



Improving physiological relevance in early phase drug discovery: A focus on 7TM receptors

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Molecular Discovery Research

Biological Reagents & Assay Development

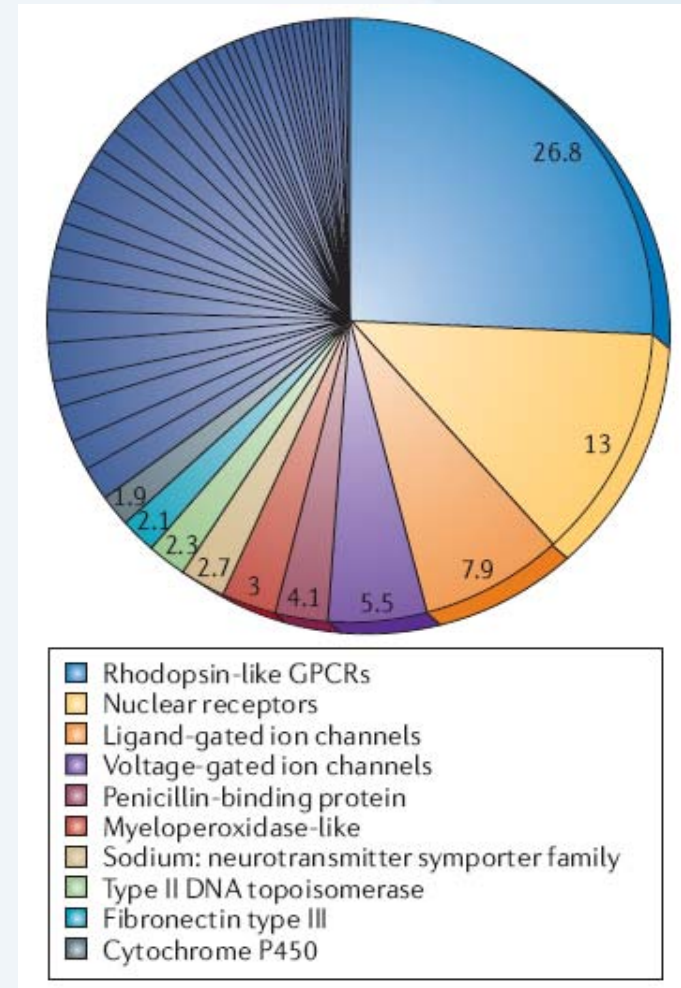
GSK - UK

Presentation outline

- Drug discovery process
- Physiological relevance of assays early phase drug discovery
- Tailoring assays to retain physiological predictability

7TM receptor targets in drug discovery

- 7TMRs are historically and intensively pursued as tractable drug targets across the pharmaceutical industry
 - Encompass ~ 30% of marketed drugs
 - 2008, FDA approved 21 new molecular entities
 - 8 act via 7TMRs (~40%)
 - Target ~ 30/370 members of the GPCR family (biogenic amines receptors)
 - Huge potential to exploit other family members & orphan receptors



Gene-family distribution of current drugs per drug substance
Overington et. al. Nat Rev Drug Discovery 2006

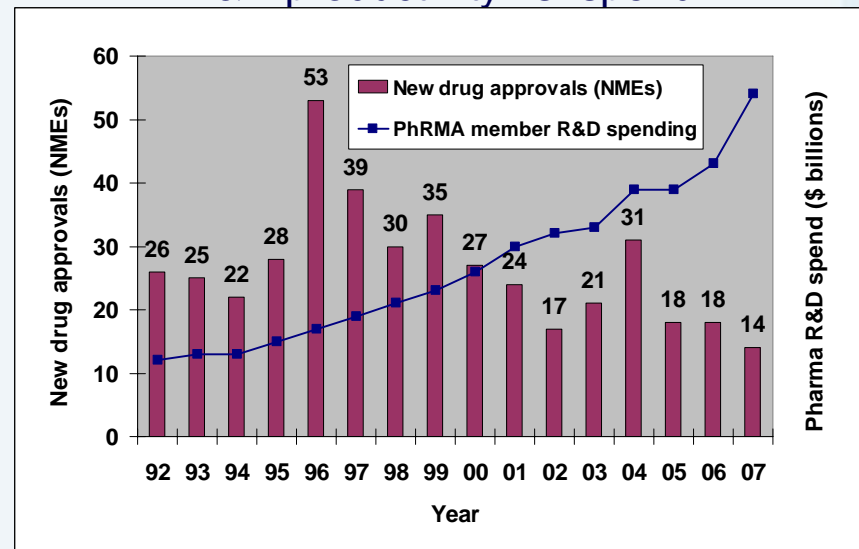
Drug Discovery Process

- Over the last few decades, there has been little change to the drug discovery (DD) process



- Despite these well established and understood processes, DD productivity has fallen
 - R&D has become less cost effective
 - Therapeutic productivity has decreased

R&D productivity vs. spend



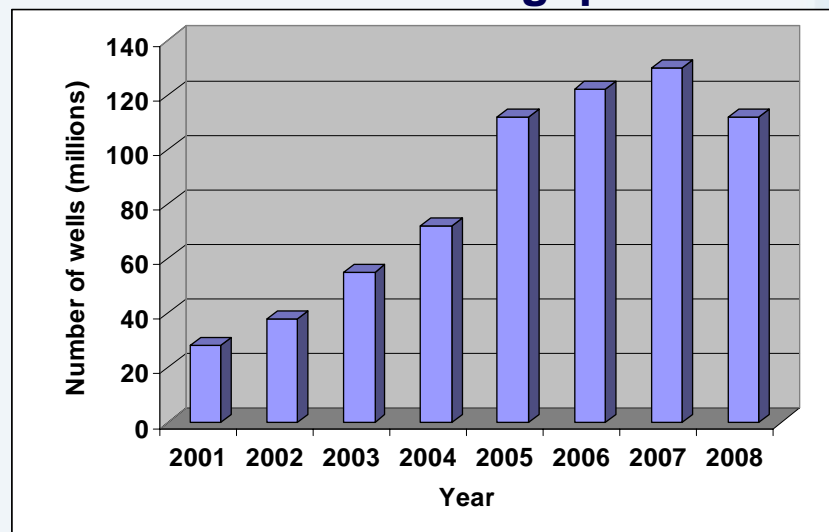
Source: Burrill & Company; US FDA

Note: NMEs do not include BLAs

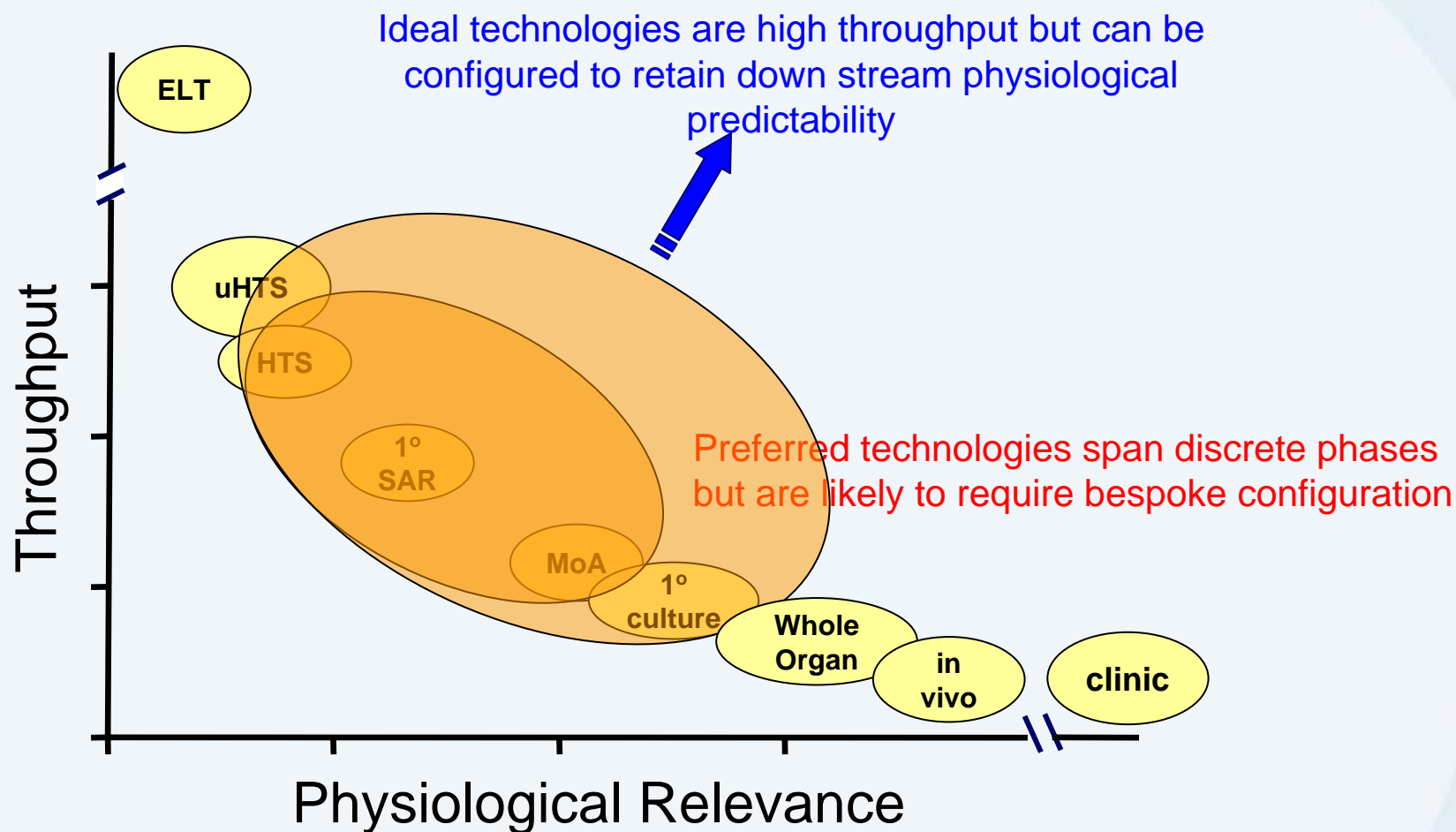
Early phase drug discovery

- Conversely, hit identification rates and costs have improved dramatically
 - molecular biology and recombinant technologies have enabled huge increases in early phase throughputs
 - late stage drug discovery process identifies
 - off-target compound activity and liability
 - a lack of efficacy in animal models and/or clinical trials
 - physiological relevance of high-throughput plate-based 1^o (HTS) and 2^o (SAR) assays?

HTS Throughput



Utility and positioning of screening technologies

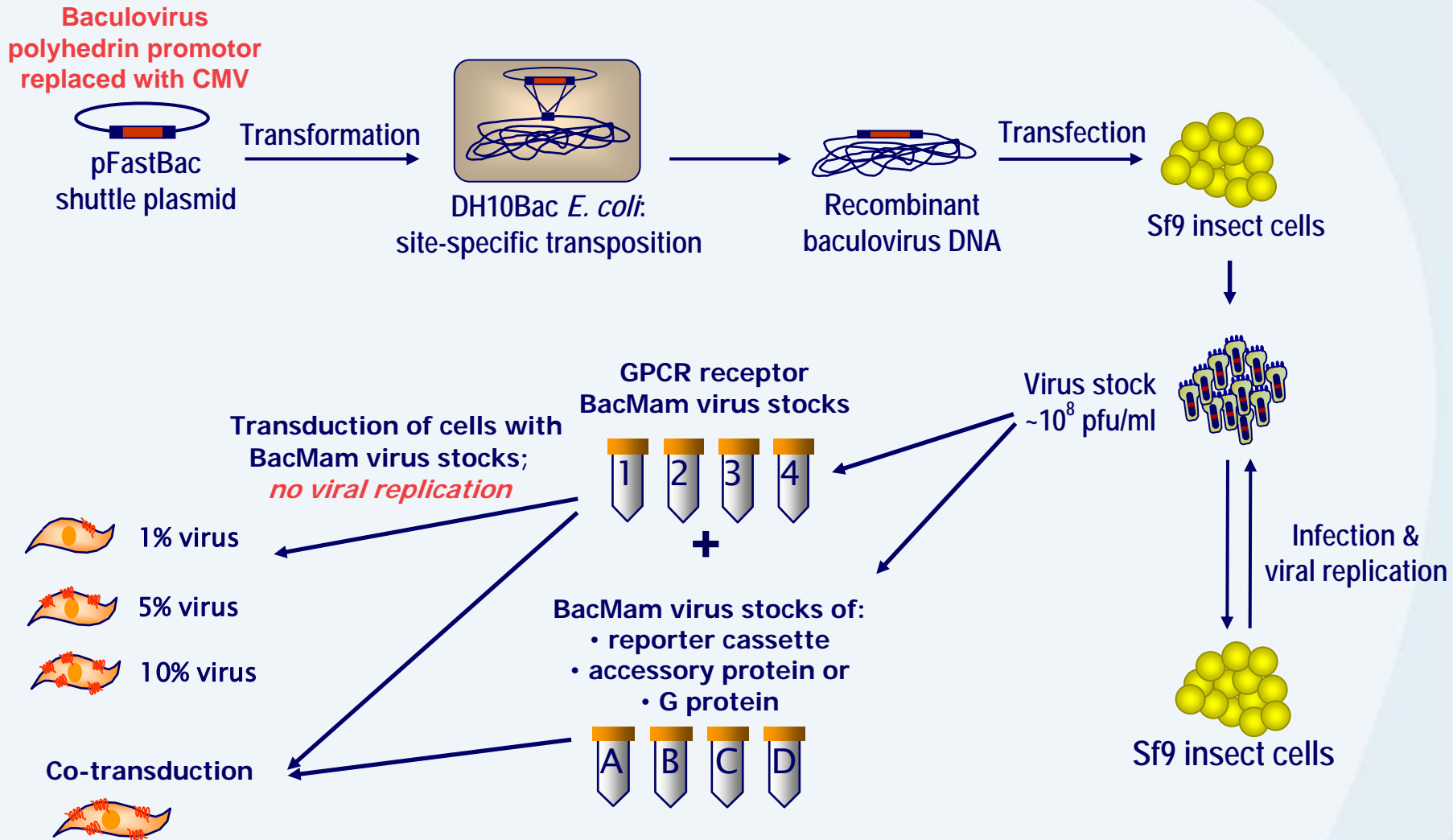


7TMR screening approaches & challenges

- 7TMR are tackled via numerous assay formats
 - Ligand binding
 - G-protein activation ($[^{35}\text{S}]$ -GTP γ S binding)
 - Second messenger generation
 - cAMP
 - Intracellular calcium (FLIPR, aequorin)
 - Ins P₃ (IP-one)
 - Reporter genes
 - yeast
 - β -arrestin
 - GIRK channels
 - etc....
- Focus should continue to be given to design and implementation of high throughput assay formats whilst tailoring outputs to specific requirements
 - Potency
 - Efficacy
 - Mechanism of action
 - Downstream predictability
 - Cost/throughput
 - **All of the above!!!**

Recombinant assays tailored to specific outputs

BacMam reagent generation and transduction



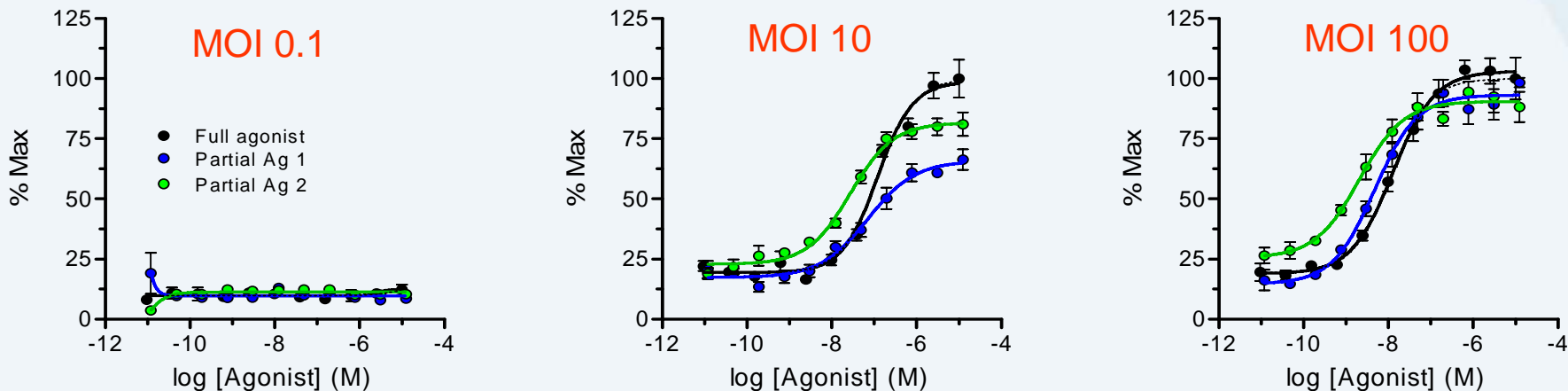
Recombinant 'efficacy' assays to drive compound optimisation

Development of a low efficacy agonist Ca²⁺/ FLIPR assay for hM1 receptor

- Successful screening campaign using BacMam transduced CHO cells
- Disconnect between FLIPR & native tissue data
 - high potency & 'apparent full agonism' across different compounds
 - believed to be 'untrue' based on native tissue & *in-vivo* data
 - existing high expression HTS reagent not longer suitable for SAR
- Native tissue assay while physiologically relevant is very low throughput
- Requirement for a new recombinant SAR screening reagent which was more predictive of native tissue data

Optimising efficacy window with BacMam

- GPCR virus titration +

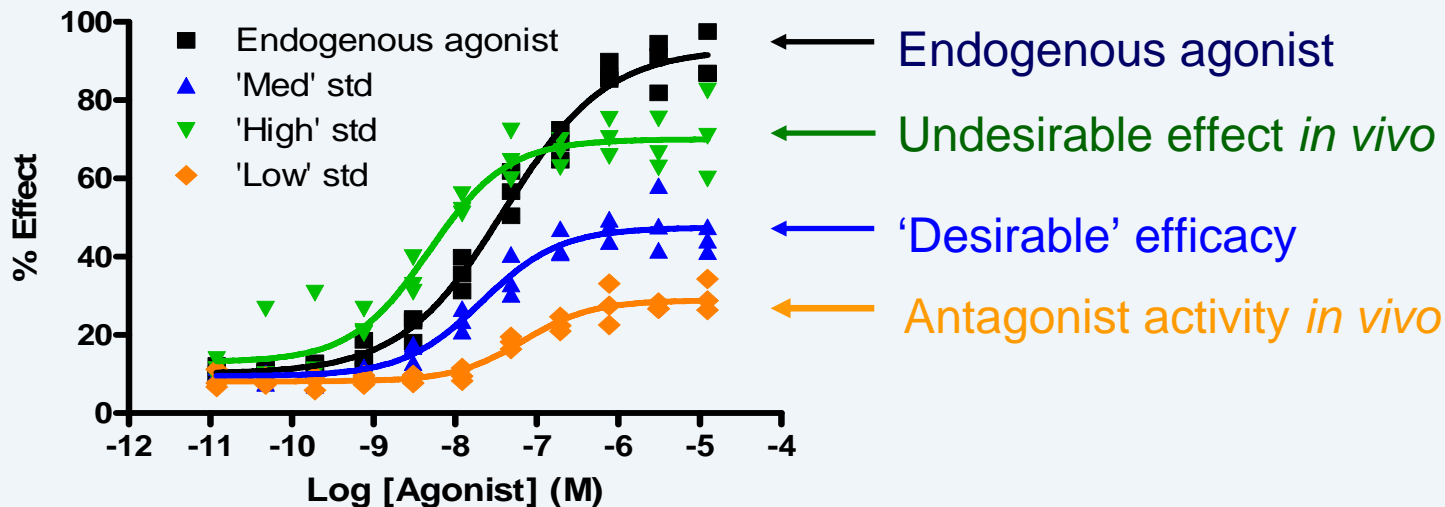


*Fresh CHO-K1 cells transduced with hM1 BacMam 24 hrs prior to assay
384W Ca²⁺/ FLIPR assay
MOI – multiplicity of infection*

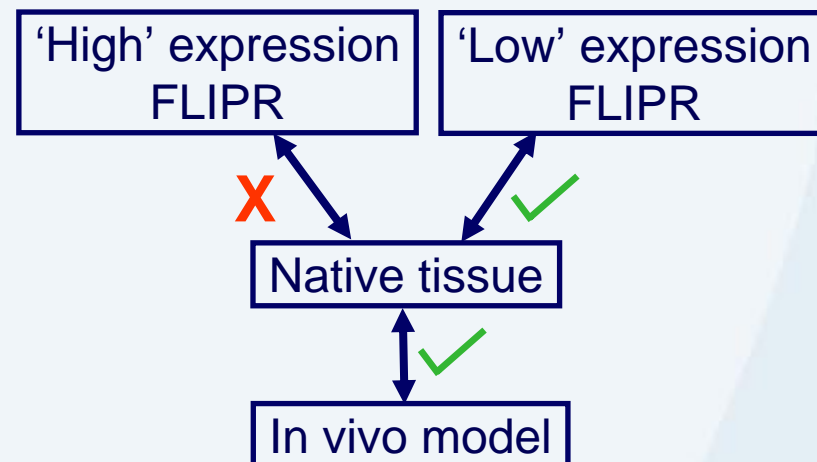
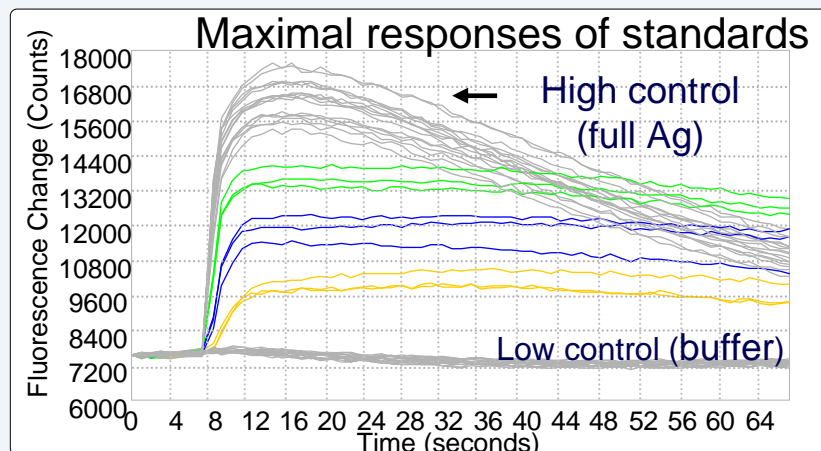
- BacMam can be used to provide differential pharmacologies
- Titration of receptor level to provide the 'desired' efficacy for key compound(s)

Desired efficacy predictive of native system

hM1 receptor MOI 10

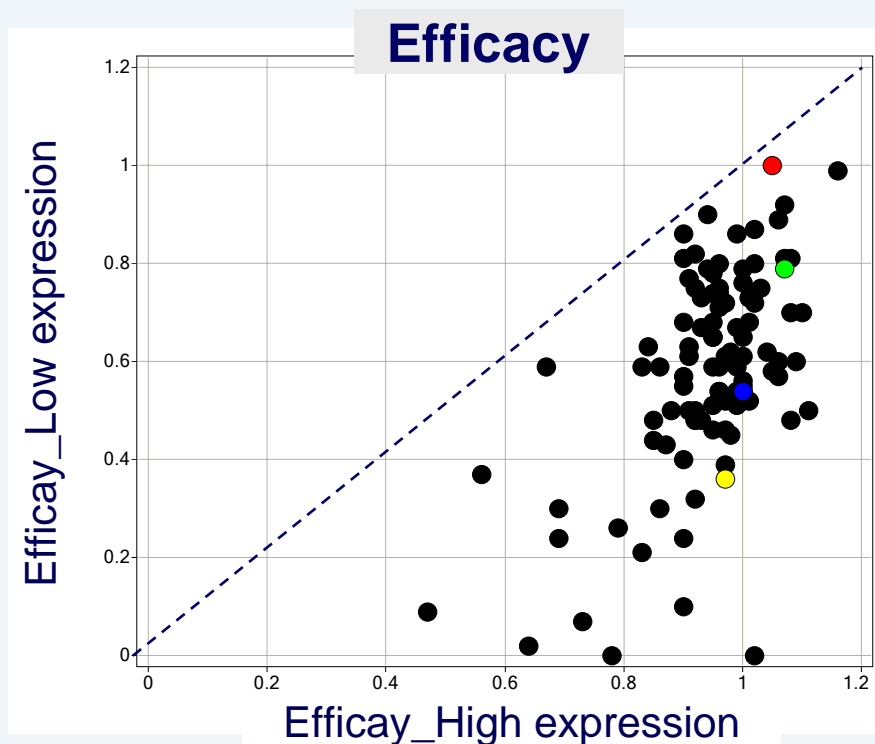


FLIPR Trace



hM1 assay performance over 8 months

Correlation of efficacy: **high vs. low expression BacMam assays**

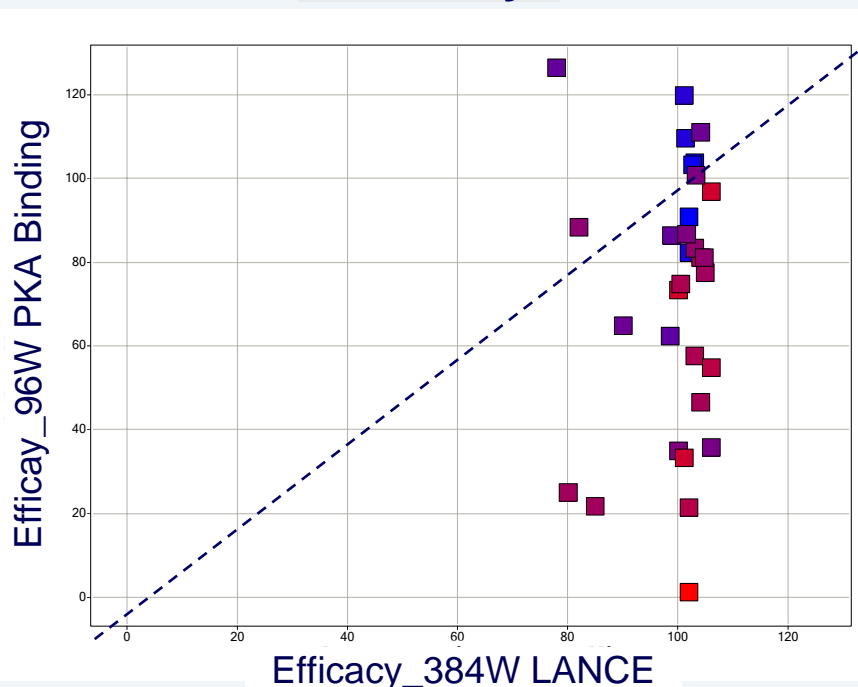


- All key standards display full agonism in the 'high expression' efficacy assay
- Low expression system allows for clear identification of compounds with 'desired' efficacies with respect to key tool compounds

Prostaglandin receptor agonist assay

Correlation between cAMP LANCE & PKA filtration binding assays:
stable cell line

Efficacy

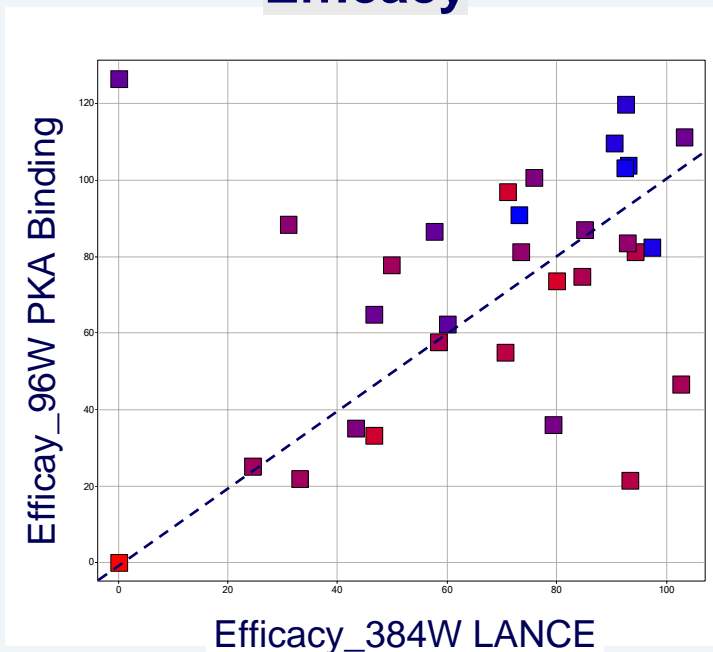


- 96W PKA efficacy correlates with *in vivo* efficacy
- Poor efficacy correlation between the two cAMP assays
- Majority of compounds are full agonists in 384W LANCE assay

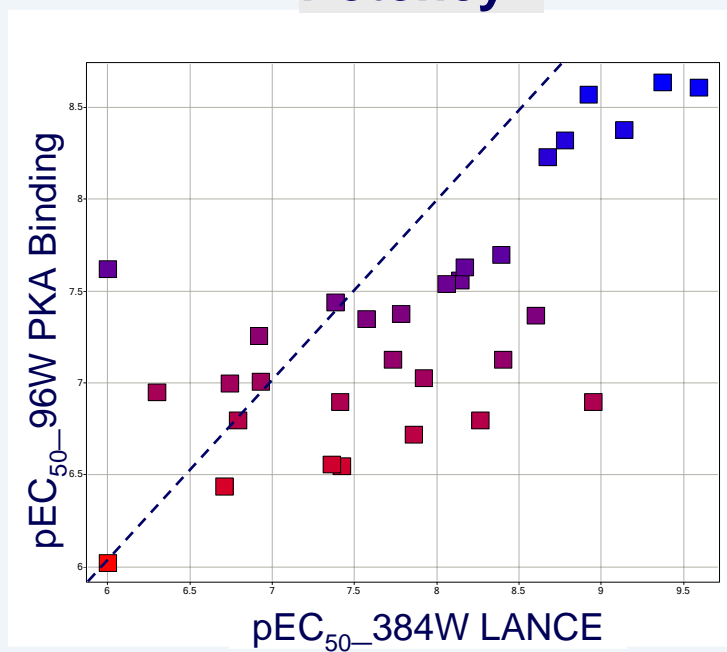
Prostaglandin receptor agonist assay

Correlation between cAMP LANCE & PKA filtration binding assays:
BacMam transduced U2OS cells

Efficacy



Potency



- Lower efficacy assay achieved using BacMam expression of receptor
- Improved correlation of efficacy between 96W and 384W assays
- High volume, lower throughput 96W assay redundant

Recombinant 'efficacy' assays to drive compound optimisation

- An efficacy readout predictive of downstream native assay systems is believed to be highly valuable:
 - To rank and/or prioritise compounds prior to tissue assays
 - Monitor **relative efficacy** with respect to known standards
 - Filter compounds to reject 'unsuitable' ones for further progression
- BacMam viral transduction methodology is enabling
 - Titrate and closely control the level of receptor expression
 - multiply cell lines & assay formats
 - Achieve a defined pharmacology for key compounds
 - A desired efficacy of a range of standard agonists can be tuned to match relevant functional tissue end points
- Third generation assays
 - *In-vivo* data on key compounds used to refine existing recombinant assay or highlight the need for new assays

Challenges selecting predicative recombinant assays

Choice of assay format

Many factors are considered and influence the choice of assay for HTS & or SAR campaigns

● Target

- Expression strategy
- Reagent generation & re-supply
- G-protein coupling
- Pharmacology of standards

● Assay

- Sensitivity
- Quality/precision
- Capacity (1536 vs. 384)
- Cost (time, FTE)
- Radioactive

Assay design to meet key criteria

Development of a functional antagonist assay for a Gi-coupled P2Y-purinergic receptor

Considerations

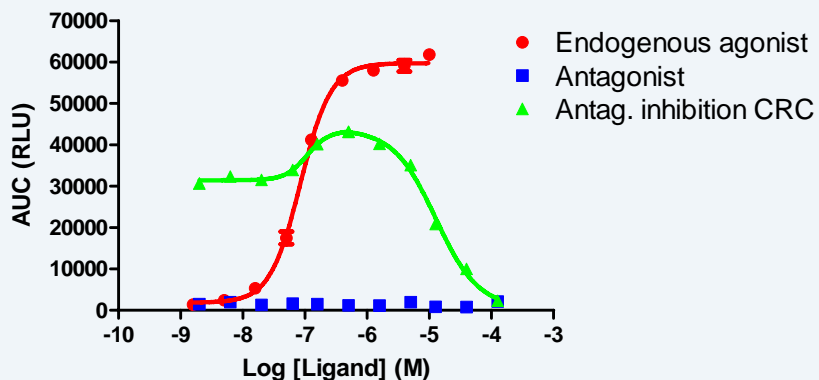
- Receptor coupling specificity
 - Native vs. non-native signalling
- Host cell line
 - Endogenous background
- Standard agonist pharmacology
- ***In vivo* profile of tool compound**
 - **Antagonist**
- HTS amenability & acceptance criteria
- Cost (time, FTE)

Options

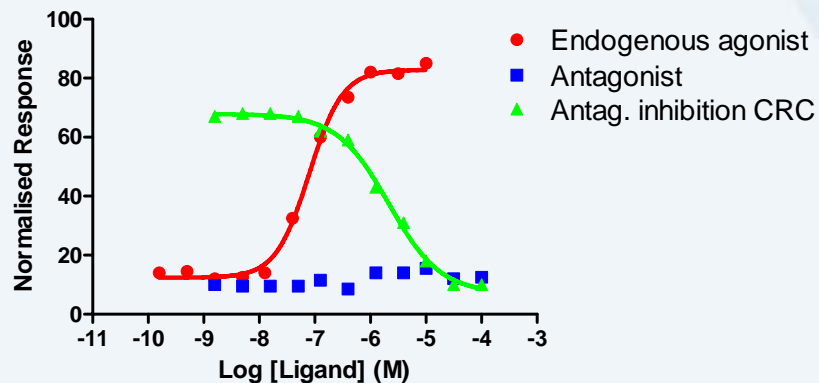
- cAMP / LANCE
 - Challenging for Gi-coupled receptors
- GTP γ S
 - Radioactive, membrane supply
 - Nucleotide agonists compete with GTP γ S
- Yeast
 - Cheap, clean background
- **Ca²⁺ (+/- chimeric G-protein)**
 - aequorin, FLIPR
- **Parallel approaches**

Comparative pharmacology – assay dependence

384W Ca²⁺ Aequorin



384W Ca²⁺ FLIPR



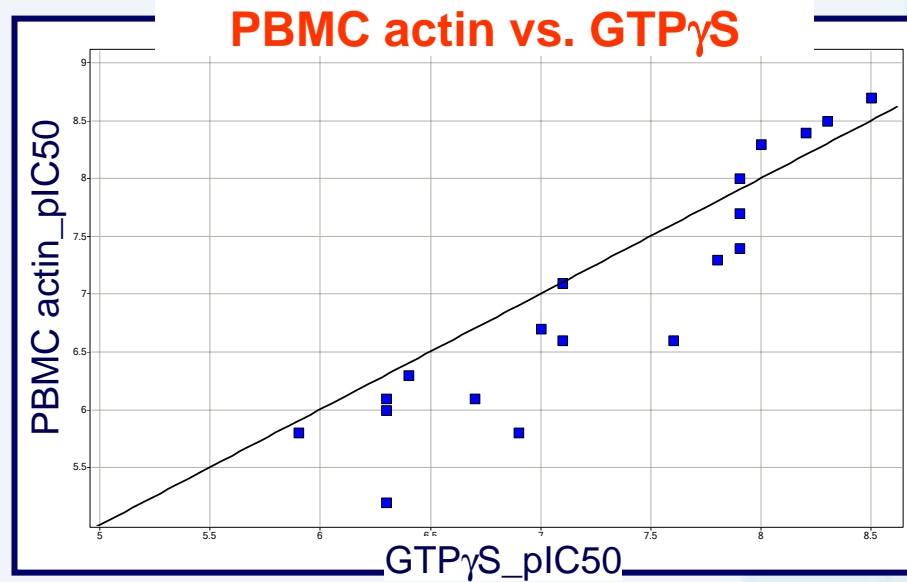
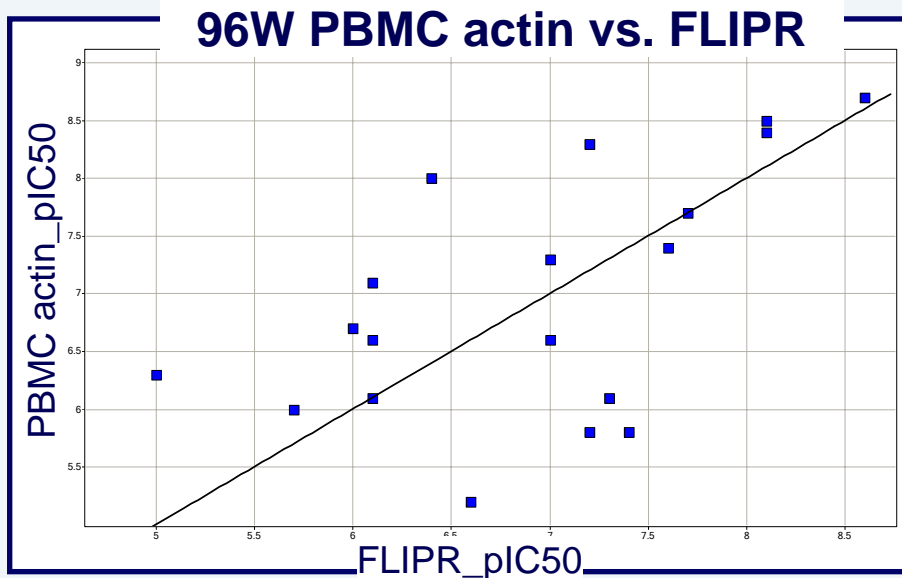
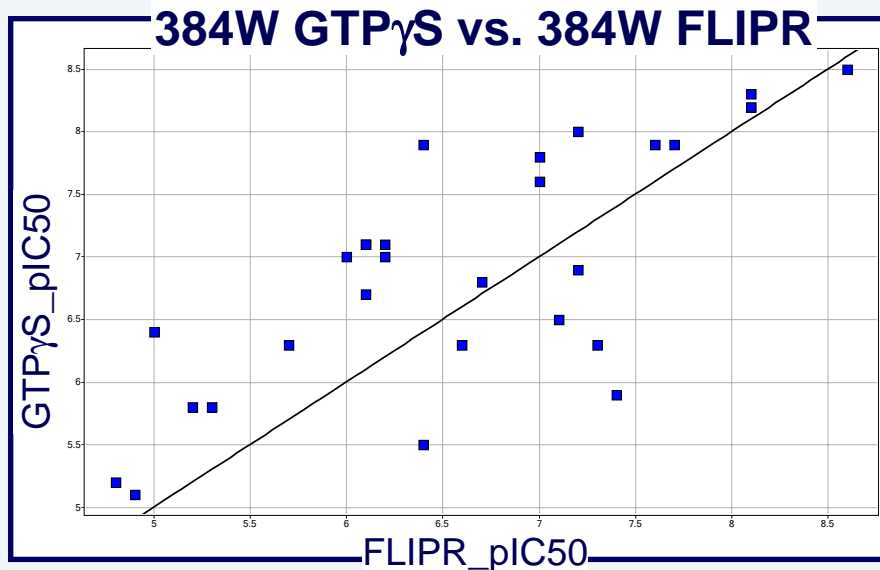
- Both assays measure changes in intracellular calcium – different detection
- Aequorin assay highlights complex antagonist pharmacology for tool compound
 - bi-phasic curve not observed in *in vivo*
- Antagonism observed in FLIPR assay agrees with *in vivo* data
 - FLIPR format assay of choice

Evolving assay design over 10 years....

Development of a functional antagonist assay for a Gi-coupled chemokine receptor

- Initial approach was to develop a 384W Ca²⁺/ FLIPR using a stable cell line (+ Gqi5)
 - pharmacology of known endogenous agonists as expected
 - no standard antagonist available
 - assay amenable to HTS environment
 - **relevant & appropriate choice of reagent & assay format**
- In-house and literature compounds were identified, a disconnect between FLIPR data and primary cell data was observed
 - disconnect between FLIPR data & other recombinant assays
 - **FLIPR assay using existing reagent considered inappropriate for HTS & SAR**
- Alternative, more relevant reagent and assay configured for HTS & SAR.

Comparative antagonist pharmacology – assay dependence



Building 'predictive' assays

- Pharmacology is both system and compound dependent
 - 'artefacts' may be real & informative – MoA, alternative coupling?
- With parallel approaches, more than one system may provide a relevant assay
- Pragmatic decisions on assay formats may need to be taken when little tool compound data is available but must be reviewed
- Advances in liquid handling technologies & miniaturisation have enabled previously undesirable assay formats to be used for HTS (e.g. GTP γ S)

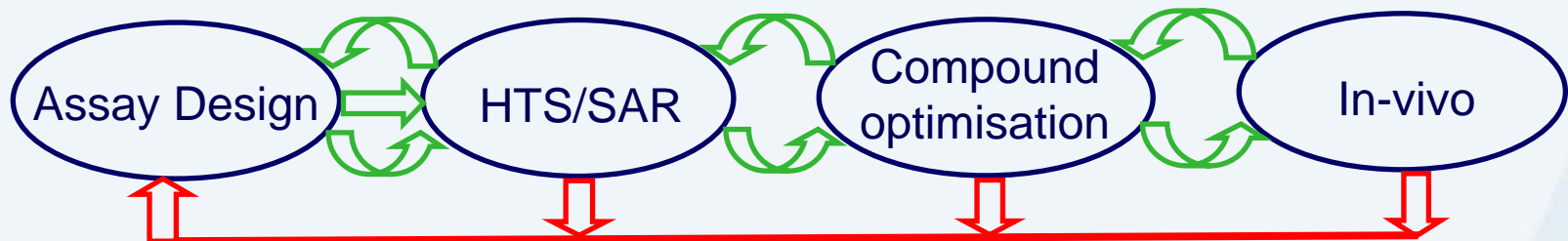
Future assay formats

Additional methodologies with increasing value to 7TMR ligand discovery

- Homogenous β -arrestin assays (e.g. PathHunter, Tango)
 - G-protein independent signalling
 - functional selectivity (β -arrestin-biased ligands)
- High content screening assays
 - Imaging assays (e.g. Cellomics Arraycsan™, Evotect Opera™)
 - spatial and temporal profiles of signalling (e.g. MAP kinase)
 - Label free detection technologies (e.g. SRU-bind, CellKey)
 - detecting whole cell responses in a label-free manner
 - Interpretation of the responses in both recombinant and native cell systems remains challenging
- The use of primary/ stem cells may provide even greater value
 - Use in the 384-well plate based assays (e.g. chemotaxis plates, HCS and label free)

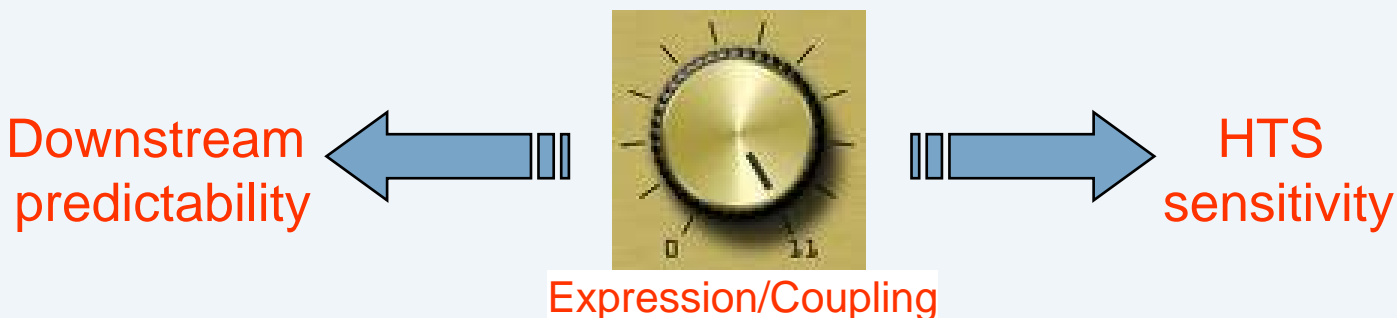
Summary

- R&D productivity is perceived to have fallen over the last few years
- To address late stage drug attrition, drug discovery process must be modified
- To exploit the advances in hit-identification rates compound profiling assays should be configured to be more predictive of downstream readouts
- Assay design and optimisation is an iterative process akin to that of compound/structural optimisation



Summary

- Assays tailored to hit identification are not guaranteed to be applicable post HTS



- Assays with physiologically predictive outputs will ensure meaningful compound triage
- Improved physiological relevance of assays in early phase drug discovery will help increase therapeutic productivity

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