“Target Validation”

An overview for sceptics

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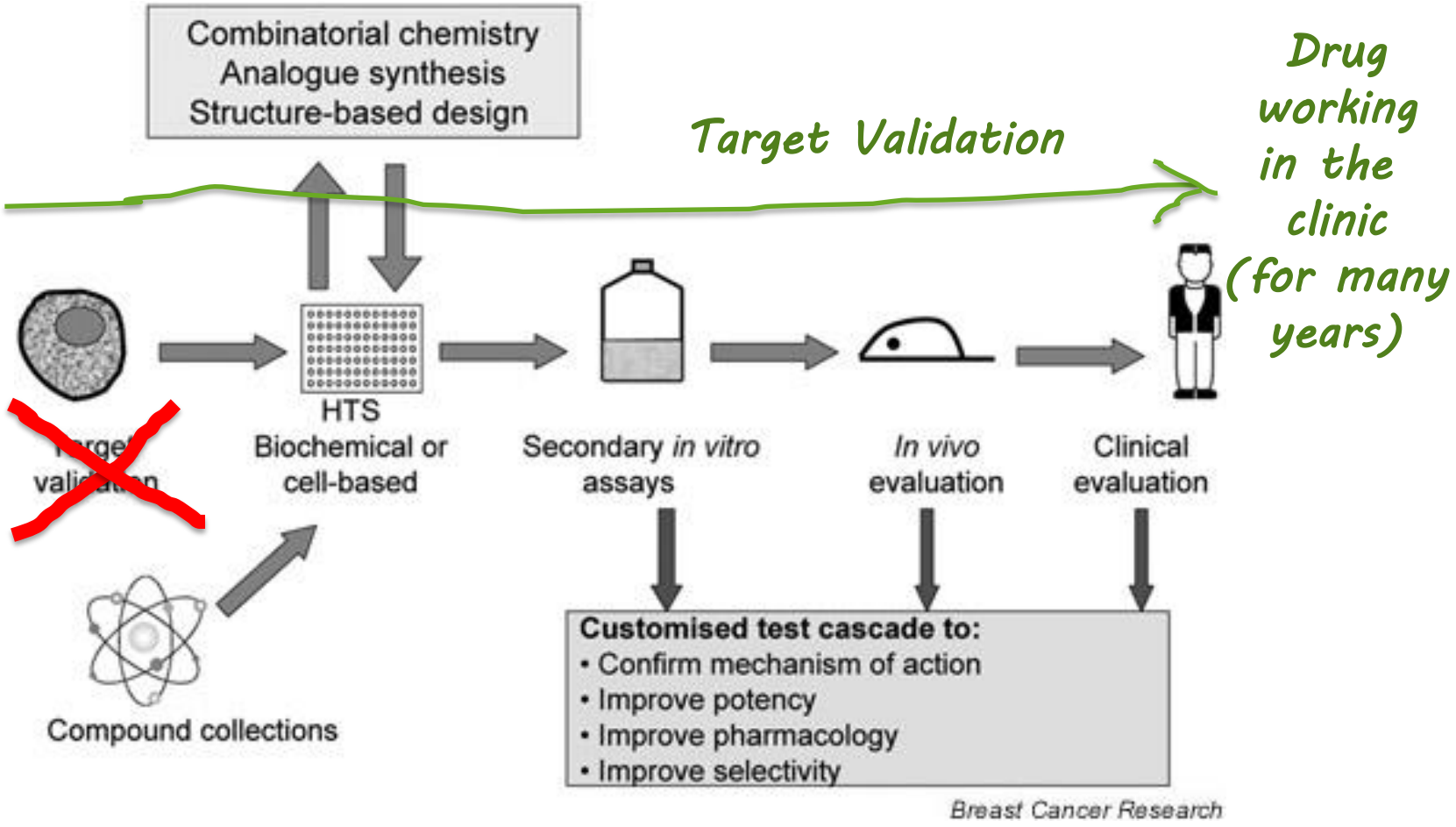
What is Target Validation?



Cox-2 : "A validated target"

Should safety be part of the target validation process?

Target Validation: The first rule

"All things are poison, and nothing is without poison; only the dose permits something not to be poisonous"

Paracelsus, 1493-1541

What makes an “ideal” drug target

Disease Validation

- Target has proven disease-modifying function
- Modulation of the target is less important under physiological conditions

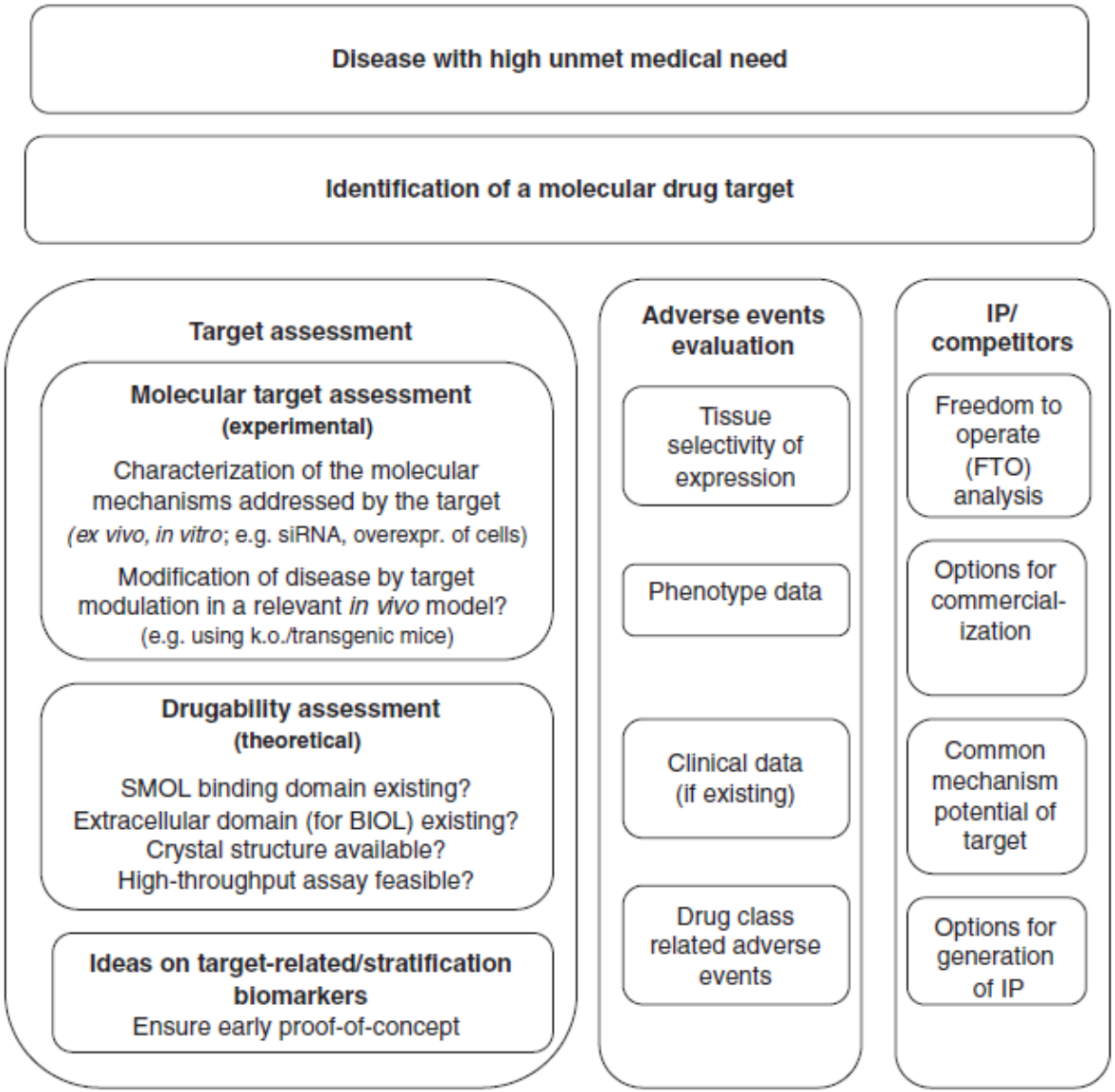
Tractability

- If the druggability is not precedented, a 3D-structure for the target protein or a close homolog should be available for a druggability assessment.
- Target has a favorable ‘assayability’ enabling high throughput screening

Safety & Efficacy

- Target expression is not uniformly distributed throughout the body
- A target/disease-specific biomarker exists to monitor therapeutic efficacy
- Favorable prediction of potential side effects according to phenotype data (e.g. in k.o. mice or genetic mutation databases).

What makes a good target? : The Bayer view



Gashaw, et al 2012
Drug Discov Today.
Feb;17 Suppl:S24-30.

Perspectives on target validation

Biology

- Biological mechanism
- Disease association
- Tissue expression
- Pathways

Variation

- Genetic variation
- Isoforms/splicing
- Epigenetics

Similarity

- Orthology (animal models)
- Homology (off target effects)

Tractability

- Druggable
- Biopharmable
- Other, e.g. siRNA

Robust Assay

Perspectives on target validation

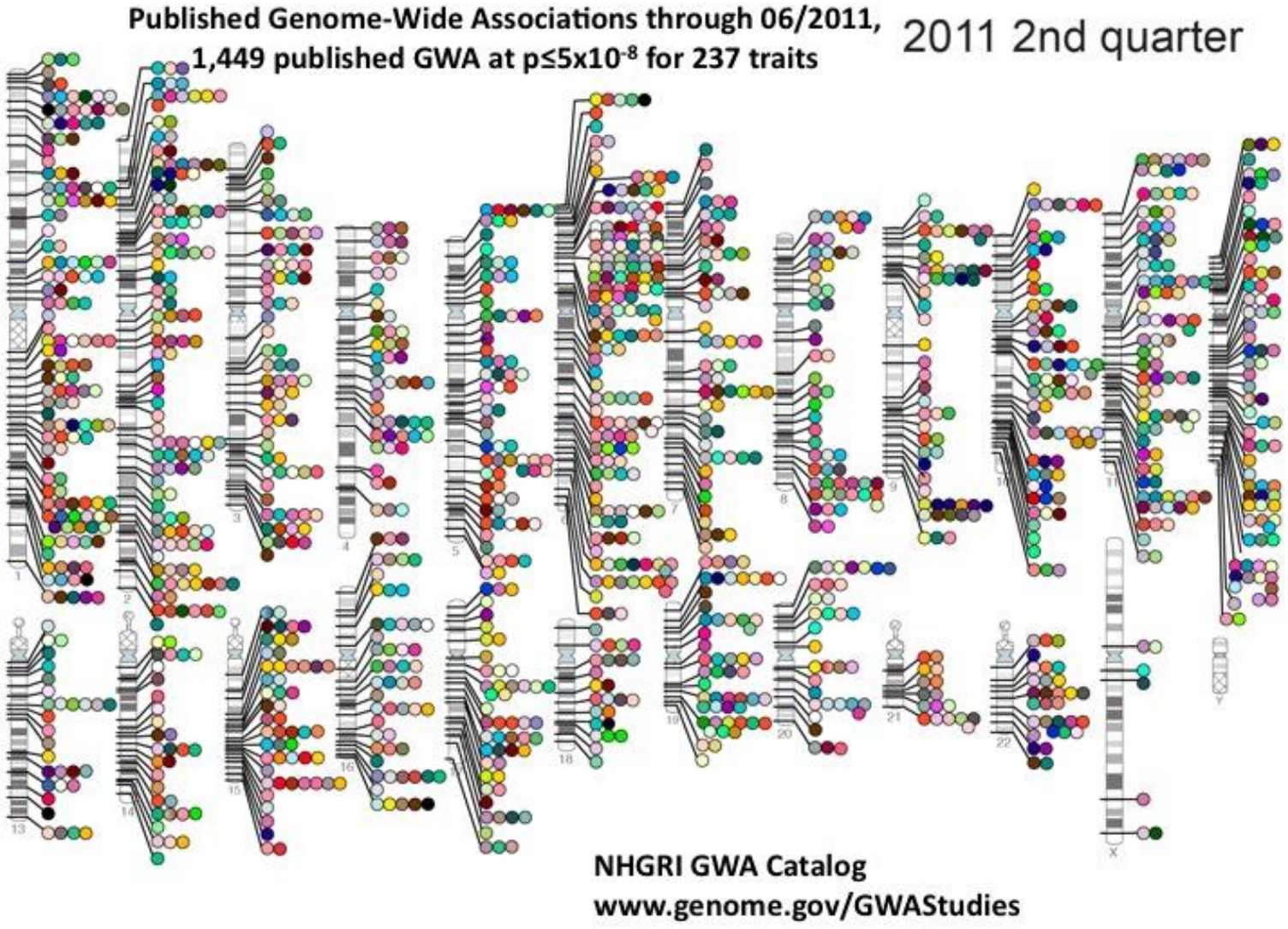
Biology

- Biological mechanism
- Disease association
- Tissue expression
- Pathways

What is a Genome Wide Association Study (GWAS)?

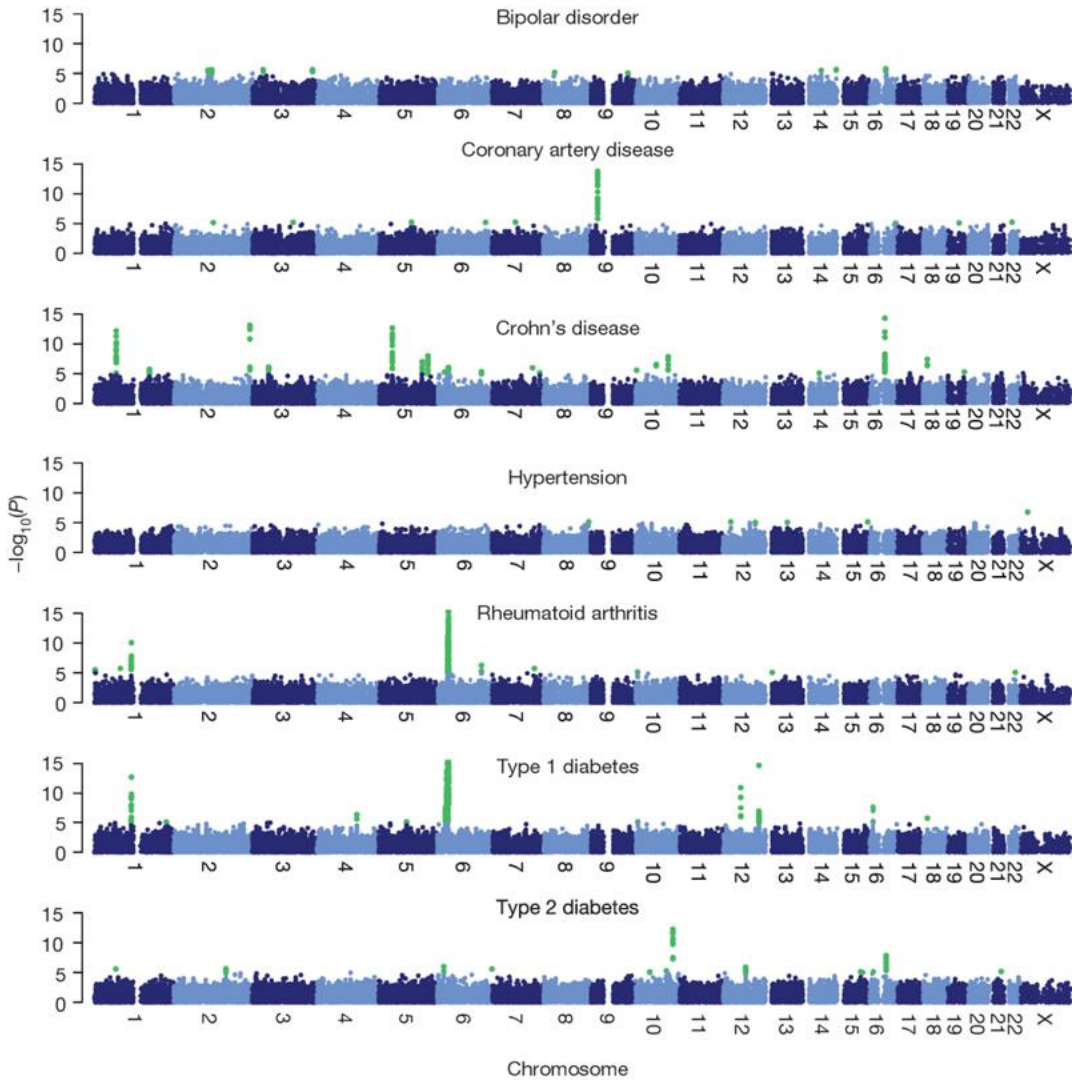
- A genome-wide association study is an approach that involves rapidly scanning genetic variants (markers) **across genome** (≈0.5M or 1M) of **many people** (>2K) to find genetic variations associated with a particular disease or trait.
- A large number of subjects are needed because
  (1) associations between causal variants in common diseases are expected to show **low odds ratios**, typically below 1.5
  (2) In order to obtain a reliable signal, given the very large number of tests that are required, associations must show a high level of significance to survive the multiple testing correction
- Such studies are particularly useful in finding genetic variations that contribute to common, complex diseases, such as asthma, cancer, diabetes, heart disease and mental illnesses
- **GWAS studies are a source of target validation in humans**

GWAS: Human disease validation



Data from NHGRI GWAS catalog (*www.genome.gov/**gwas**tudies/*)

Genetics: Mining Genome Wide Association Studies (GWAS)



WTCCC (2007) Nature 447(7145):661-78

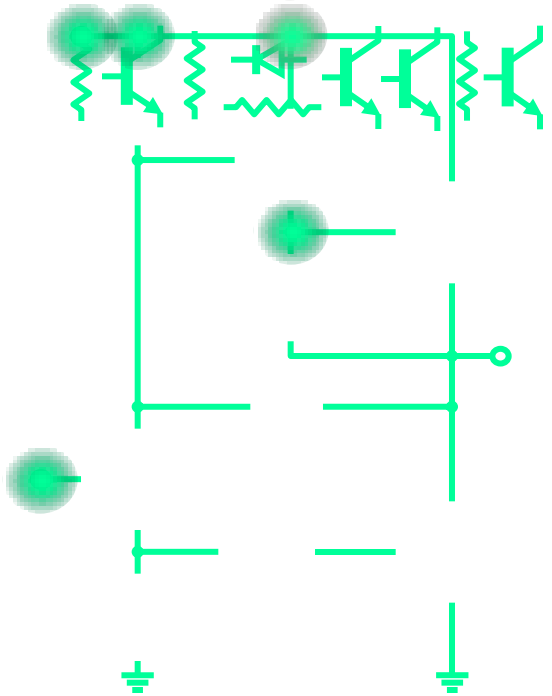
Wellcome trust case/control consortium

GWAS for seven common diseases

+ Genome-wide corrected P values $<1\ 10^{-5}$ in green
+ Crohn's disease (CD) shows multiple genome wide significant associations
+ Several CD genes were involved in the process of autophagy, **suggesting deregulation of this pathway ?**

Target validation in a pathway context

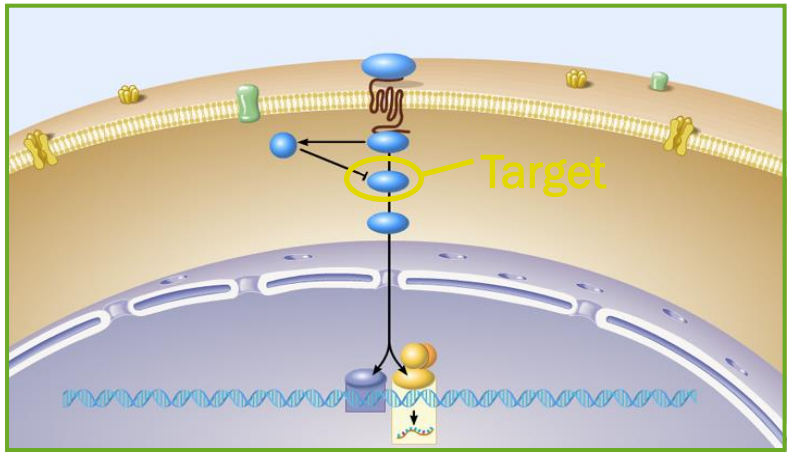
- An appealing and widespread metaphor for biological systems is that of electronic circuits
  - The genome, as 'parts list,' comprises the components
    - Do we *really* know all the parts?
  - Next, we try to understand the connectivity or 'wiring diagram' of a given system
  - Finally, we want to be able to specify and predict the system's dynamic behavior

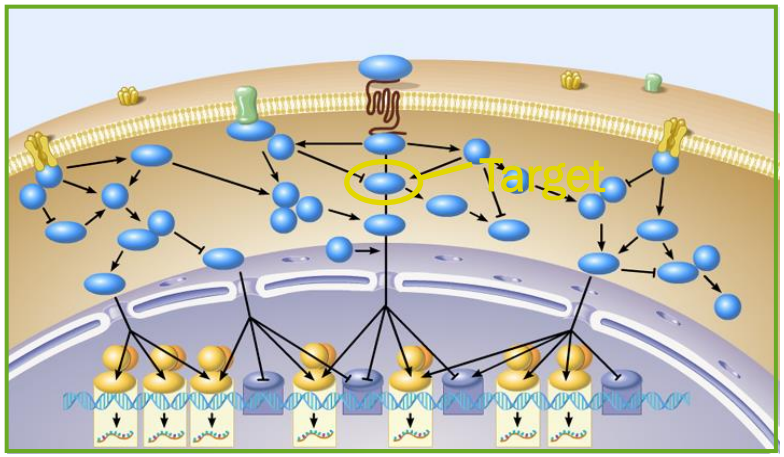


[Bhalla (2003) *Prog. Biophys. Mol. Biol.* **81**(1):45-65]

Slide: David Searls

Pathway Informatics & Target Discovery





Target discovery focused on identification of a pathway and macromolecular target involved in disease. The assumption is:

- One target ⟶ One consequence

In reality:

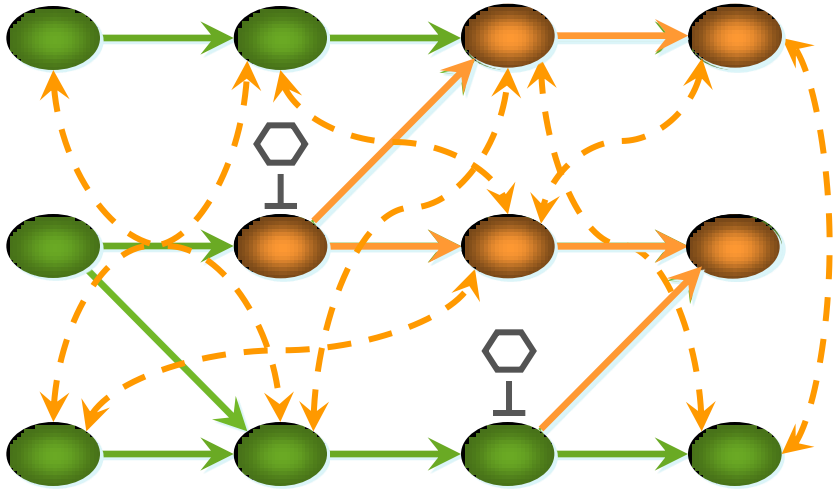
The target is usually one component of a complicated biochemical network

- A target acting as a single critical node may control or influence many processes.
- Network interactions can be redundant. "Work arounds" limit efficacy of a drug.
- Drugs can interact with multiple targets.
- Efficacy and safety are often a consequence of interaction with multiple targets

Slide: David Searls

Pathway Pleiotropy & Redundancy

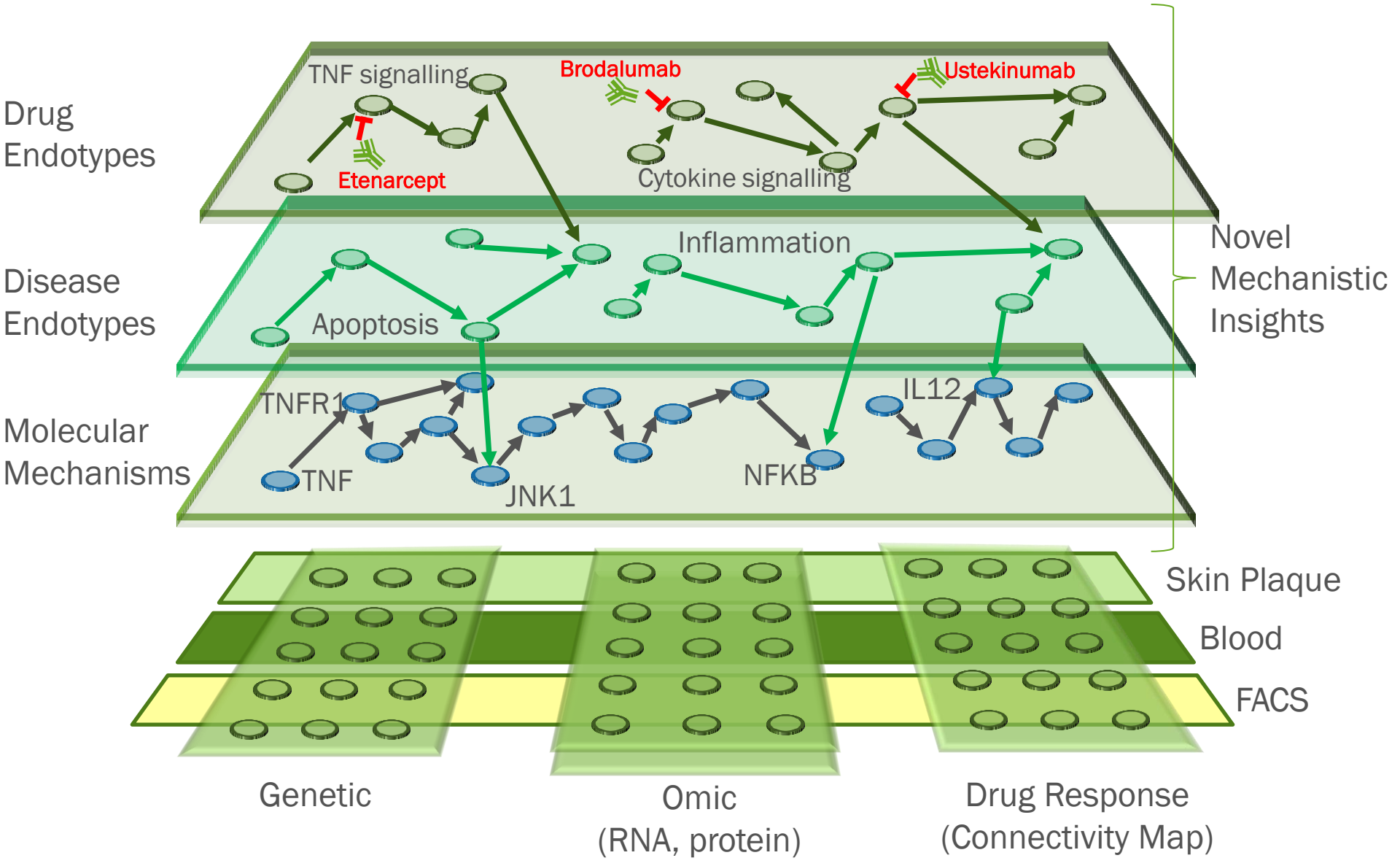
- In drug discovery, pleiotropy and redundancy are crucial considerations
  - Intervention is easier in 'simple' pathways
  - Crosstalk between pathways introduces complications to aspects of drug action
    - Safety: unexpected target-related effects
    - Efficacy: may call for combination therapy or polypharmacology
  - More holistic effects may be a challenge



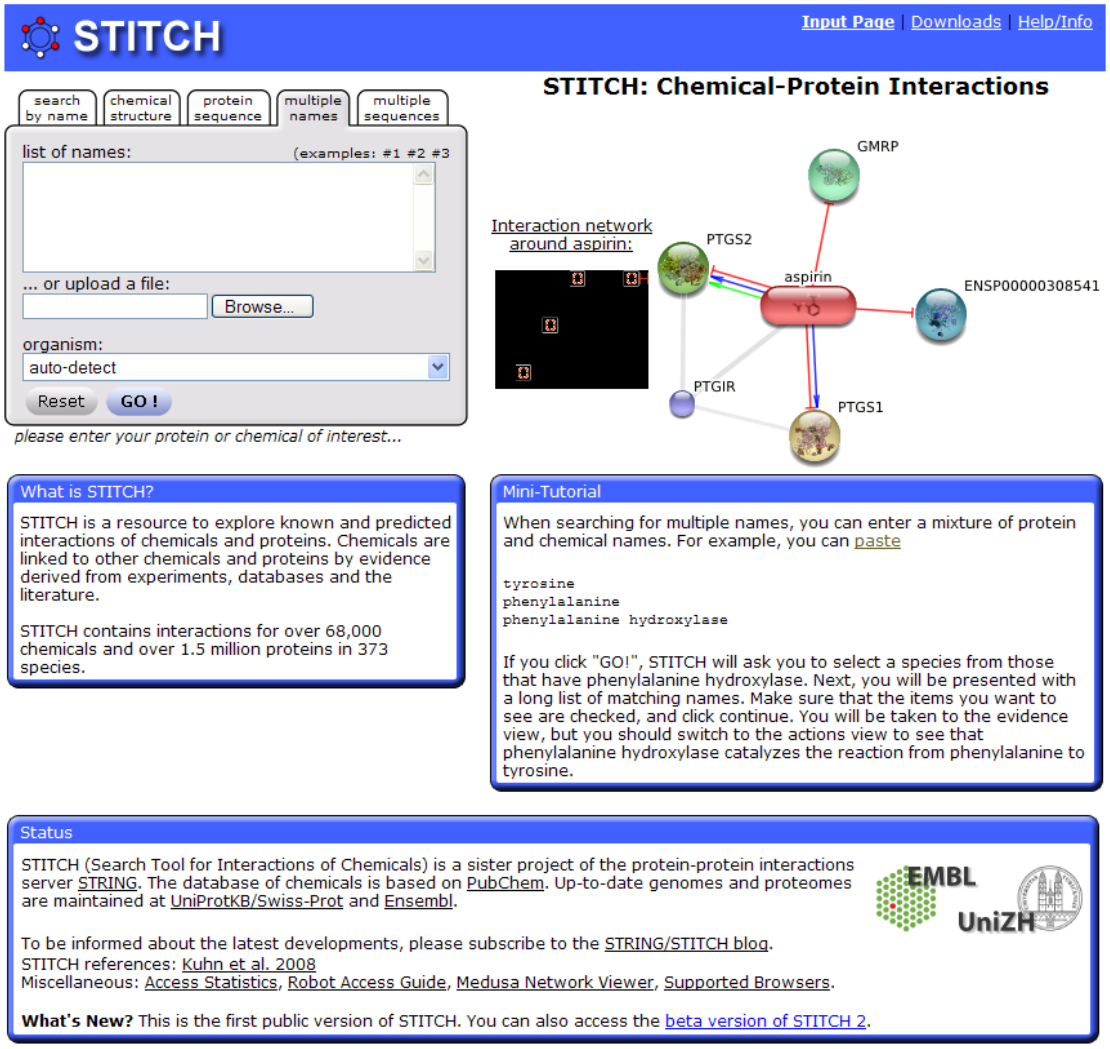
Slide: David Searls

Nature Rev. Drug Discov. **2**, p613 (2003)

Genomics: A Systems Approach



STITCH: Pathway Analysis (http://stitch.embl.de/)

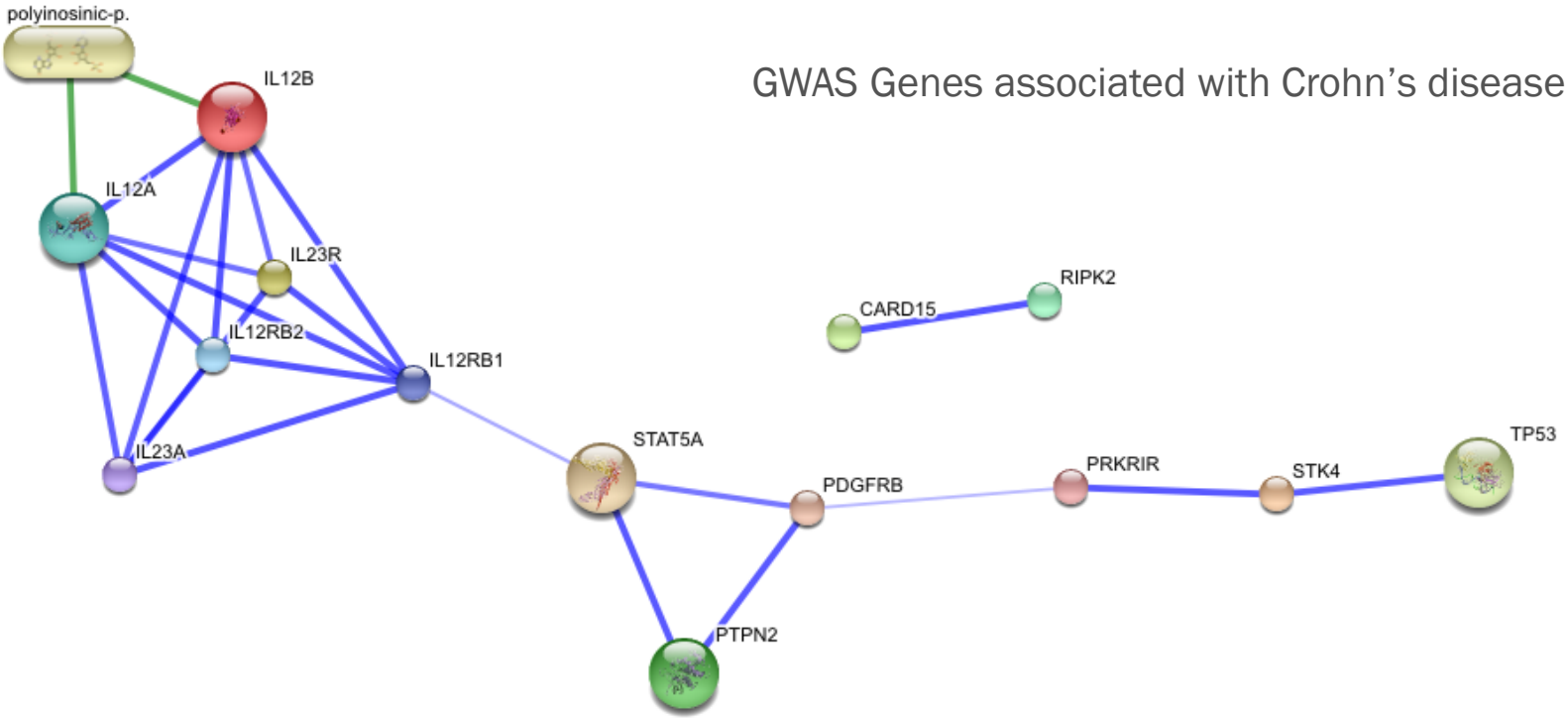


SIMPLE INTERFACE

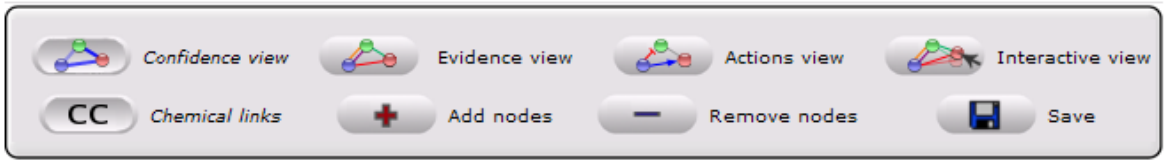
- Add multiple genes
- Identify interactions
- Identify known drugs
- Expand network

STITCH: Pathway Analysis (http://stitch.embl.de/)

GWAS Genes associated with Crohn's disease

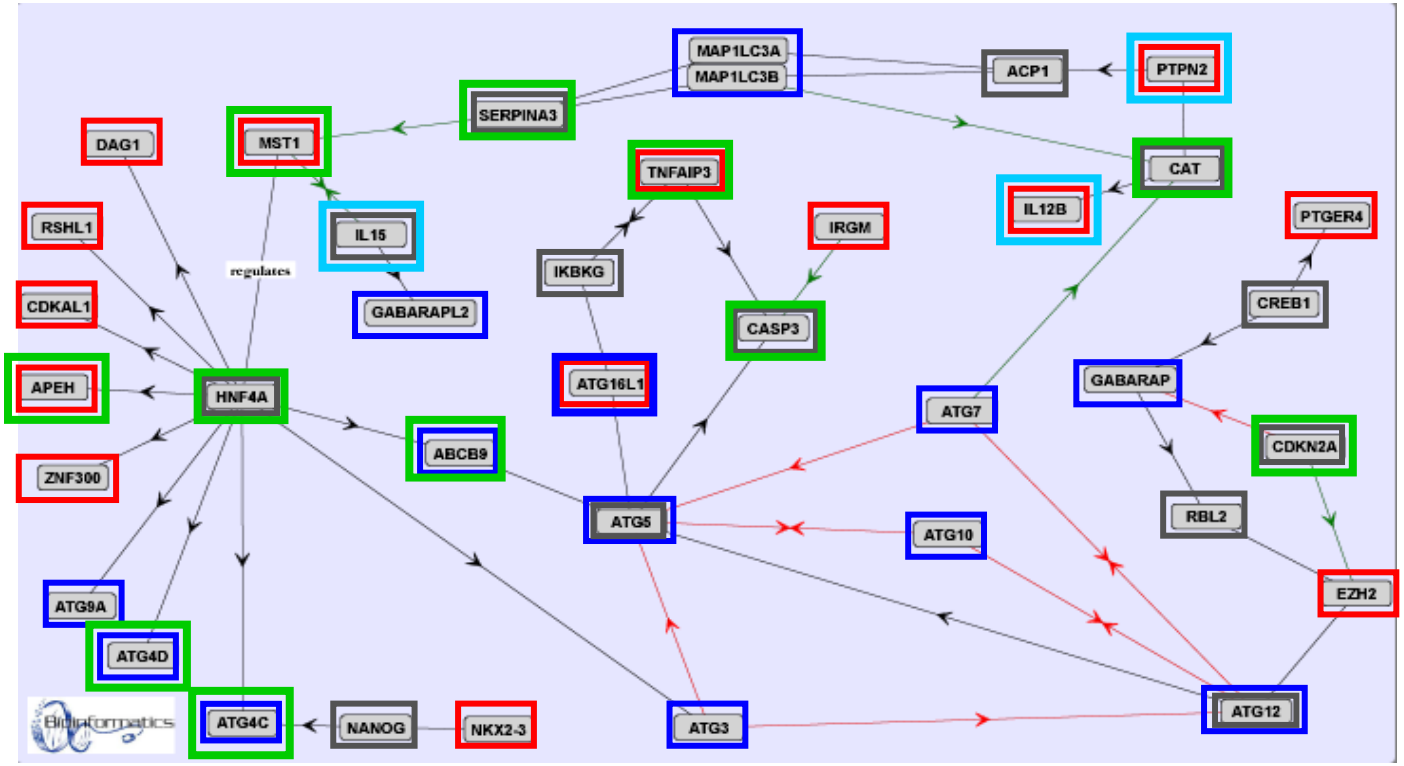


This is the **confidence view**. Stronger associations are represented by thicker lines.
Protein-protein interactions are shown in blue, chemical-protein interactions in green and interactions between chemicals in red.



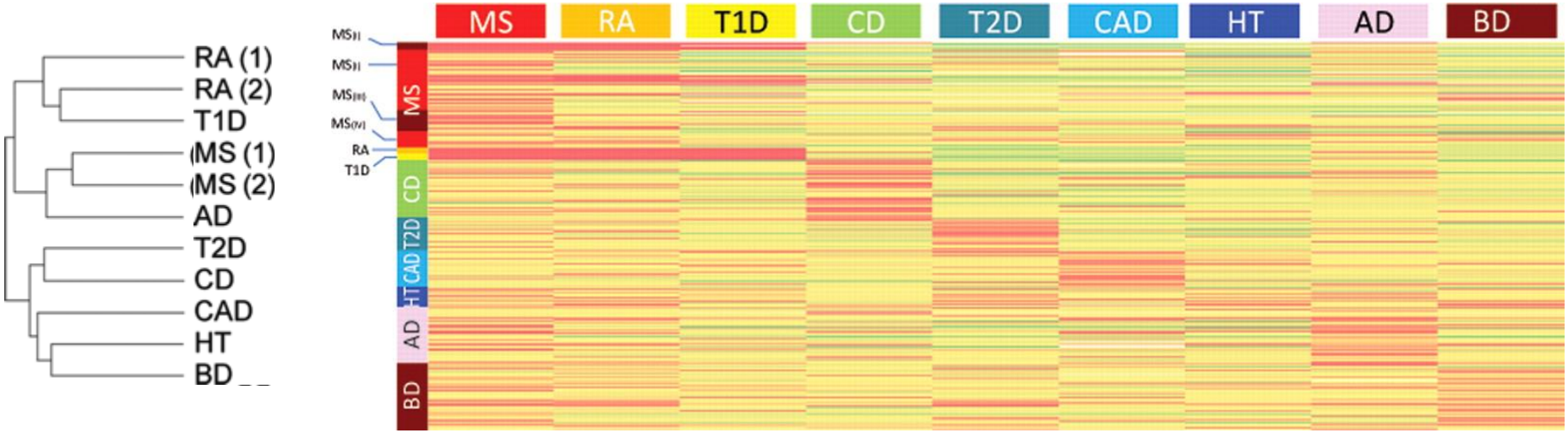
New Drug Mechanisms from Pathways: Crohn's disease GWAS

- Wellcome trust genome scan identified and replicated 23 genes in CD
  - 14/23 associated genes were linked to the autophagy mechanism
  - Reduced autophagy identified as a key mechanism in Crohn's disease
    - Rapamycin is a known drug which downregulates autophagy
    - Rapamycin is now in phase II trial for Crohn's disease



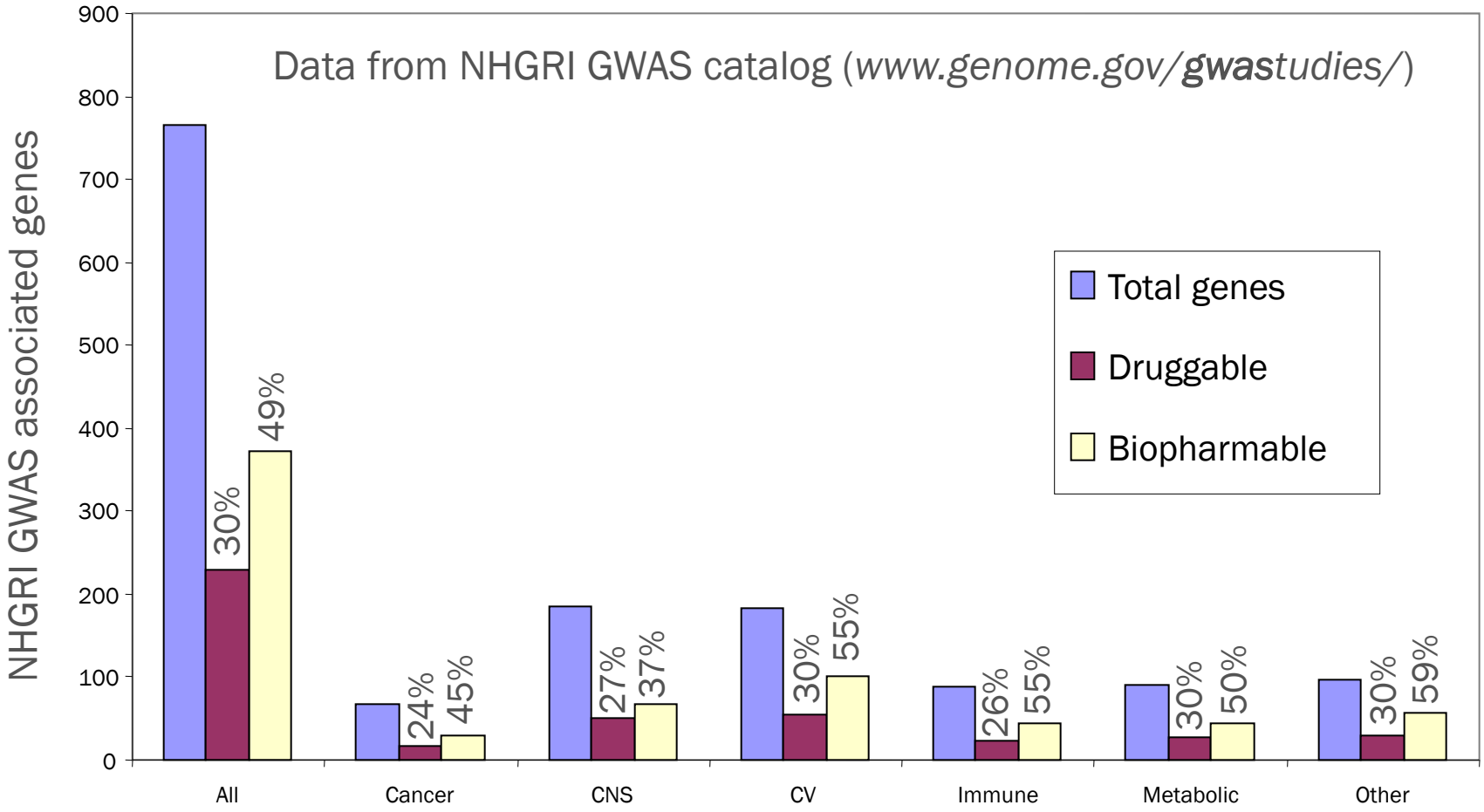
COMMON DISEASES SHOW COMMON PATHOLOGIES

- Pathway analysis applied to the seven WTCCC GWAS studies and a GSK Alzheimer's GWAS
- Common modules emerged and clustered across all the disease GWAS, hinting at linked pathology
  - Suggests repositioning opportunities?



Baranzini S E et al. Hum. Mol. Genet. 2009;18:2078-2090

Therapeutic opportunities from GWAS



Sanseau et al 2012, Nature Biotechnology 30,317–320

GWAS: Confirming drug indications

Table 1 Selected examples of matches between GWAS trait and drug indication[a]

Drug name or class	Most advanced development phase (for the indication)	Gene	Drug indication	GWAS trait	GWAS reference
Statins	Launched	HMGCR	Hypercholesterolemia	LDL Cholesterol	1
Ustekinumab	Approved	IL12B	Psoriasis	Psoriasis	13
Ustekinumab	Phase 2	IL12B	Crohn's disease	Crohn's disease	2
Anti-IL2 receptor mAb	Phase 2	IL2RA	Ulcerative colitis	Crohn's disease	2
AMG -785/ CDP-7851	Phase 2	SOST	Bone regeneration/ osteoporosis	Bone mineral density	14
Znt8 agonists	Preclinical	SLC30A8	Type 2 diabetes	Type 2 diabetes	15

[a]Examples are ranked from most advanced drug (launched) to less advanced (preclinical). The associated gene between each GWAS and the drug is shown. The drug indication and the phase of development for each drug are derived from the Pharmaprojects database. In each example the GWAS trait is identical (rows 1, 2, 3 and 6) or closely related (rows 4 and 5) to the drug indication. For the full list, see **Supplementary Table 3**. In many cases, more drugs for the gene are listed in the database at different phases. The GWAS references are from the catalog of GWAS data (http://www.genome.gov/gwasstudies).

Sanseau et al 2012, Nature Biotechnology 30,317–320

Repositioning opportunities from GWAS

Table 2 Selected examples of mismatch between GWAS trait and drug indication[a]

Drug name	Most advanced development phase (for the indication)	Gene	Current drug indication	GWAS trait (new potential drug indication)	GWAS references
Denosumab/AMG-162	Launched/registered	TNFSF11	Osteoporosis/bone cancer	Crohn's disease	2
RPI-78M	Phase 3	IL27	Adrenoleukodystrophy	Crohn's disease/inflammatory bowel disease	2,16
Nepicastat	Phase 2	DBH	Cocaine addiction/post-traumatic stress disorder	Smoking cessation	7
Biib-033	Phase 1	LINGO-1	Multiple sclerosis	Essential tremor	4,5
AMG-557	Phase 1	ICOSLG	Systemic lupus erythematosus	Crohn's disease/celiac disease/ulcerative colitis	17–19
Cwp-231	Preclinical	TCF4	Cancer	Fuchs's corneal dystrophy	20

[a]Examples are ranked from most advanced drug (launched) to less advanced (preclinical). The associated gene between each GWAS and the drug is shown. The drug indication and the phase of development for each drug are derived from the Pharmaprojects database. For the full list, see **Supplementary Table 4**. In many cases, more drugs for the gene are listed in the database at different phases. The GWAS references are from the catalog of GWAS data (http://www.genome.gov/gwasstudies).

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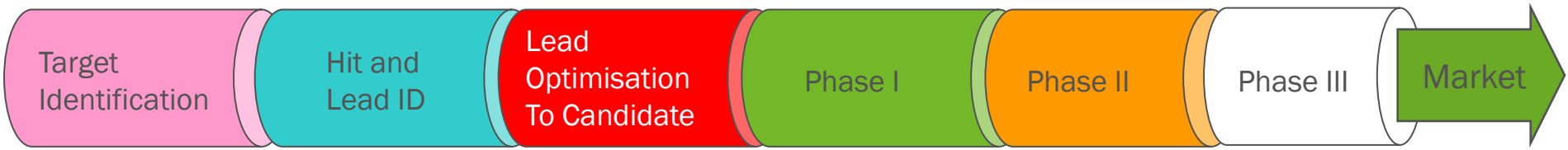
Target biology informs drug discovery



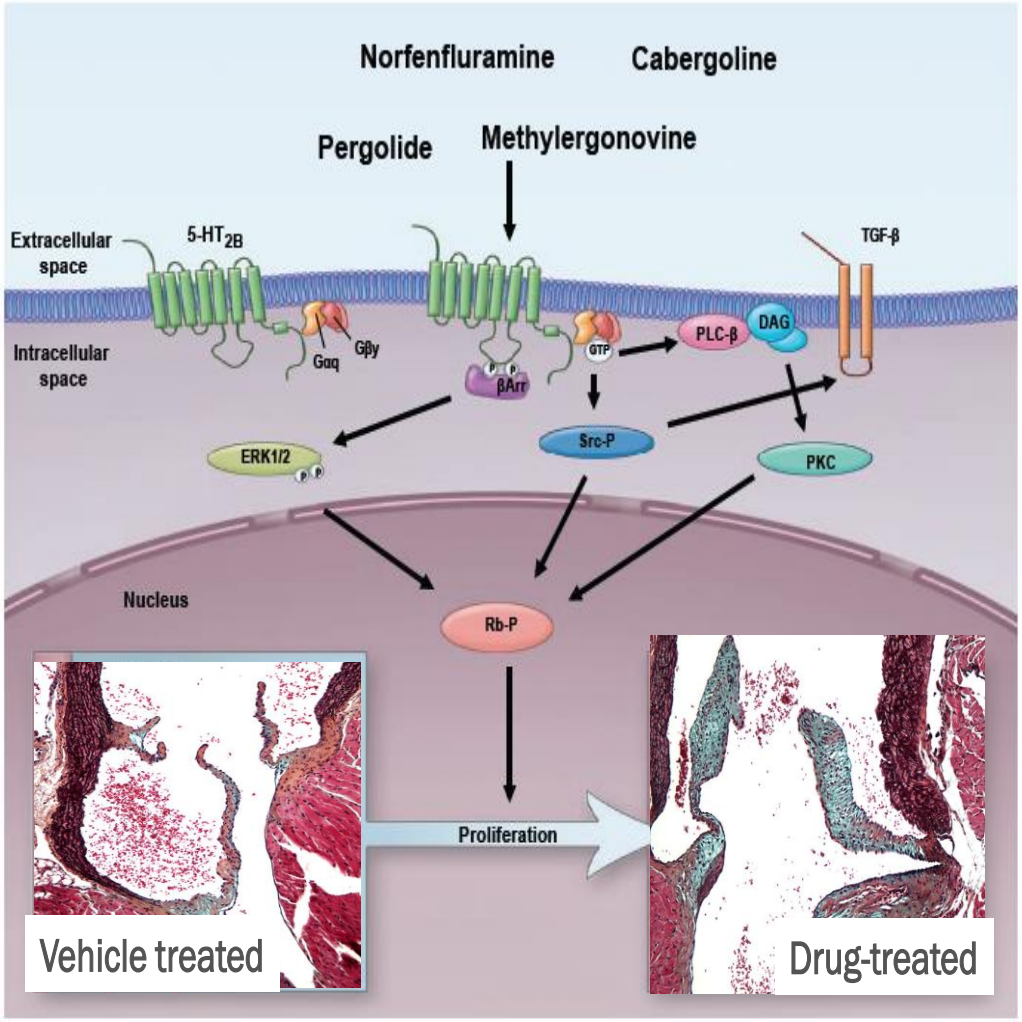
- Target pathway
- Near targets/pathways
- Target distribution
- Functional genomics
- Tool compounds

Candidate Selection
therapeutic index ?
- (Toxic dose/Effective dose)

Risk Assessment
Extrapolation from animal model to man (relevance, sensitivity)

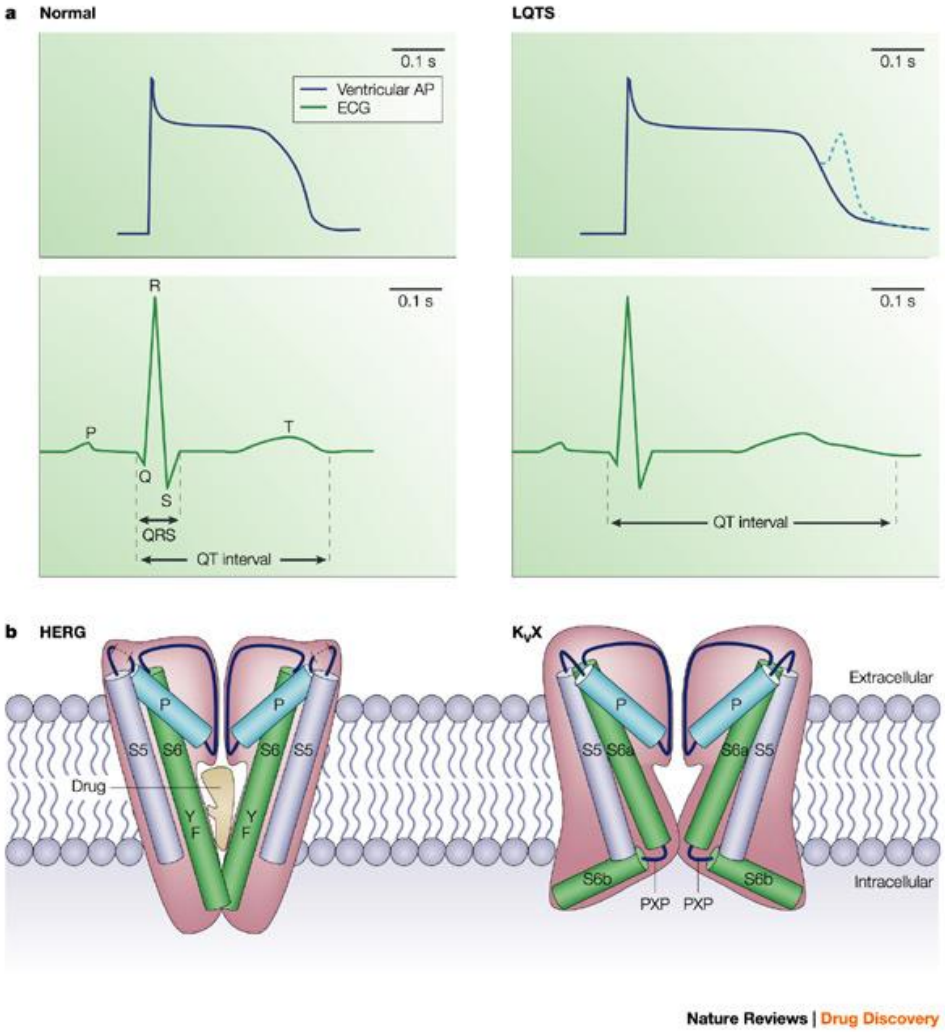
Drug Development
- Species
  - sensitive?
  - relevant?
- Endpoints?
- Timing?

Common liabilities: On Target



- e.g. Neuropsychiatric HTR2B agonists cause heart valve pathology
- Proliferation of cardiac fibroblasts on the tricuspid valve, known as cardiac fibrosis
- Emphasises ideal of restricted expression of target to disease tissue

Common liabilities: Off Target



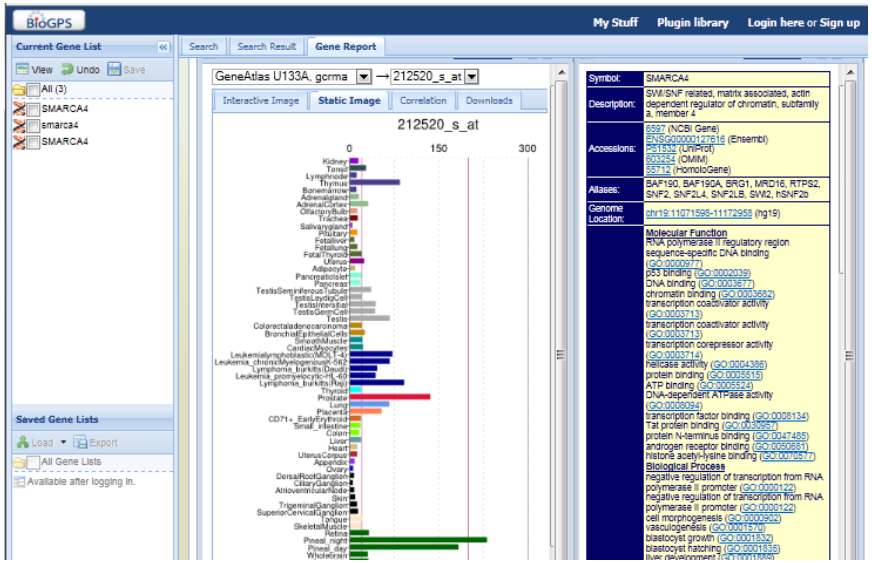
Nature Reviews | Drug Discovery

- Many drugs, eg. Antihistimines induce Long QT syndrome by HERG action
- HERG channels contain two aromatic residues (Y652 and F656) in the S6 helices (green), which line the pore cavity and can therefore form cation–interactions with multiple drugs.
- HERG also has a larger cavity than other KV channels, and so can accommodate a wide size range of drugs

Target Expression

BioGPS

- www.biogps.org

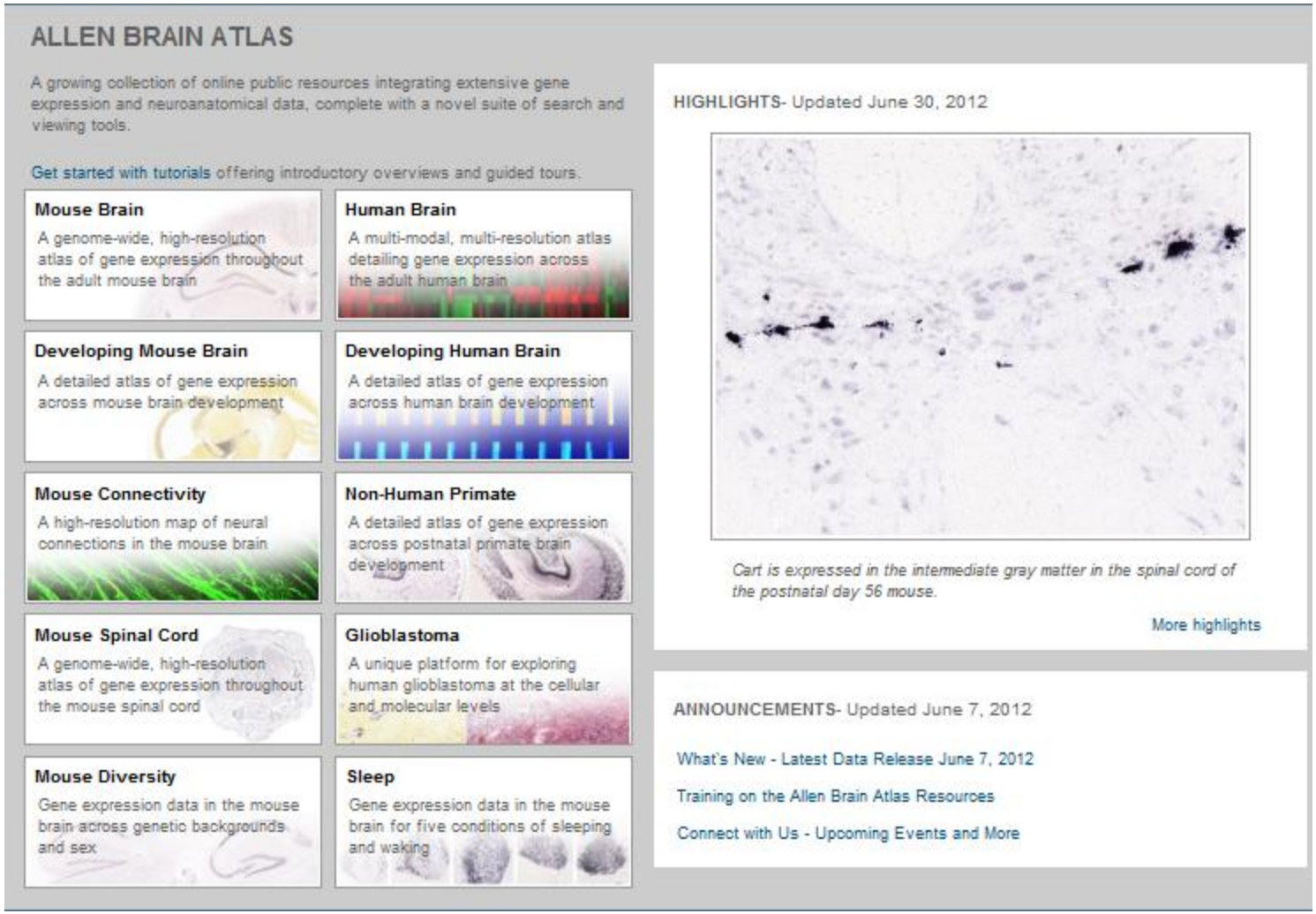


EBI Gene Expression Atlas

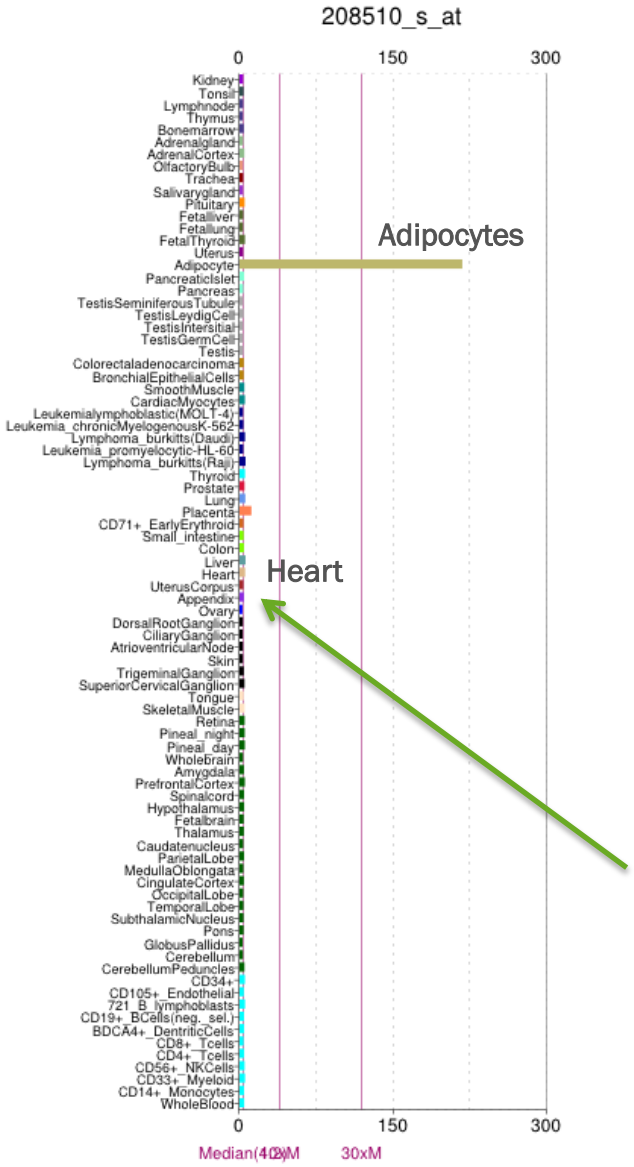
- **Comprehensive view of public expression data**
- View the Novartis data in BioGPS here
  - http://www.ebi.ac.uk/gxa/experiment/E-AFMX-5

Target Expression : Allen Brain Atlas

- http://www.brain-map.org/



Target Expression : PPARG agonists



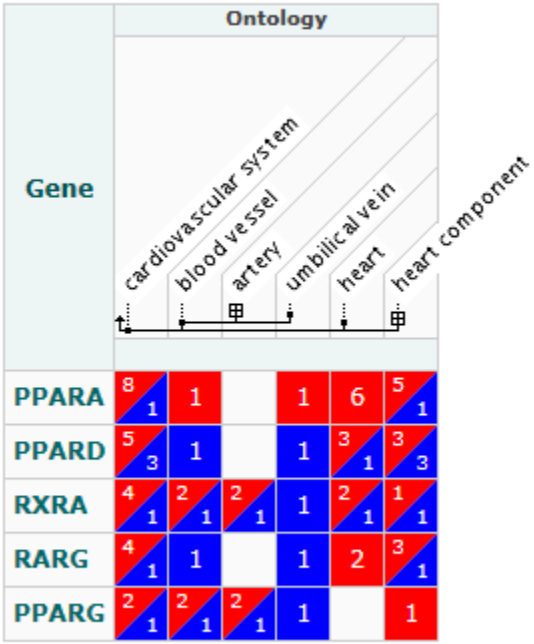
- Type II Diabetes target
- Nuclear hormone receptor
- Highly expressed in adipocytes
- Rosiglitazone (Avandia) was a blockbuster for GSK….

- ….until emergence of cardiotox
- Seems unlikely on the basis of expression!
- Could it be an off-target effect?

Target Expression : PPARG agonists

Genes 1-5 of **5** total found • Download all results • JSON XML

Legend: 3/1 - number of studies the gene is **over**/**under** expressed in



Processing time: 1.55 secs.

- Chembl query shows Avandia activity at PPARG homologues
- Notably all are expressed at higher levels than PPARG in heart according to EBI Gene Expression Atlas

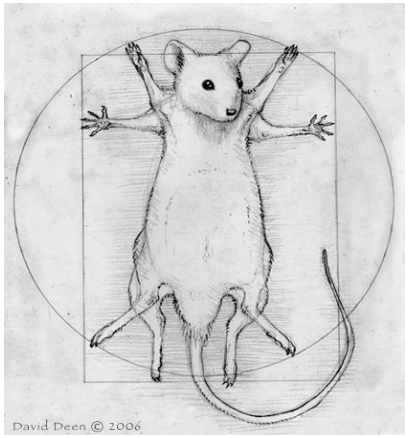
Perspectives on target validation

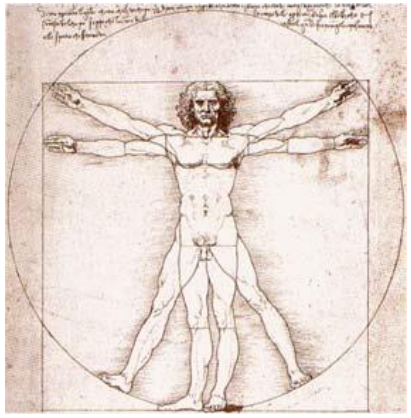
Similarity

- Orthology (animal models)
- Homology (off target effects)

What Makes a Good Animal Model?

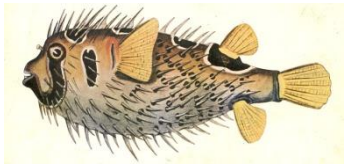
- A good animal model should have the following characteristics:
  - Should closely reproduce the disease or condition under study
  - Should be an industry/academic accepted model
  - The data should be robust enough to be duplicated by a 3rd party
  - Should show statistical significance – feasible to use sufficiently large number of animals to demonstrate statistical significance
  - The data endpoints should be convincing enough to justify transition into man





Selecting Species for Preclinical Studies

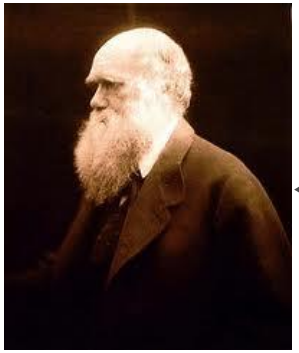
- Preclinical studies in animal models aim to accurately predict drug action and safety in Man
  - Preclinical studies are only valid if the function of the gene is equivalent (orthologous) between the model and man
- Three types of evolutionary selection pressure:
  - **Negative selection:** Deleterious mutations are selected against to conserve function
  - **Neutral selection:** Neutral mutations are unaffected by selection
  - **Positive selection:** Advantageous mutations are selected for. Positive selection is indicative of functional change



Histones have equivalent function in humans and fish

Negative selection

(Genome integrity)



Alanine:glyoxylate aminotransferase functions differently between humans and cats

Positive selection

(Carnivorous diet)

Molecular Evolution in Drug Discovery

- Evolution of targets between and within species
  - Loss of classic dose-response paradigms
- Humanisation of antibodies
  - Intra-species variation
  - Immunogenicity in animals may differ to human
- Evolution Increases uncertainty in the preclinical transition to man
  - Recently underlined in the disasterous TGN1412 trial
- A good understanding of the preclinical model is imperative for a safe transition to man

Marmoset Models in Neuroscience Drug Discovery

- *Callithrix jacchus*
- Key animal model in psychiatry
  - Tiny radio implant measures EEG/EMG/EOG traces 24/7
  - Records sleep levels.....

Response to threats...

.....social and grooming behaviour

GSK Orexin Receptor Antagonists

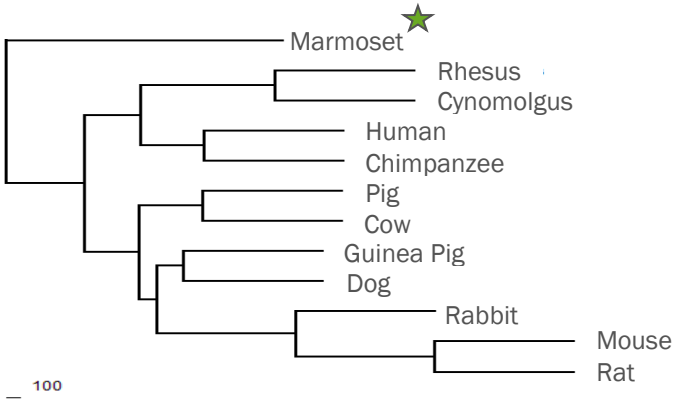
- Orexin signalling is key in satiety, sleep and mood
- Neuroscience programmes in Sleep & Mood
  - Orexin Receptor antagonist pharmacology appeared normal in Marmosets.....

Orexin receptor evolutionary analysis predicts receptor pharmacology

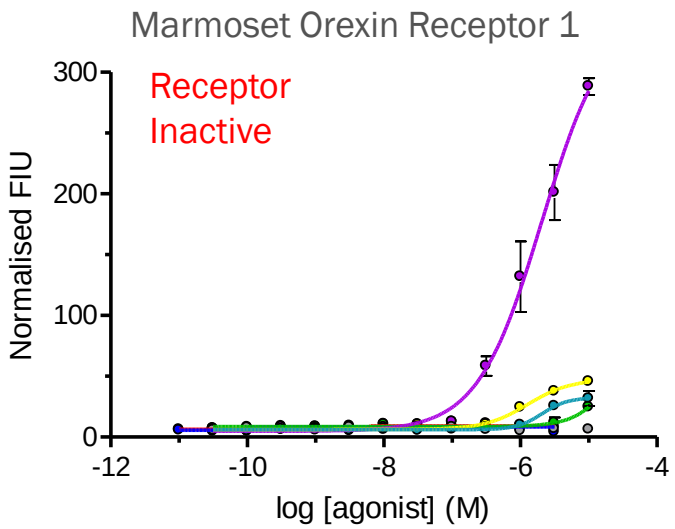
Orexin Receptor Antagonist Development

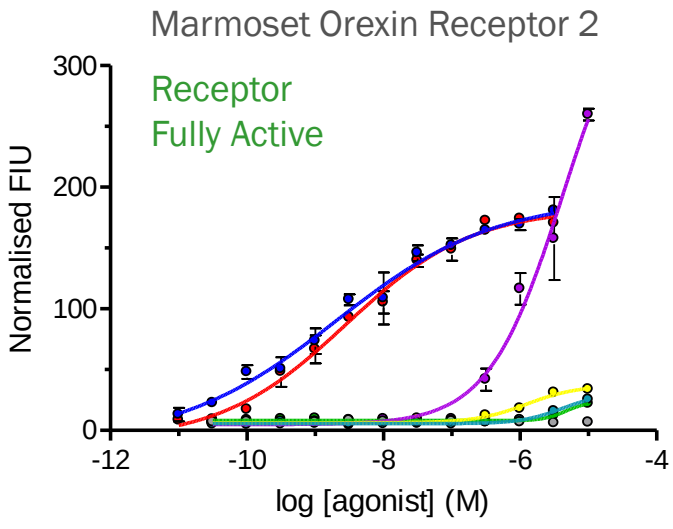
- Marmoset Orexin R1 protein highly divergent
- Orexin R2 & ligands also showed modest sequence divergence in Marmoset
- Orexin R1 receptor pharmacology investigated
  - Marmoset Orexin R1 shown inactive *in vitro*
- Demonstrates that Orexin action in Marmoset is mediated entirely by Orexin R2

Orexin Receptor 1 protein phylogeny



***in vitro* response of Marmoset Orexin receptors to Orexin ligands A & B (FLIPR)**



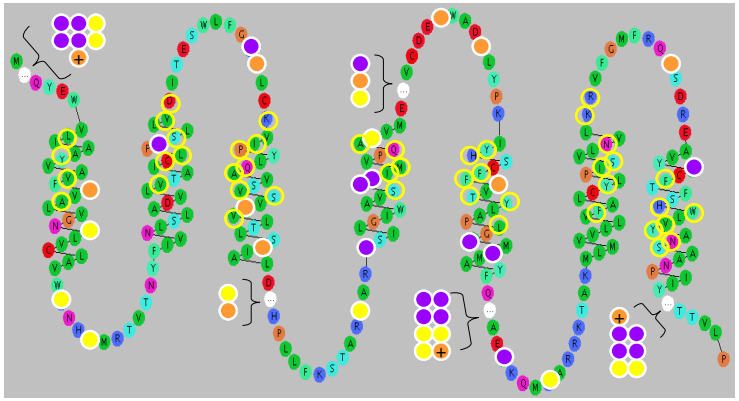


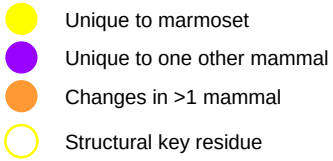
- Orexin A
- Orexin B
- Carbachol
- Histamine
- Ach
- UTP
- Buffer

Topp et al, 2011,

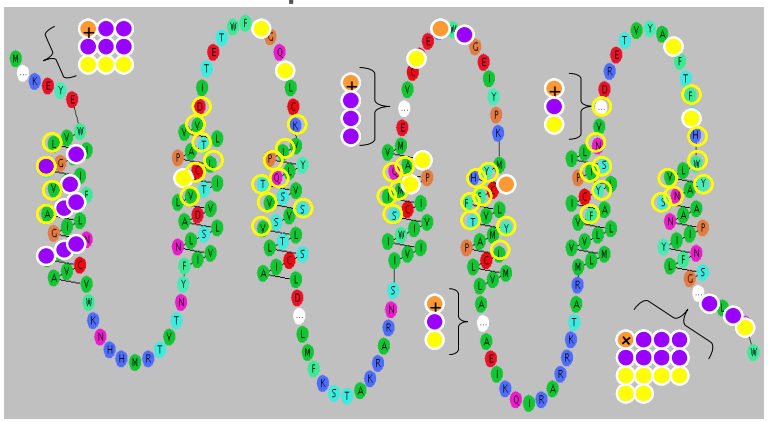
EVOLUTION OF Orexin Signalling in Marmosets

Orexin Receptor 1





Orexin Receptor 2

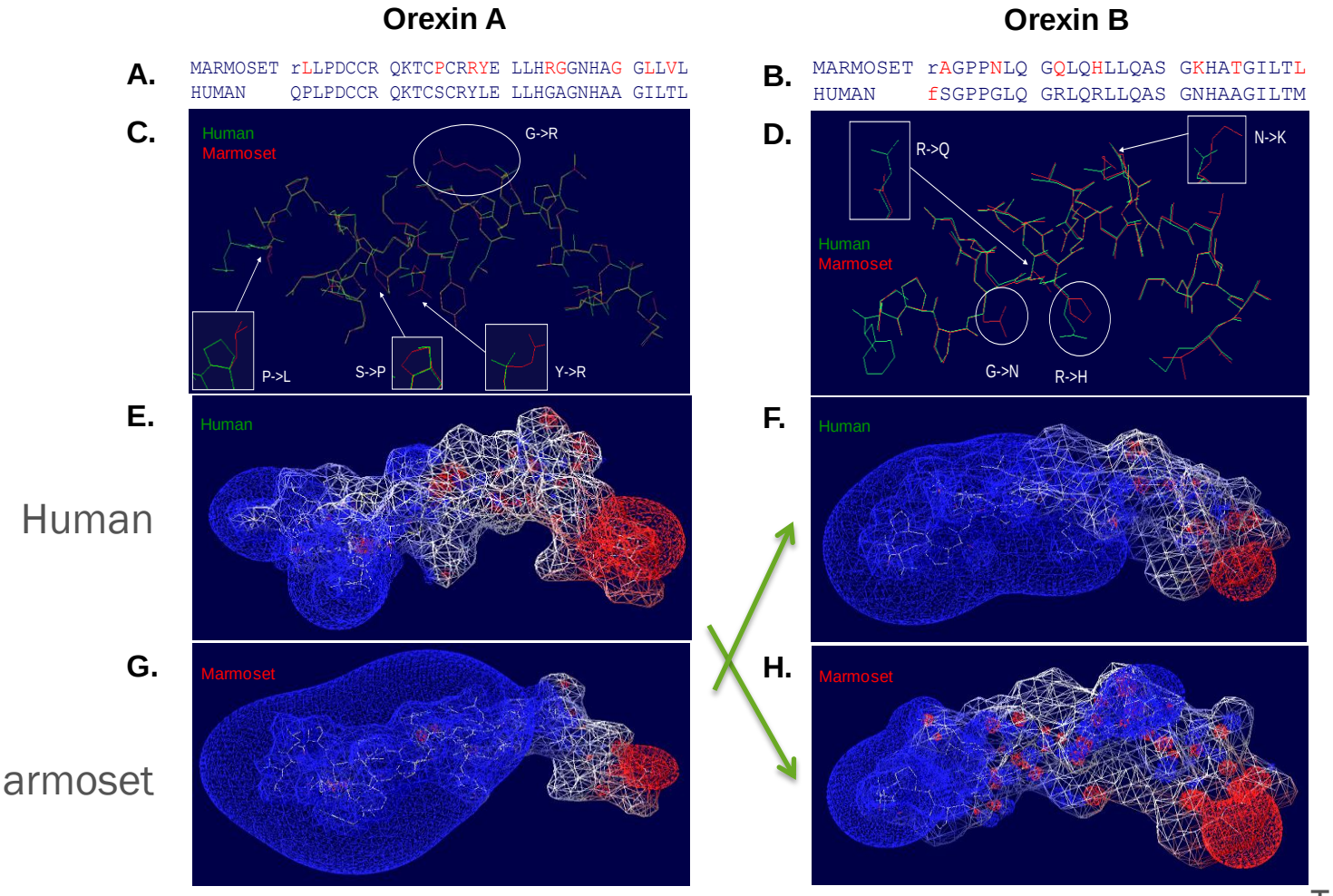


- Analysis of Orexin receptor structure showed most changes in Orexin R1 are intracellular (signalling?), changes in Orexin R2 are mostly extracellular (ligand binding?)
- Evolutionary selection analysis was performed on the Orexin receptor and ligand genes using PAML (Yang, 1997)
  - Both receptor & ligand lineages show evidence of positive selection
  - Positive selection at specific-site residues is restricted to the mature peptide regions of the orexin ligands A & B.
    - Suggests altered ligand function to compensate for receptor loss?

Topp et al, 2011,

Structural & electrostatic modelling of Marmoset and Human orexin ligands

- Orexin A most active against Orexin R1 (which is inactive in Marmoset)
- Marmoset Orexin A has evolved similar electrostatic properties to human Orexin B

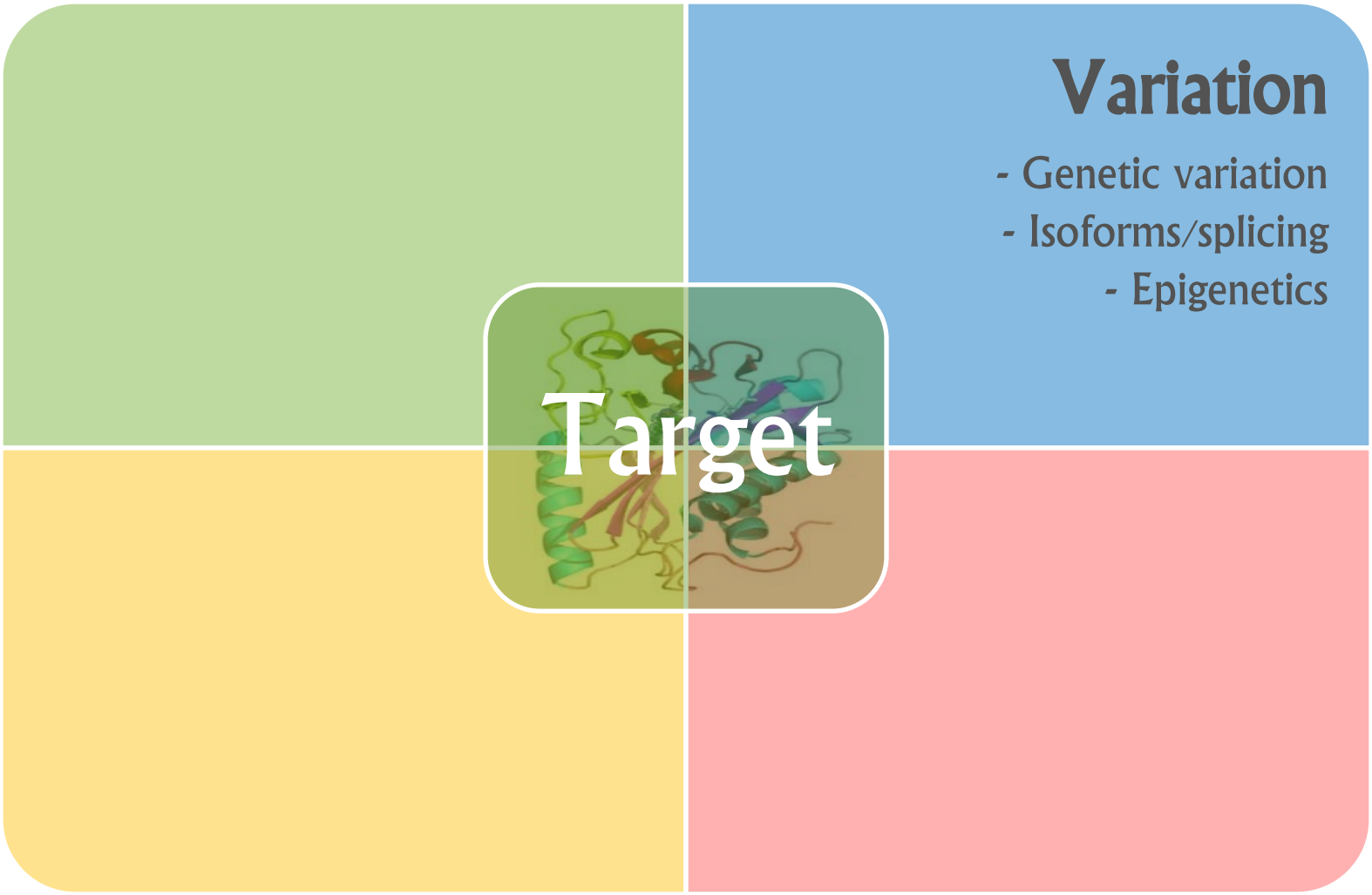


Topp et al, 2011,

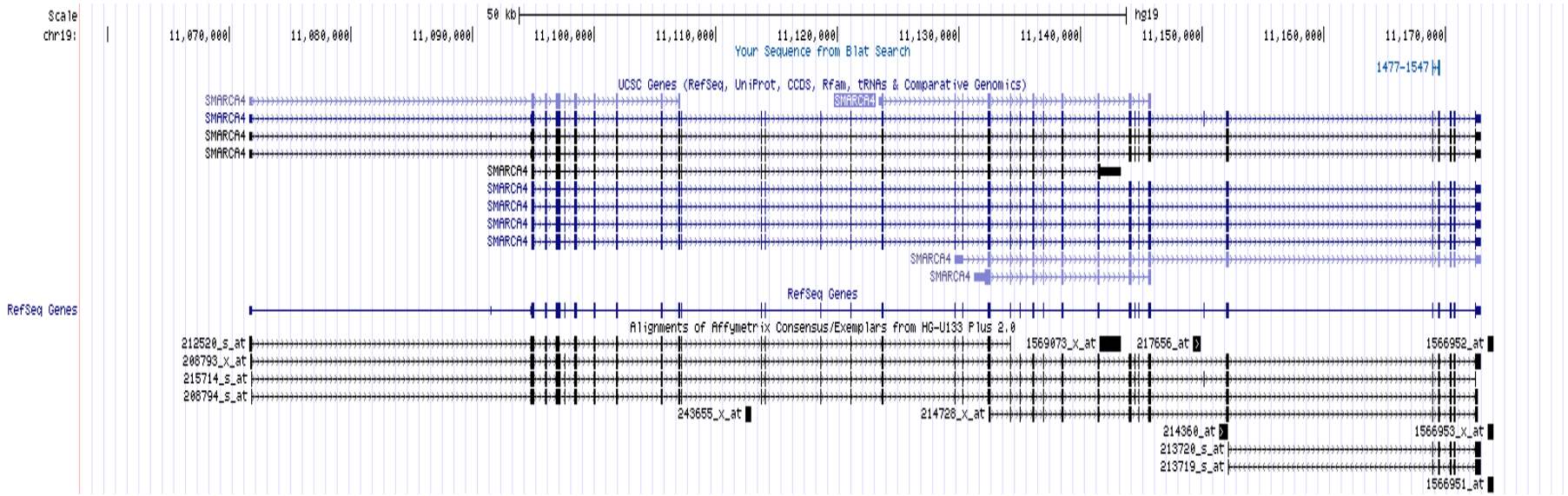
Conclusions: Marmoset orexin signalling

- Orexin ligands and receptors have both diverged significantly in Marmosets
  - Suggests co-evolution of ligand & receptor
  - Orexin A ligand may have evolved to act on remaining active orexin receptor 2
- Orexin antagonist results in marmosets should be treated with caution
  - No divergence seen in old world primates
  - These would be better models
- Evolutionary biology and phylogenetics are key tools for drug discovery

Perspectives on target validation

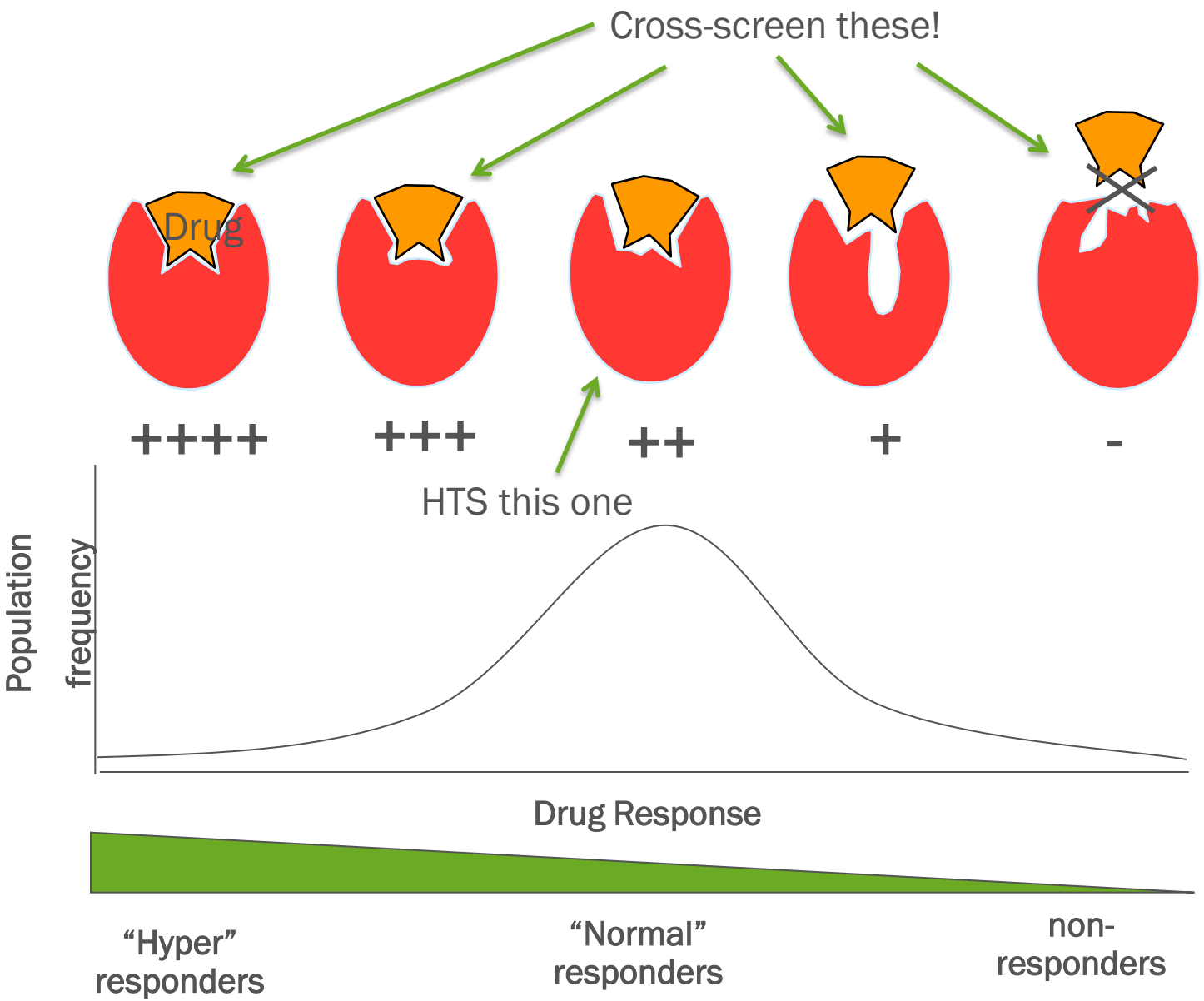


Target Expression and Splicing



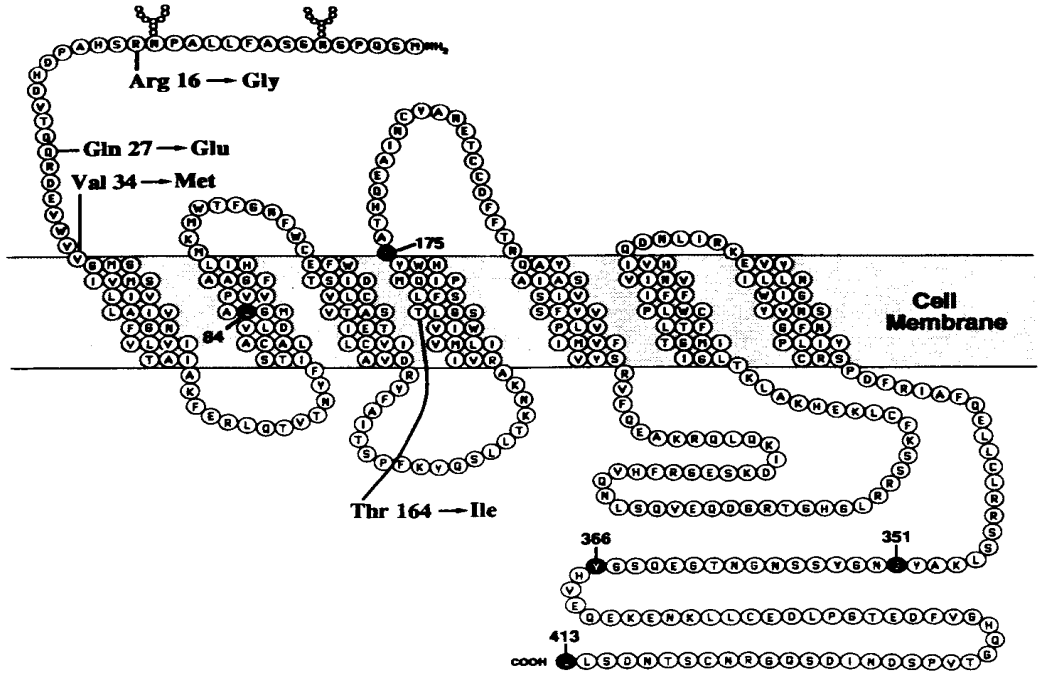
- Target validation needs to consider the most biologically relevant transcript
- **UCSC genome browser** presents a view of target splice isoforms and gene expression probes (“Affy U133Plus2” track)

The Classical PGX paradigm –
Target polymorphism and Therapeutic Response



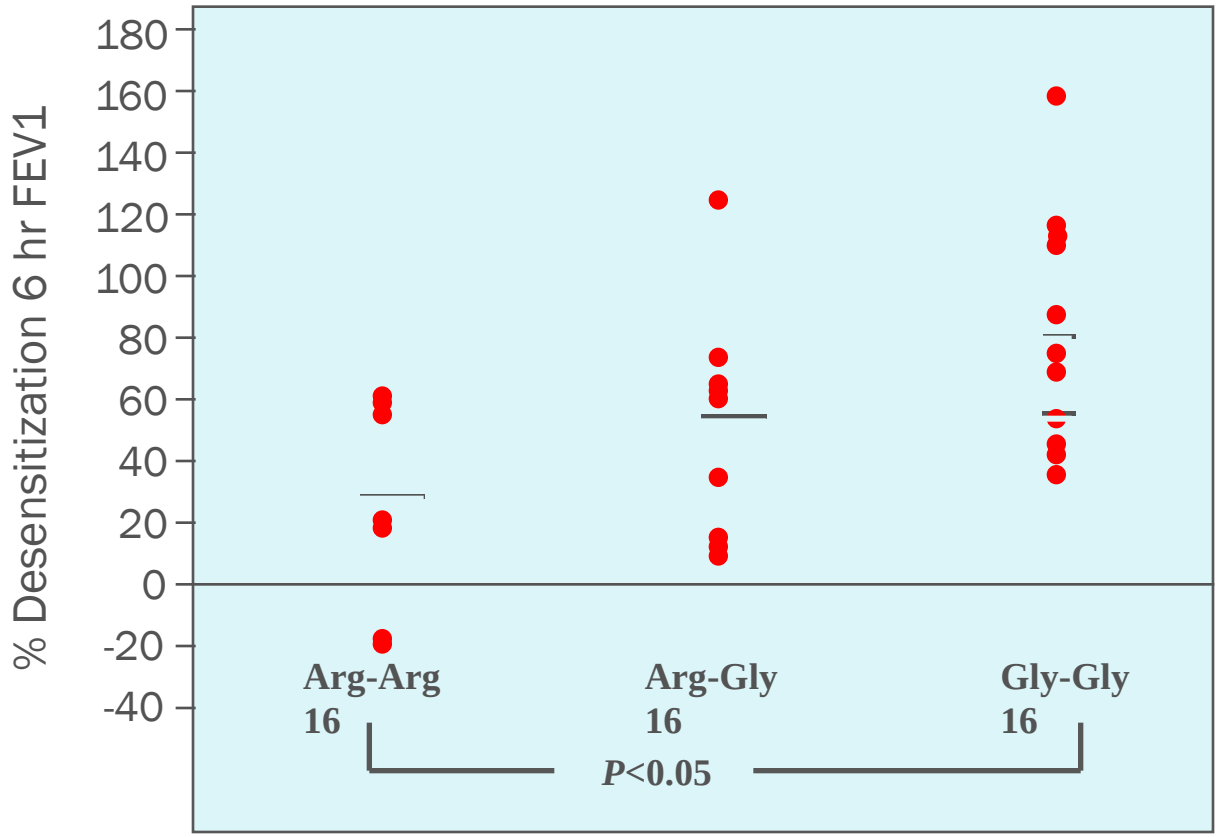
β_2-Adrenergic Receptor Polymorphisms

- beta2-adrenoceptor variants can lead to desensitization with regular formoterol therapy (Tan *et al.* 1997)



Tan *et al. Lancet* 1997;**350**:995–999.

Drug Target Pharmacogenetics – Formoterol and Desensitization



Adapted from: Tan *et al. Lancet* 1997;**350**:995–999.

Getting a view of target variation

Uniprot

- "Chemist friendly"
- Good context
- Not comprehensive

NCBI dbSNP

- Comprehensive / Exhaustive
- Rare variants (<1% freq) may not be an issue for target validation, but could be a safety issue

Tan *et al. Lancet* 1997;**350**:995–999.

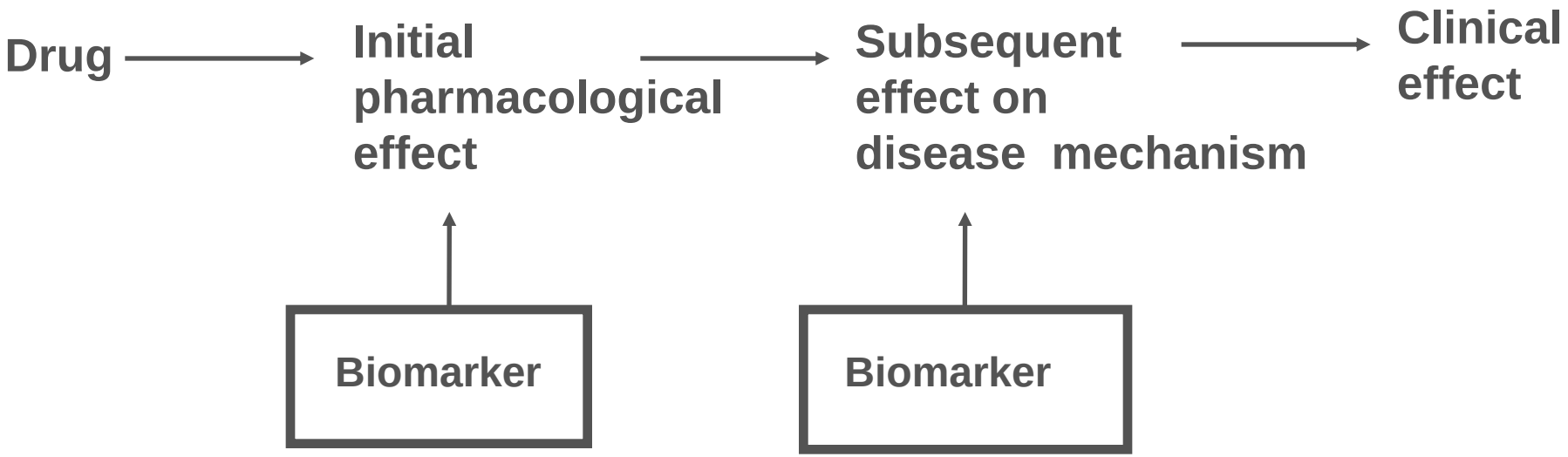
Perspectives on target validation

Druggability

- See Anna's Talk

Robust Assays need Biomarkers

- Biomarkers are critical to demonstrate efficacy



Type 1
Drug-specific

Type 2
System property

Types of Biomarker in Drug Discovery

Type 1

- confirmation of primary pharmacology
- supports the predictions of preclinical models
- allows PK /PD relationship assessment
- but is not really closer to predicting efficacy
- drug specific
- easy to validate

Type 2

- biological / disease response
- an effect downstream from primary pharmacology and which is likely to result in clinical benefit
- potential diagnostic or surrogate
- system not drug specific
- because of system complexity may need to be an experimental model
- hard to validate

So you have a target? What next?

Understand target biochemistry

- kinetics – will help to inform assay development and drug design
- substrate specificity – may aid design of inhibitors
- inhibitors / agonists – may be starting point of medicinal chemistry
- protein structure – aids rational design and modelling

Clone and express gene(s) to develop systems to assay target

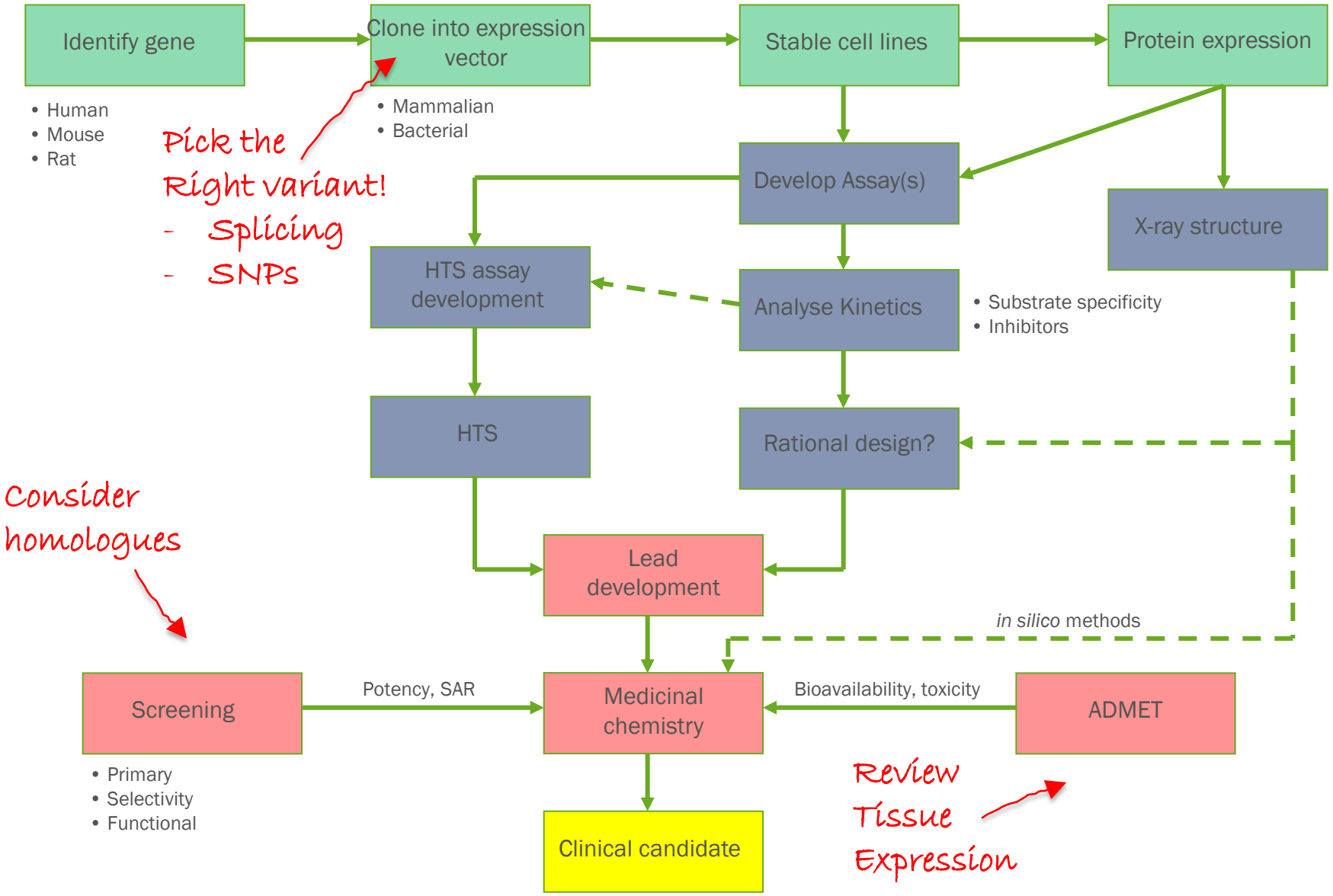
- mammalian cells – use to develop whole cell assays
- yeast or *E. coli* – used to express protein

Assay development

- primary – target assay, kinetic or binding, solution-based (?)
- whole cell - may be primary assay
- functional - required to assess effect of target inhbition
- selectivity – required to determine specificity of leads

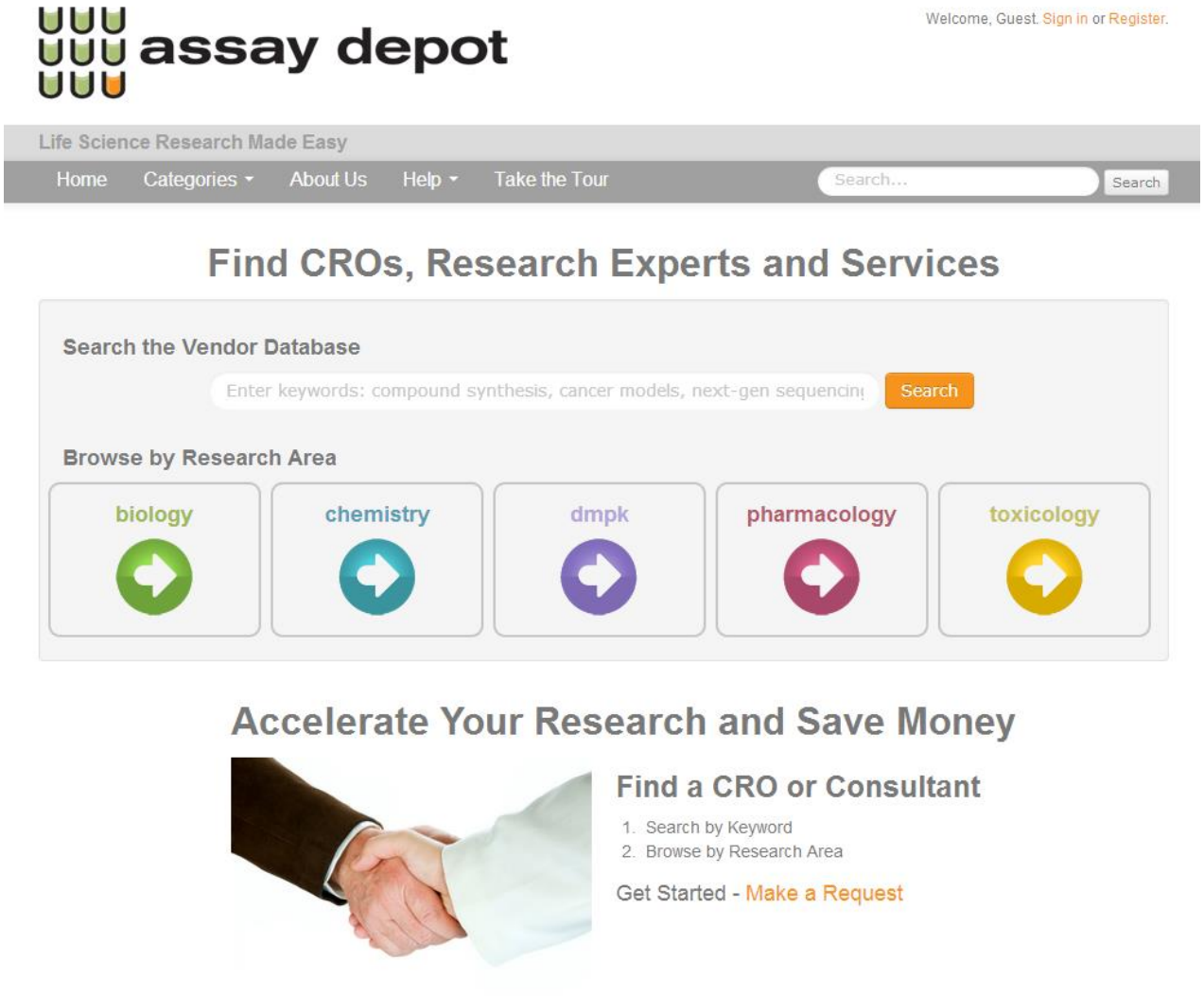
High throughput screening (HTS)

Defining a critical path to the clinic



Time for some wet work

Time for some wet work....



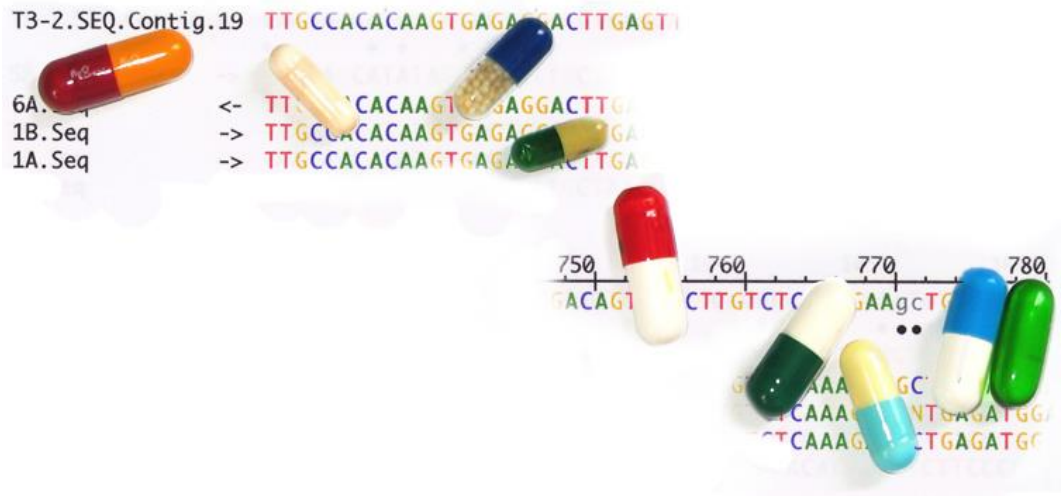
Seeking the Holy Grail of Drug Discovery

PROOF OF CONCEPT

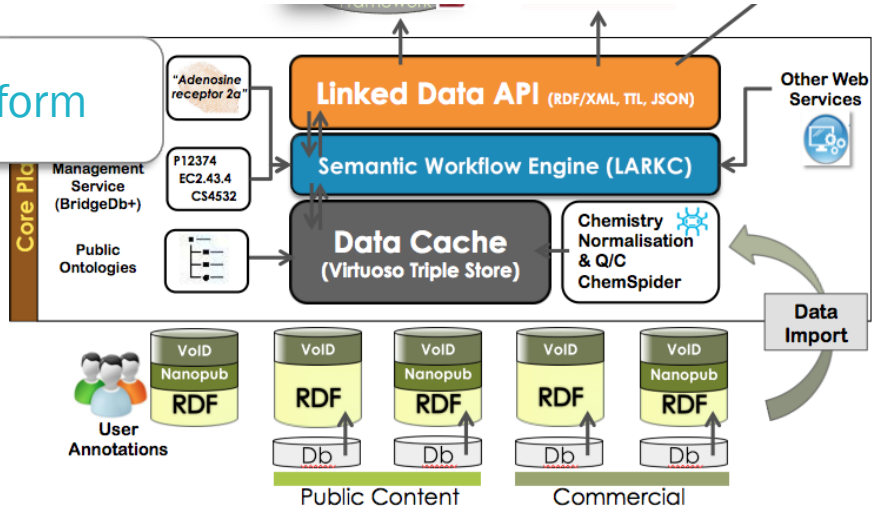
POC: The Holy Grail of Target Validation

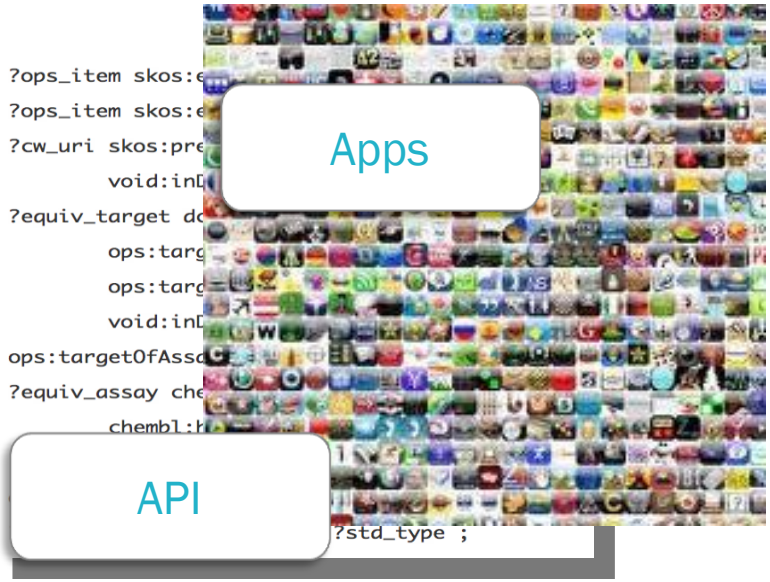
- PoC is a *critical* stage in drug discovery
  - At PoC huge resources are committed to take a drug to market
  - PoC is usually judged at Phase II
- Proof of Concept should show the following:
  - That human data reflects the data generated in the animal models
  - No unexpected side effects
  - demonstrate efficacy in the target disease
  - Ideally secondary benefits or indications
- PoC studies need to satisfy the following:
  - Endpoints of study should be minimum size to demonstrate efficacy with statistical significance
  - Placebo effect can not be ignored.

Tools for Target Validation

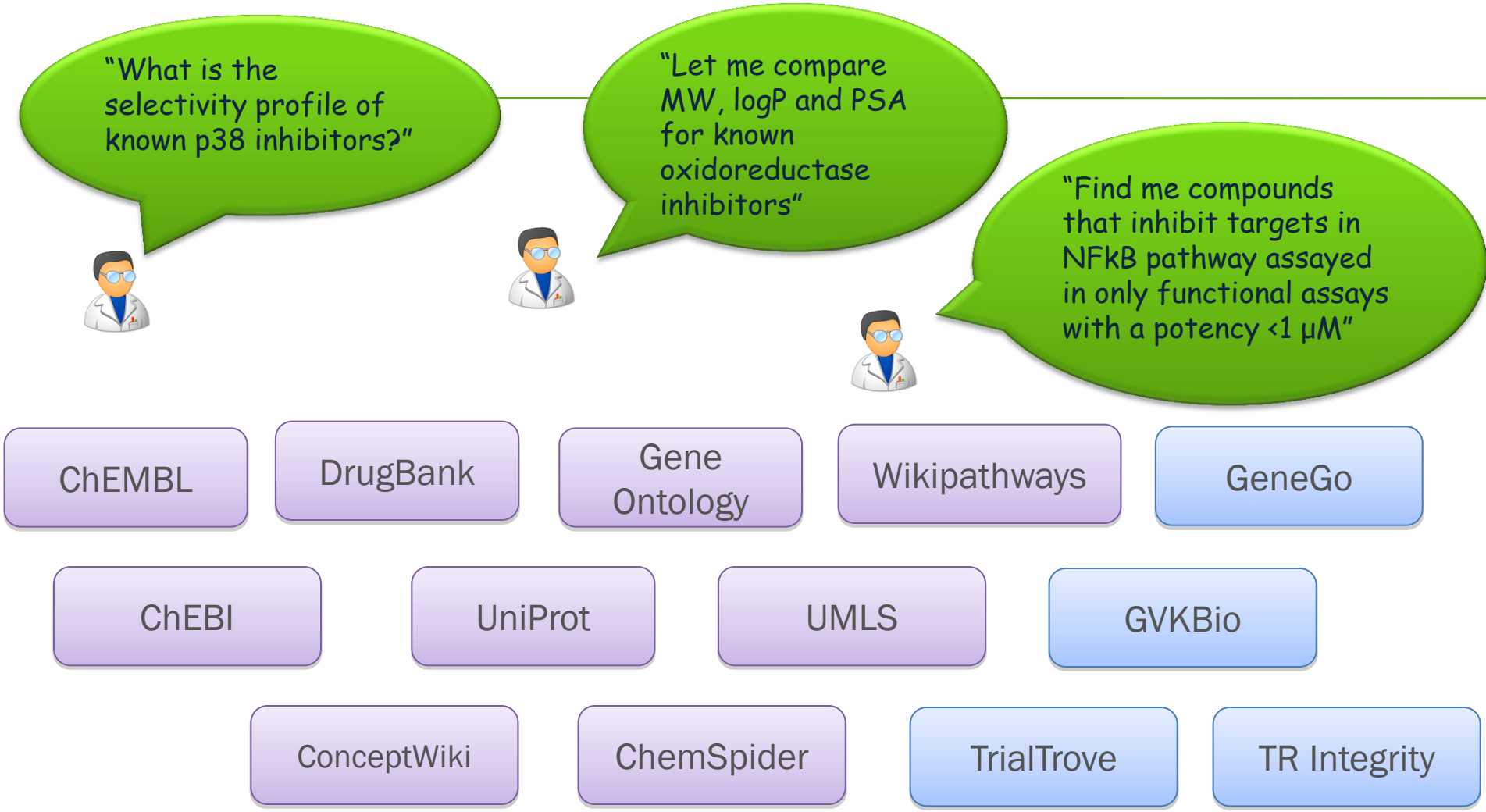


Open PHACTS





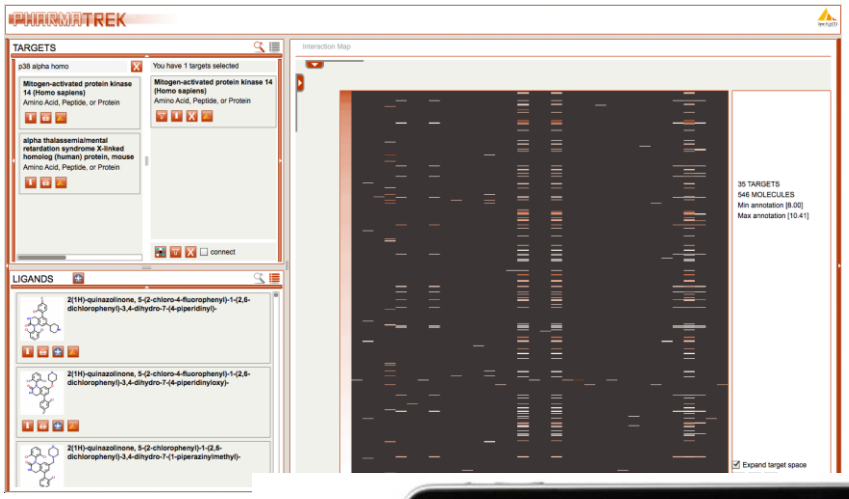
Slide: Paul Groth



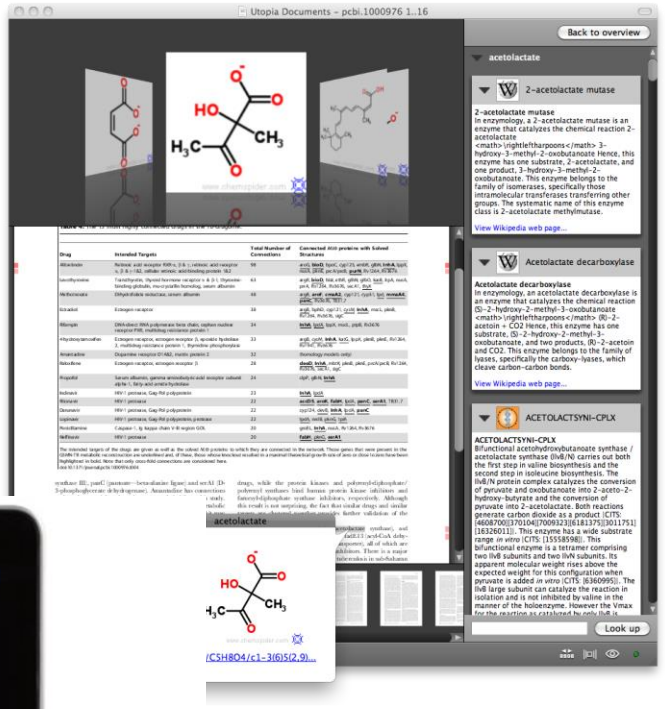
Slide: Paul Groth

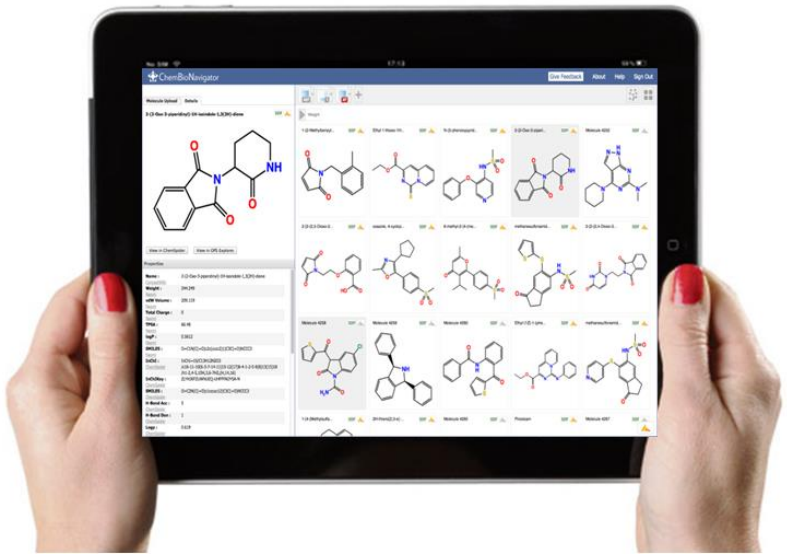
Open Phacts Apps (www.openphacts.org)

Pharmatrek



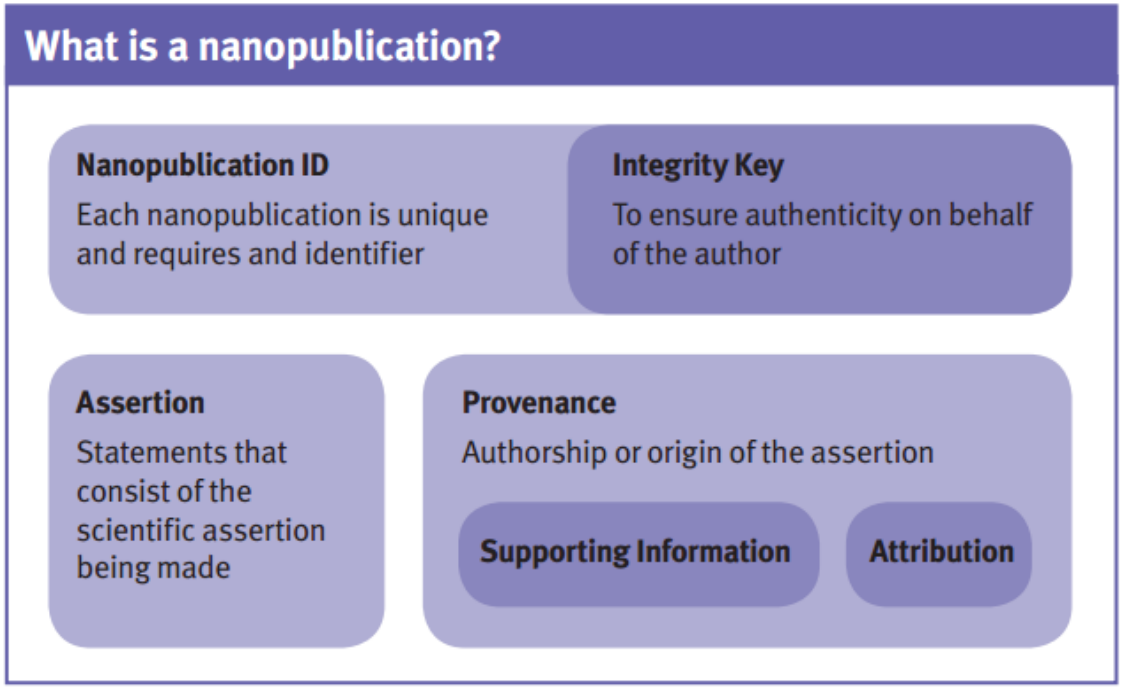
Utopia Docs





ChemBioNavigtor

Semantic interoperability approach

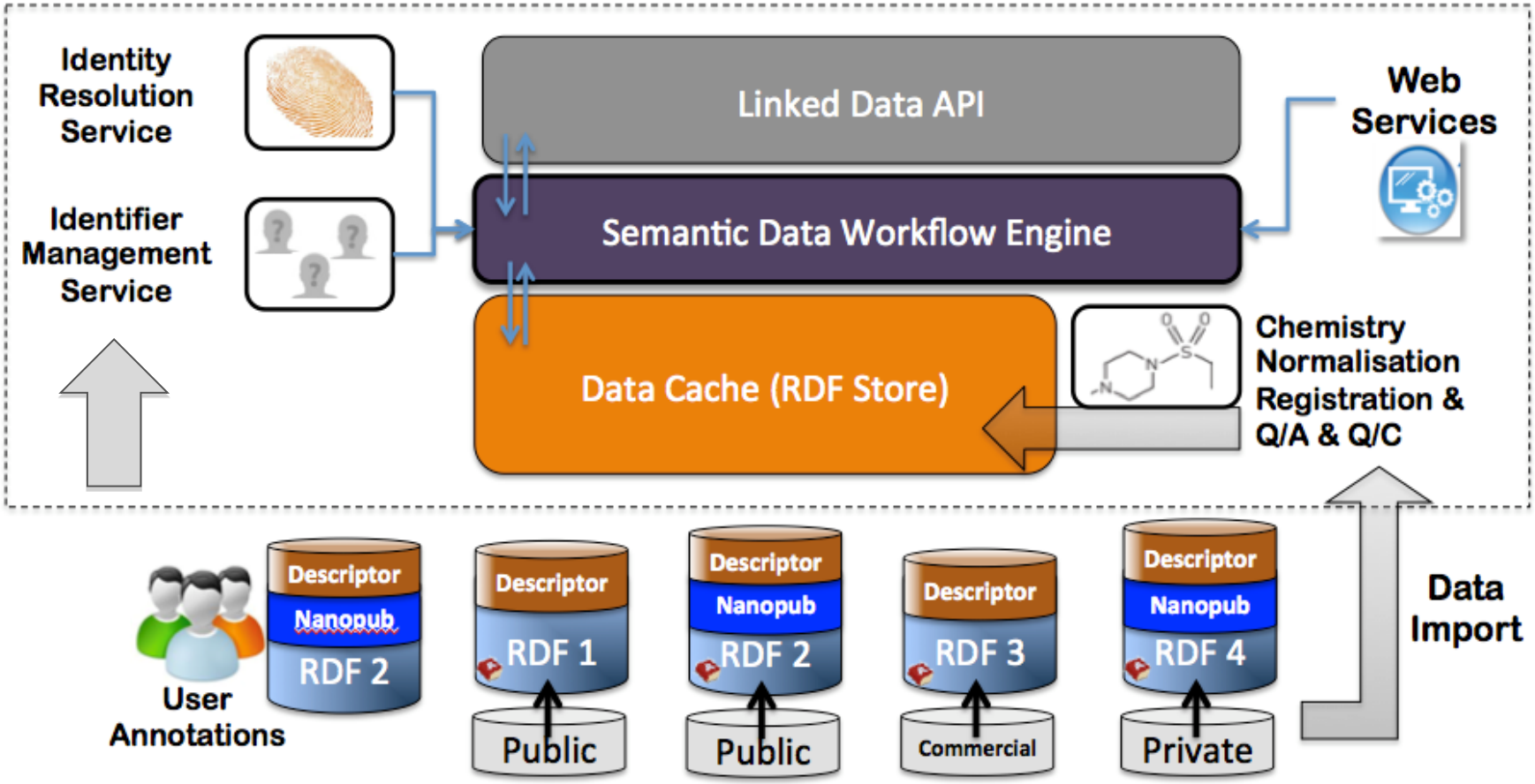


Principles

- Respect data providers
- Make it easy for application developers

Slide: Paul Groth

Semantic interoperability approach



Slide: Paul Groth

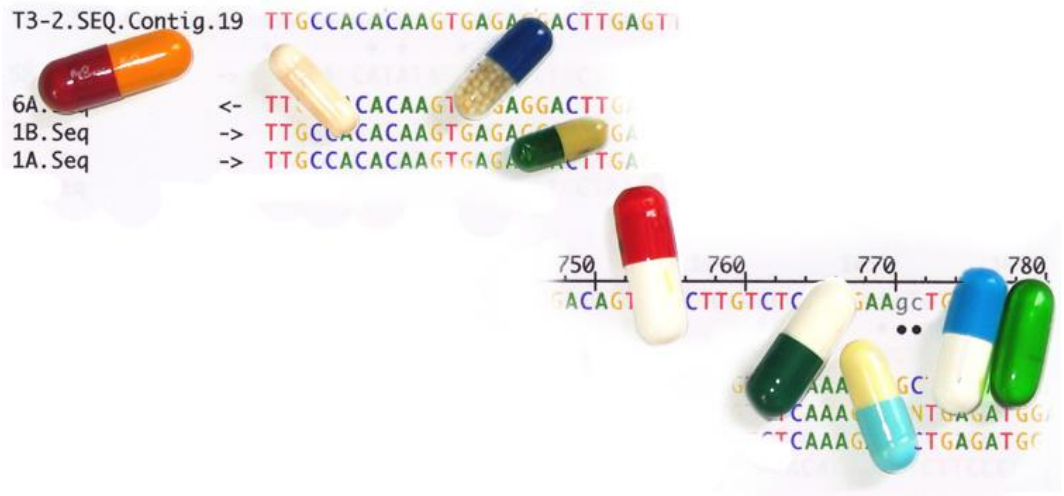
Semantic Resources – Data sets

814,535,923 triples

Source	Version	Supplier	Downloaded	Initial Records	Triples	Properties
Chembl	Chembl 13 RDF (11-Jun-2012)	Maastricht	08 Aug 2012	1,149,792 (~ 1,091,462 compounds, 8845targets)	146,079,194	17 (for compounds) 13 (for targets)
DrugBank	Aug 2008	Bio2Rdf (www4.wiwiss.fu-berlin.de)	08 Aug 2012	19,628 (~14,000 targets, 5000 drugs)	517,584	74
SwissProt	2012_07 (July 11, 2012)	SIB	07 Aug 2012	536,789	156,569,764	78
ENZYME	July 11, 2012	SIB	07 Aug 2012	6,187	73,838	2
ChEBI	Release 94	EBI	08 Aug 2012	35,584	905,189	2
ChemSpider ACD Labs		ChemSpider	08 Aug 2012	1,194,437	161,336,857	22 ACD 4 CS
ConceptWiki		NBIC	07 Aug 2012	2,828,966	3,739,884	1

Slide: Paul Groth

Tools for Target Validation



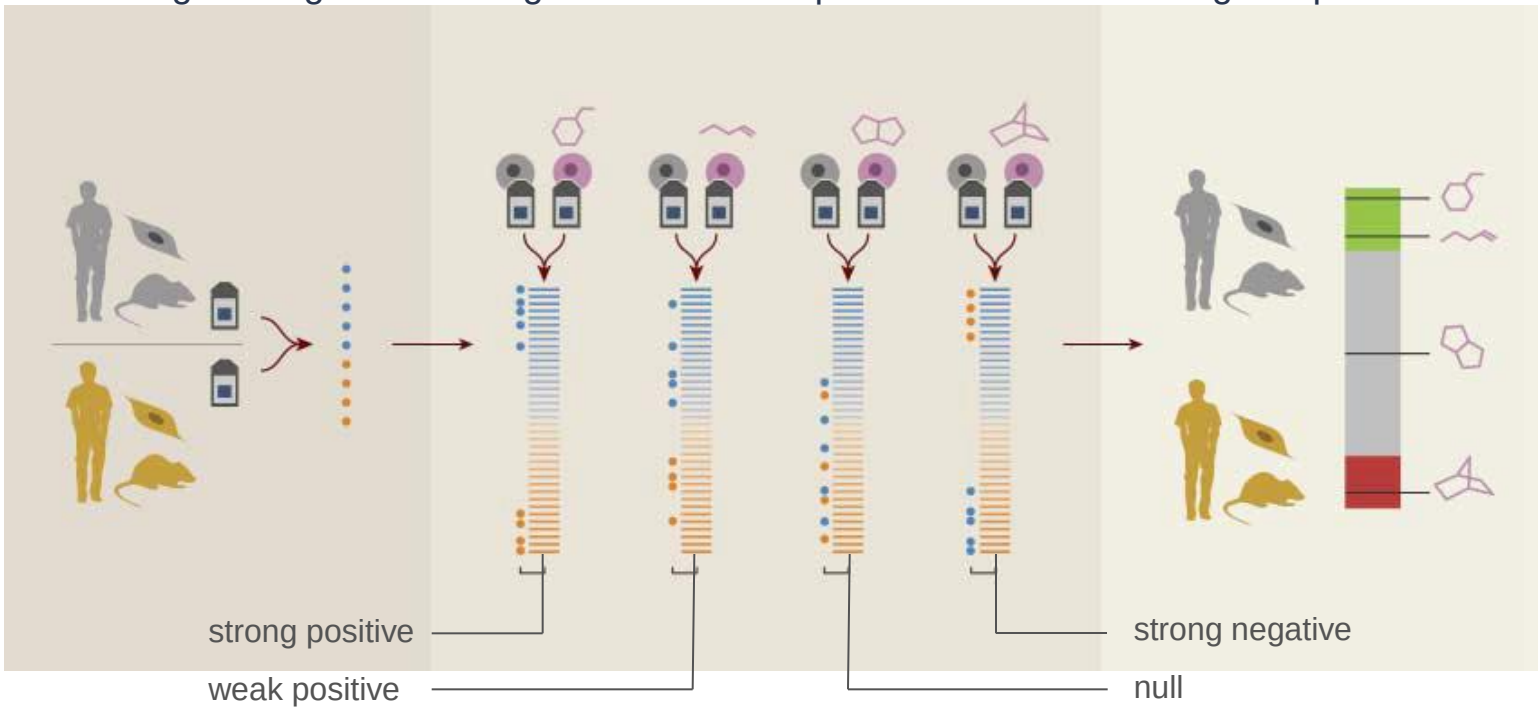
Connectivity Map

Search database by comparing a query signature with reference signatures

Query signature – list of up- and down-regulated genes

Reference signatures - ranked gene lists for compounds

Output – lists of high and low scoring compounds



CMAP v2.0

- Early access portal
- Drugs of interest
  - Methotrexate
- siRNA of interest
  - TNF/TNFR
  - IL12/IL12RB
  - IL17/IL17RA
  - Pathway components
- Lincscloud.org