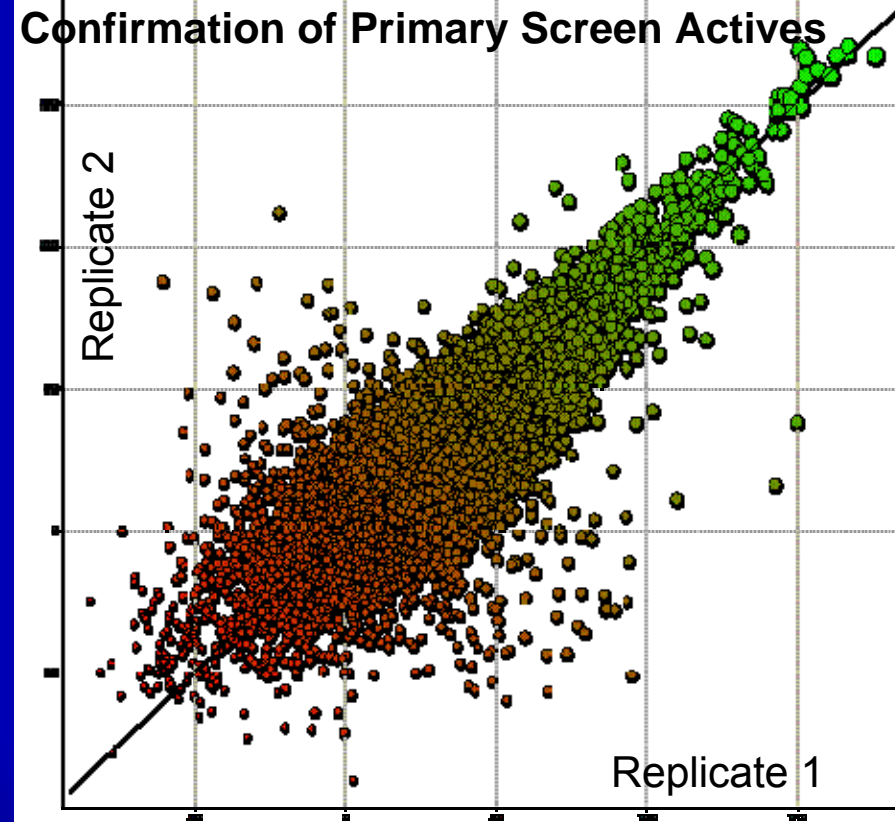
# CR1 GTP $\gamma$ S and $\beta$ -arrestin (PathHunter, DiscoverRx) Assays



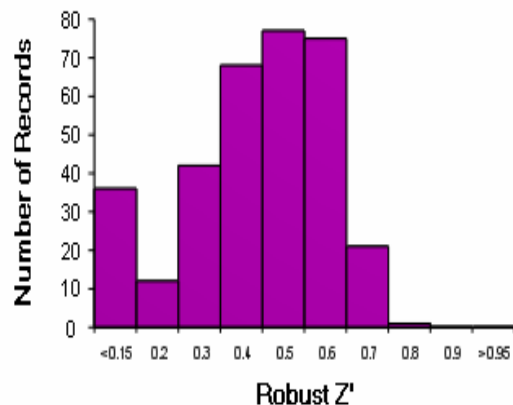
- Good correlation of pharmacological tools
- Hit rate  $\beta$ -arrestin: 1.3%
- Hit rate GTP $\gamma$ S : 0.7%
- Specific and common hits

# CR1 $\beta$ -arrestin HTS

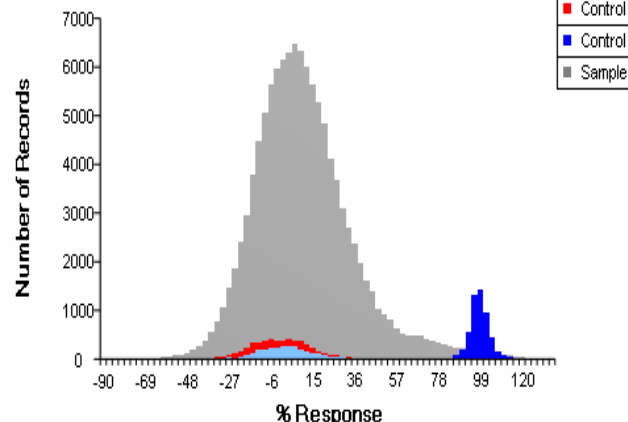
- ~ 2m compounds assayed
- Robust assay performance
- 2.6% hit rate
- 4k compounds progressed to conc response



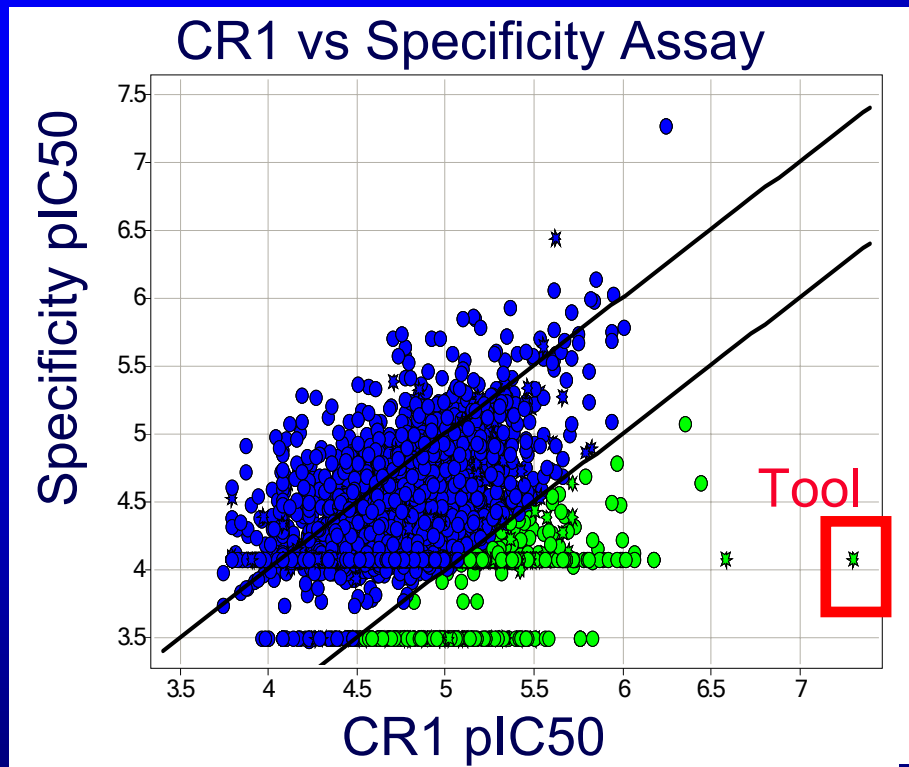
#### Robust Z' Distribution



#### Distribution of Sample and Control Populations



# CR1 $\beta$ -arrestin HTS Output

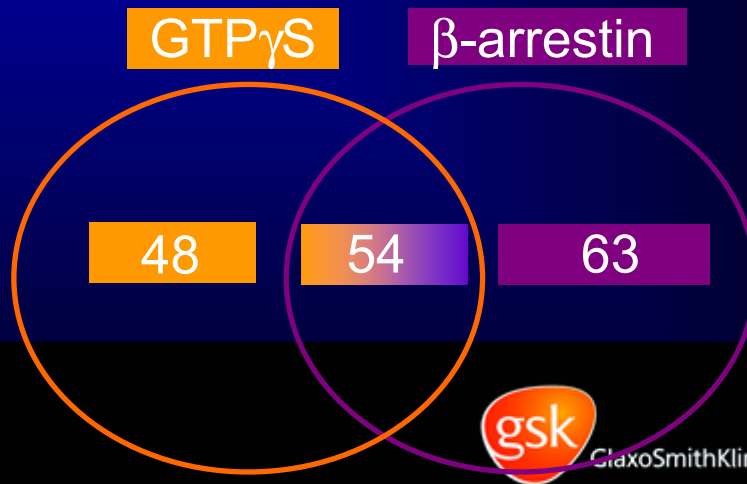
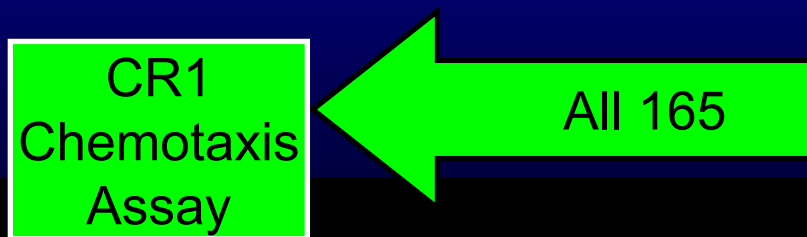


4k were profiled as IC50s

622 target mediated hits identified  
( $>1$  log unit over Specificity)

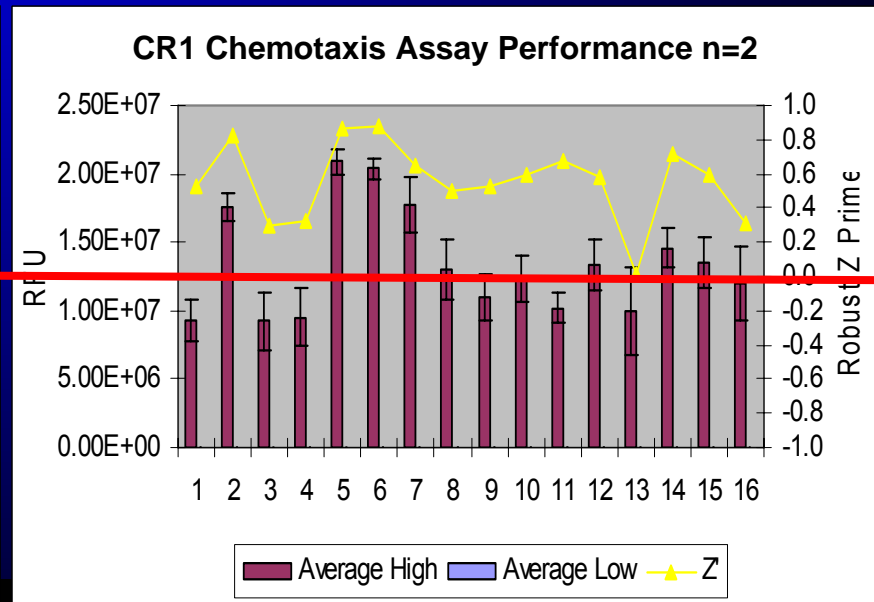
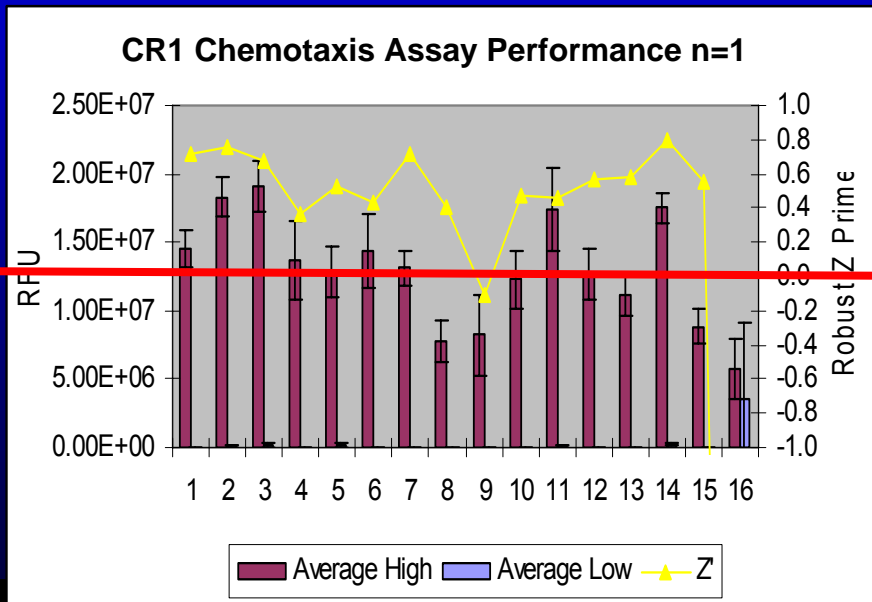
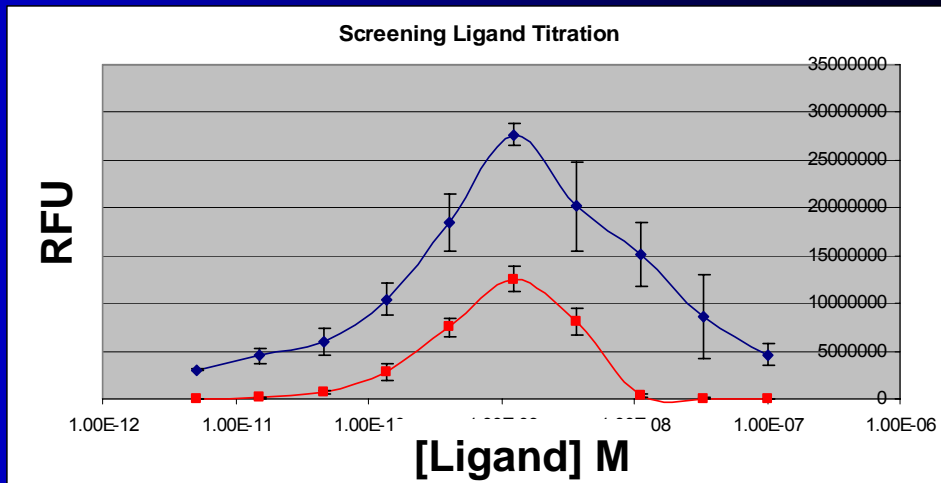
Most potent compound was a  
pharmacological tool

Selection for progression based on  
structure

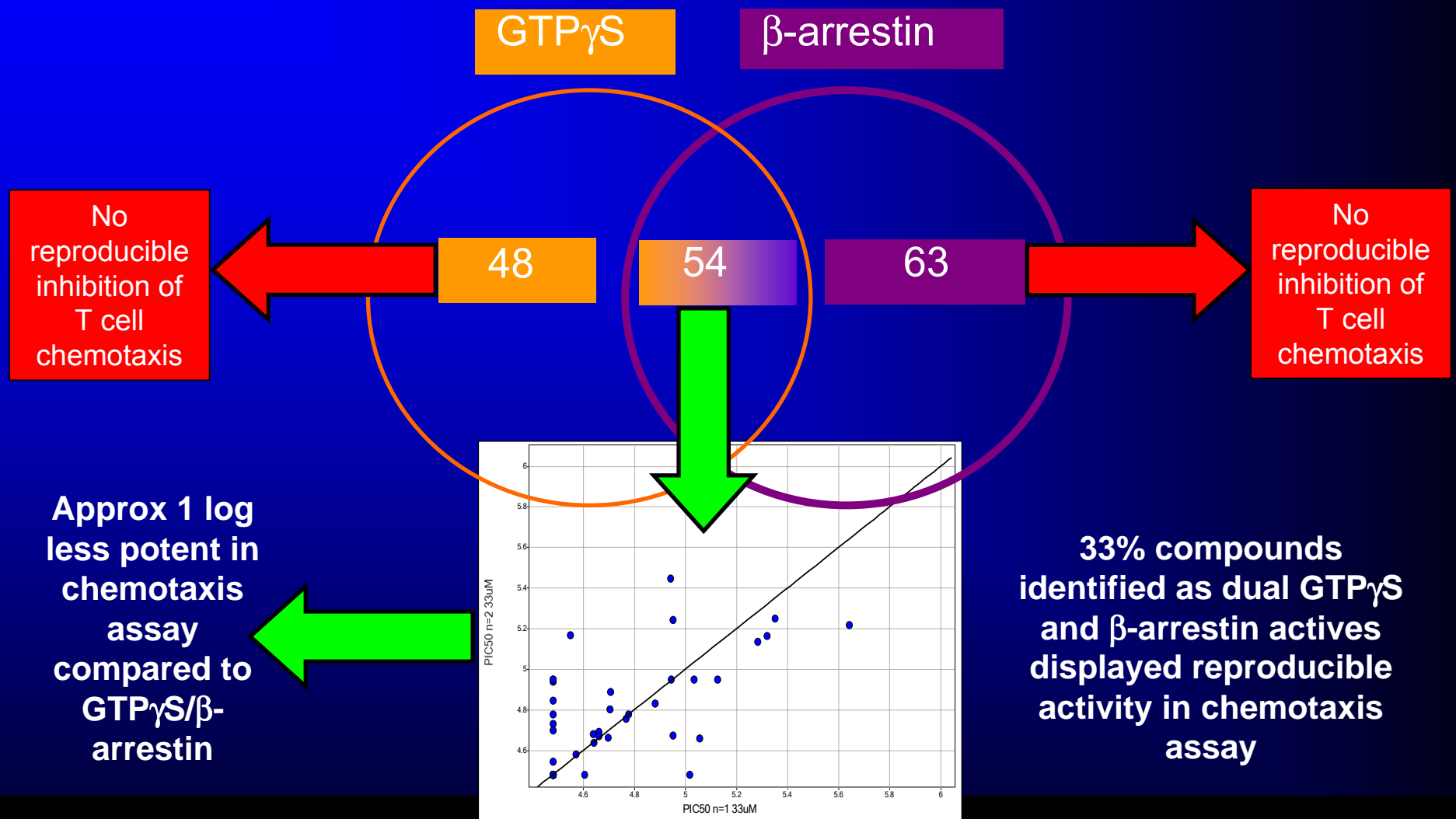


# Validation of CR1 GTP $\gamma$ S and $\beta$ -arrestin HTS outputs in Chemotaxis Assay

- 95% assay plates passed quality control
- Mean Z Prime = 0.56 +/- 0.19 (n=30)
- Ligand titration displayed expected max chemotaxis concentration

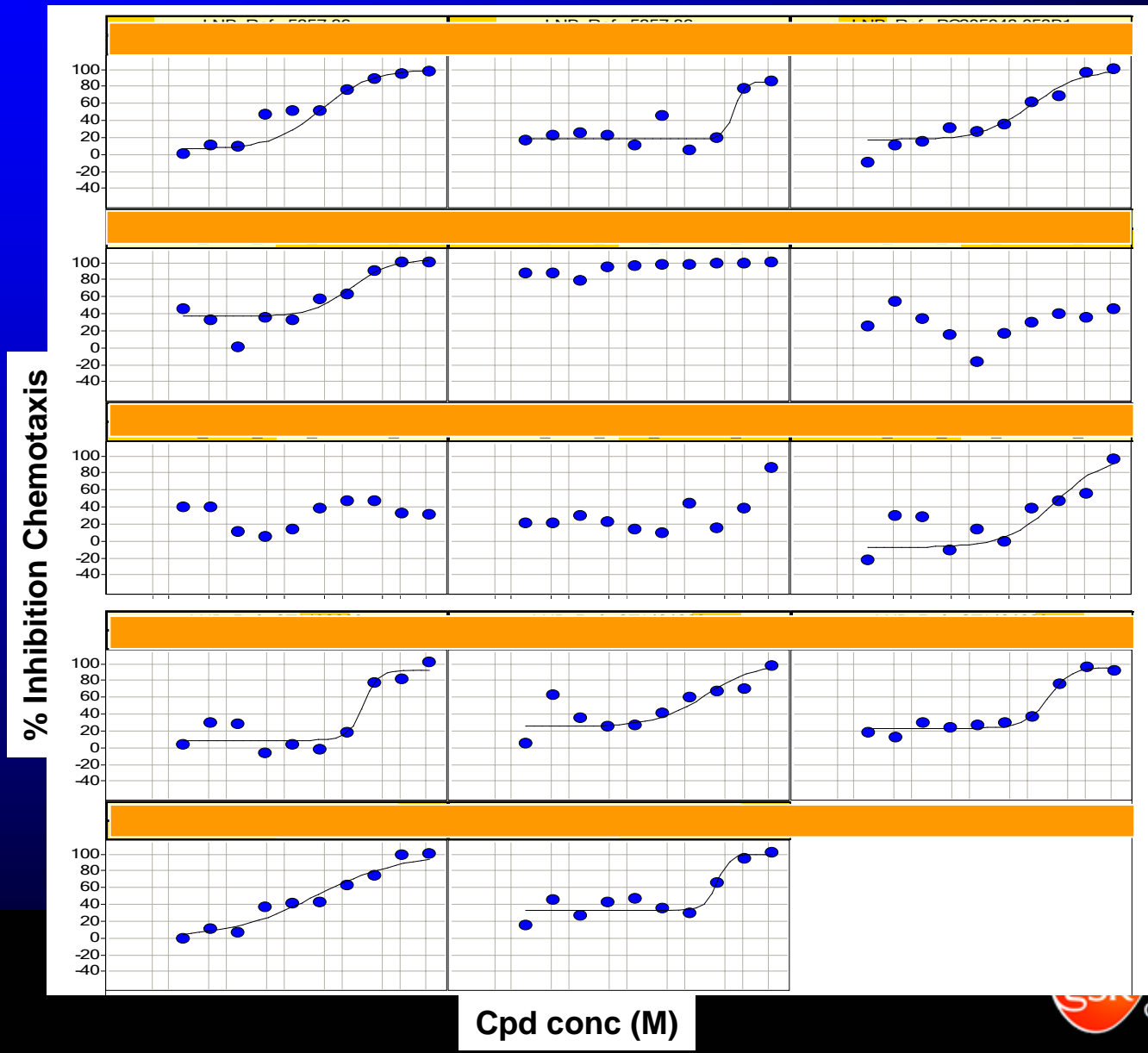


# Validation of CR1 GTP $\gamma$ S and $\beta$ -arrestin HTS outputs in Chemotaxis Assay



Potency Correlation of Dual GTP $\gamma$ S/ $\beta$ -arrestin actives in CR1 chemotaxis assay

# Exemplar Curve Fits of GTP $\gamma$ S/ $\beta$ -arrestin actives in Chemotaxis Assay



# Summary of Data – Inferred Conclusions

- Pre-treatment of cells with pertussis toxin blocked ligand induced chemotaxis response in this CR1-ligand system
- Compounds which blocked  $G\alpha_i$  signalling **OR**  $\beta$ -arrestin recruitment did not inhibit chemotaxis
  - $\beta$ -arrestin assay measuring G-protein dependent and G-protein independent pathways
    - $G\alpha_i$  selective blockers - do not block G protein independent  $\beta$ -arrestin recruitment
    - $\beta$ -arrestin recruitment selective blockers –  $\beta$ -arrestin recruitment not a requirement for chemotaxis
- Compounds which block CR1 receptor mediated  $G\alpha_i$  signalling **IN ADDITION TO**  $\beta$ -arrestin recruitment were shown to inhibit chemotactic response
  - Compounds blocking  $G\alpha_i$  activation and both  $\beta$ -arrestin recruitment pathways
  - Pertussis Toxin MOA
- Further investigation of PT treatment in  $\beta$ -arrestin assay

# Conclusions

- Complex interaction of G proteins and  $\beta$ -arrestin in each assay system
  - $\beta$ -arrestin assays can identify novel chemotypes demonstrating differential signalling properties
  - Many assays required to dissect biology and ligand signalling pathways
- Importance of native tissue phenotypic assay system to elucidate efficacy
  - CR1 requires inhibition of multiple pathways for efficacy
  - Relationship between ligand bias and efficacy may alter with different receptor system

# Acknowledgements

**Simon Hodgson**

**Alison Ford**

**David Hall**

**Maite De Los Frailes**

**Fernando Ramon**

**Ben Powney**

**Lee Collier**

**Keri Hildick**

**Toni Lewis**

**Steve Ludbrook**

**Steve Rees**