Chemical and Biological Data – From Compound Selection to Mode of Action Analysis (and Back Again)

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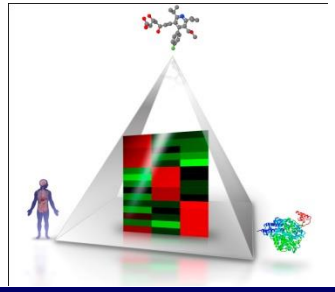


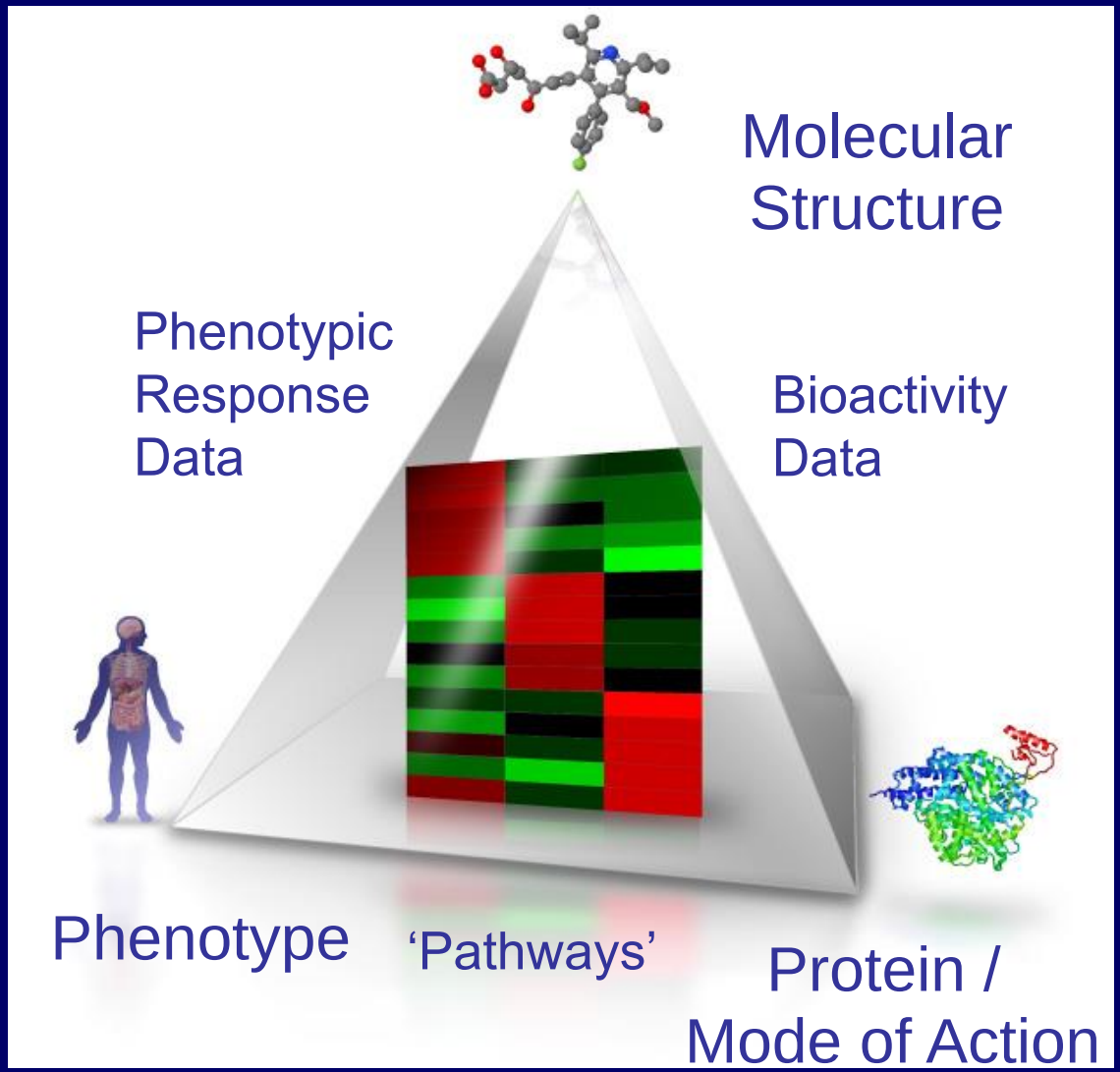
Outline

- Chemical and biological data – what is out there (and how can we use it?)
- Understanding modes of action of phenotypic readouts
- Why we need a chemical *and* biological view of modes of action
- Using chemical *and* gene expression data for MoA analysis and compound selection

Outline

- Chemical and biological data – what is out there (and how can we use it?)

Core Data Considered: Chemistry, Phenotype, Targets / Mode of Action



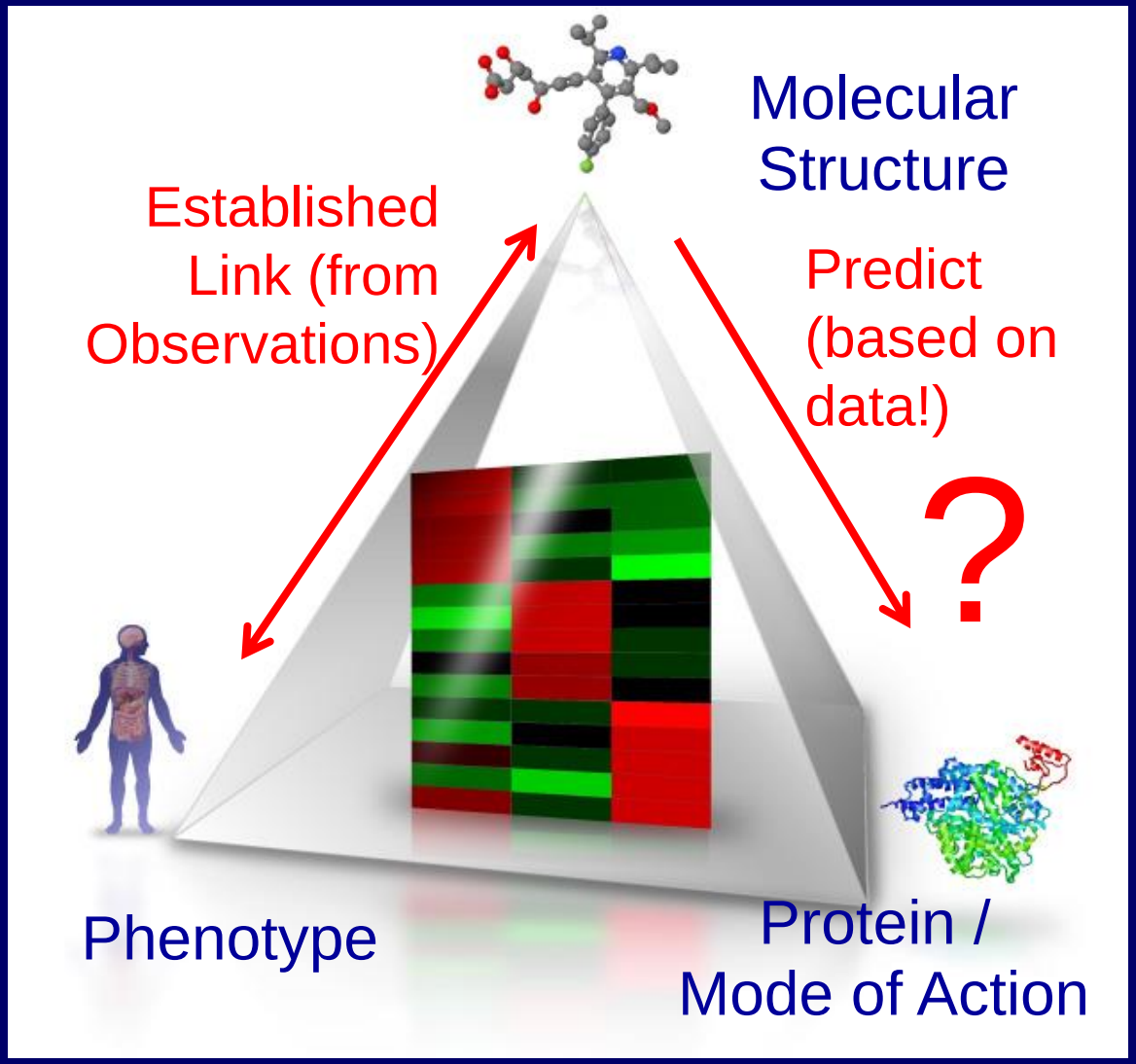
So what's the point of it all?
We would like to answer questions!

- "What is the reason upon treatment with A for phenotypic effect B?"
  -> *Mode of Action*
- "Which compound should I make to achieve effect C in a biological system?"
  -> *Chemistry*
- "Does patient D or patient E respond better to drug F?"
  -> *Phenotype / Phenotype Change*

Outline

- Understanding modes of action of phenotypic readouts

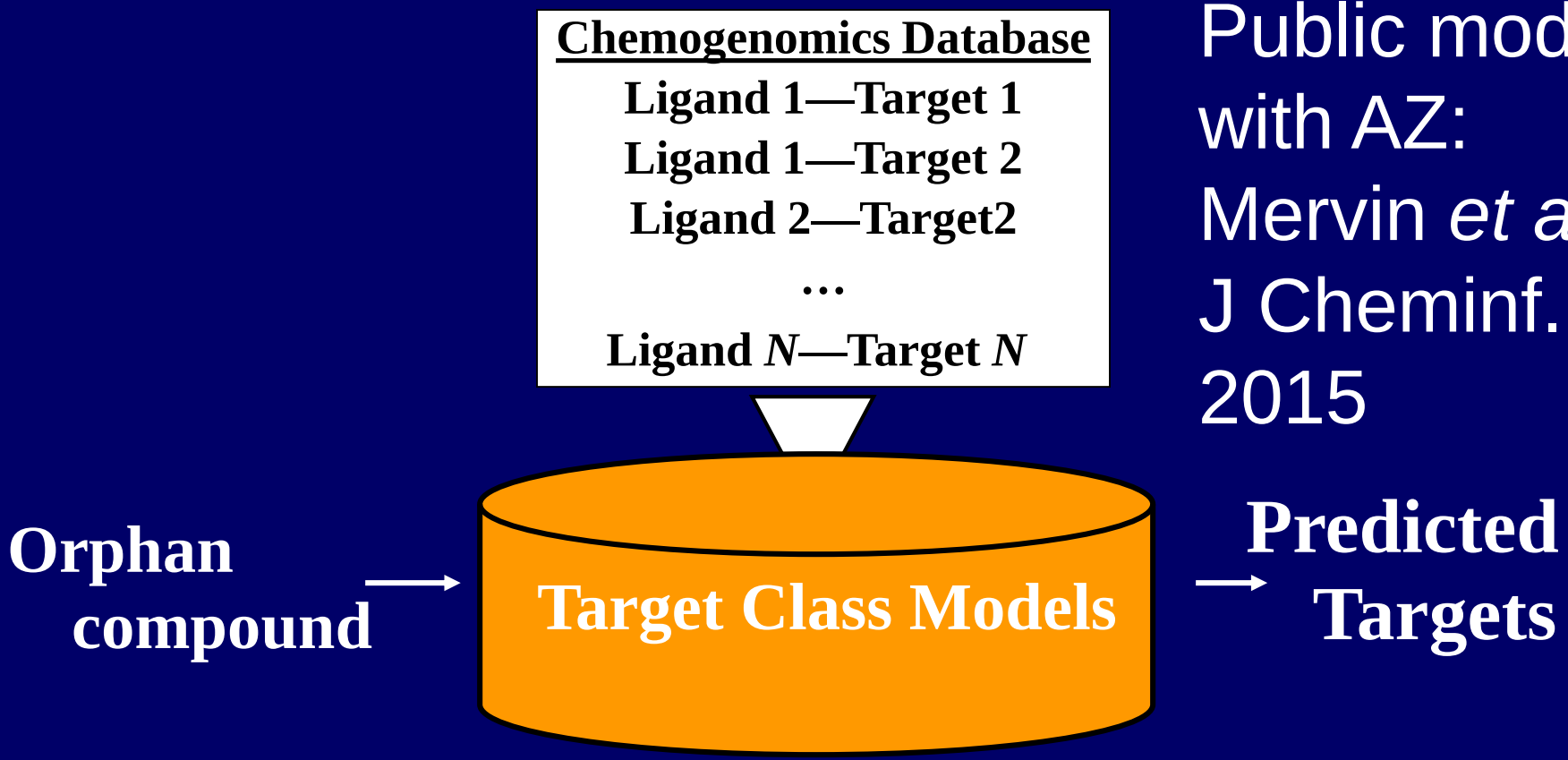
Starting from *in vivo* efficacy we can predict the MoA, based on ligand chemistry



A. Koutsoukas *et* al., J Proteomics 2011 (74) 2554 – 2574.

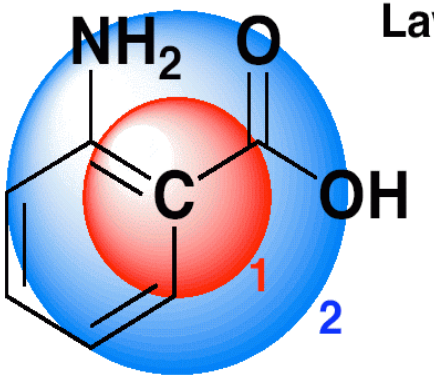
Exploiting known bioactivity data for new decisions: Target predictions

• The models enable automated prediction of the targets or target families of orphan ligands given only their chemical structures.



Public model with AZ: Mervin *et al.*. J Cheminf. 2015

How do you describe molecules? E.g. using ‘Circular fingerprints’



Layer:	0	1	2
	C.ar (sp^2)	C.ar (sp^2)	C.ar (sp^2)
		C.ar (sp^2)	C.ar (sp^2)
		C (sp^2)	N (sp^3)
			O (sp^2)
			O (sp^3)

- Each fingerprint feature represents a *central atom and its neighbors*
- Abstract enough due to losing connectivity (but keeping atom types quite concrete); disjoint (but ‘overlapping’ features) … weakness in symmetry and repeat units (terpenes, etc.)

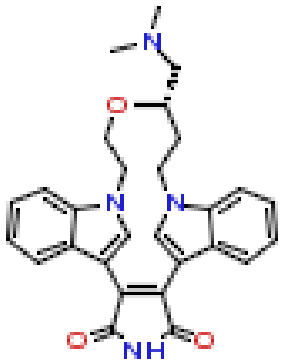
RC Glen, A Bender, CH Arnby, L Carlsson, S Boyer, J Smith
IDrugs 2006, **9**:199-206

Prediction Examples: Gleevec, Ruboxistaurin

- Gleevec (Novartis),
  - Launched
  - Targets Bcr-Abl, c-kit, PDGFRb

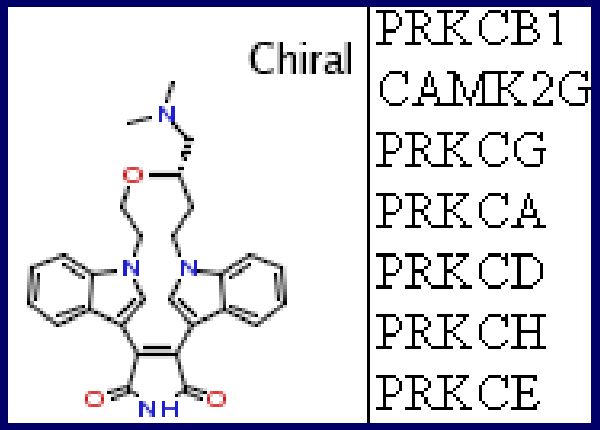
- Ruboxistaurin (Lilly/Takeda),Phase III
  - PKCb

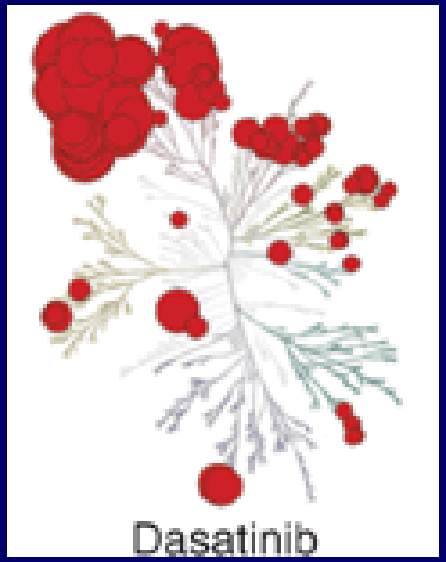
Molecule	Targets	Scores
	ABL1	46.50
	PDGFRB	28.99
	KIT	22.02
	CDK9	21.30
	BRAF	16.13
	FLT1	13.09
	PLK1	8.05
	BTK	5.44

Molecule	Targets	Scores
Chiral 	PRKCB1	95.81
	CAMK2G	87.48
	PRKCG	66.35
	PRKCA	56.99
	PRKCD	52.44
	PRKCH	51.41
	PRKCE	50.42
	PRKCZ	42.48

Case study of *in silico* mode-of-action analysis

- Rat (organism-level) screen, with Eli Lilly





Understanding rat sleep data

- Project with Eli Lilly
- Male Wistar rats

Work by Georgios Drakakis
ACS Chem Biol. 2017

- Treated with ~500 sleep-inducing compounds, dozens of readouts from EEG/EMG, Abdominal Minimitter, Cage that define ‘good sleep’

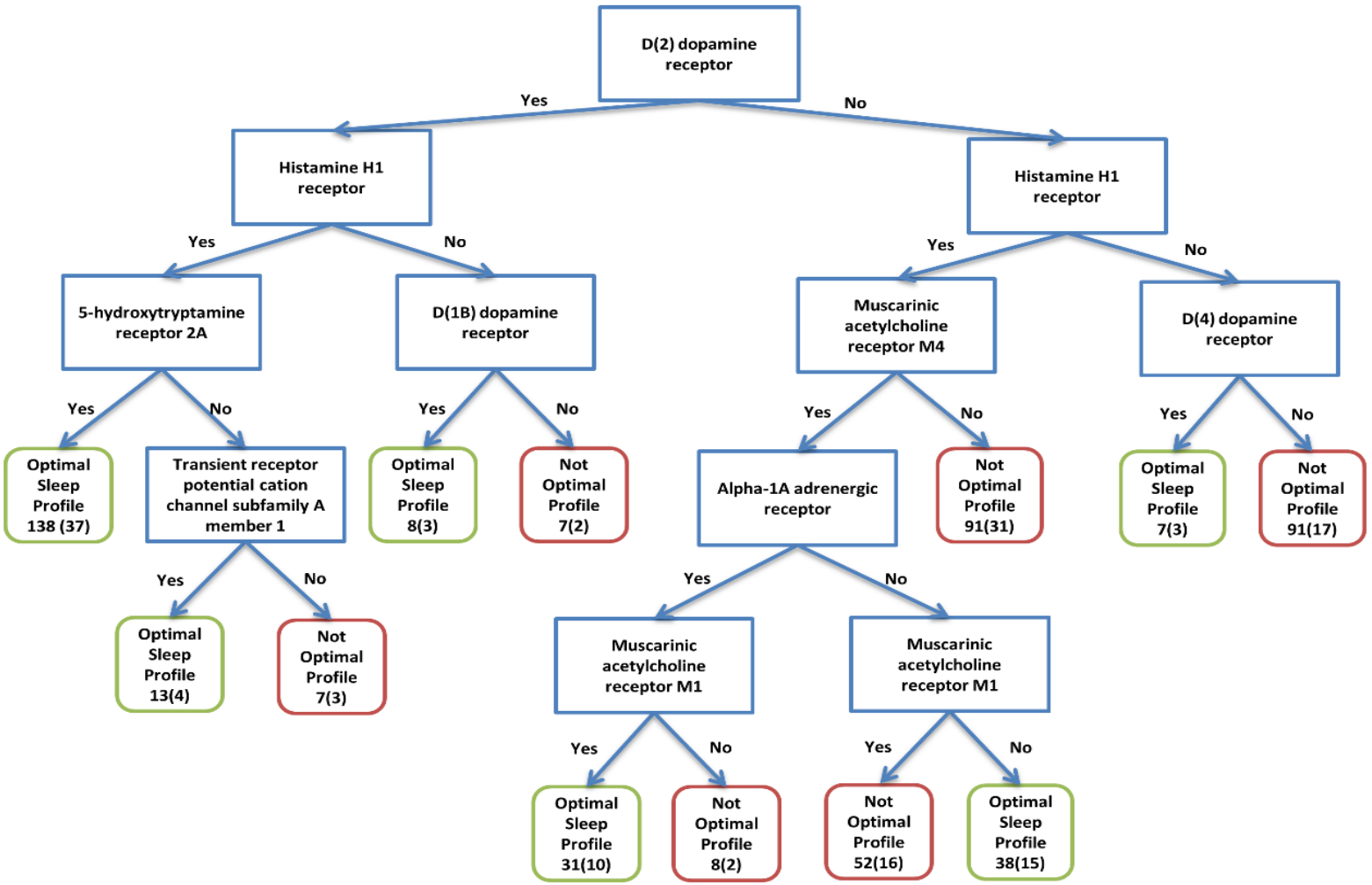
- **Q: What are bioactivity *profiles* associated with compounds inducing good sleep?**

- Going from single to multiple targets (polypharmacology), and from single to multiple simultaneous MoA hypotheses for given phenotype

Efficacy *and* side-effect readouts used to define desired phenotype

Variable Name	Variable Description	Variable Type
NREM6hr	Cumulative non-REM sleep in the first 6 hours post dosing compared to vehicle	Efficacy
Sleep6hr	Cumulative total sleep in the first 6 hours post dosing compared to vehicle	Efficacy
LBout	Longest sleep bout in the first 6 hours post dosing compared to vehicle	Efficacy
AvgAvgBout	Average of the first 6 average hourly sleep bouts post dosing compared to vehicle	Efficacy
RebIns	Rebound insomnia; the cumulative non-REM sleep between hours 6-9 hours post dosing compared to vehicle	Side-effect
REMinh	REM sleep inhibition; the cumulative REM-sleep in the first 12 hours post dosing compared to vehicle	Side-effect
LMinh	Locomotor inhibition; the cumulative locomotor Activity per minute of Wake (MOW) time in the first 6 hours compared to vehicle	Side-effect

Decision trees learn receptor bioactivity profiles associated with ‘good’ and ‘bad’ sleep



Bioactivity profiles give 6 MoA hypotheses for prospective testing (5 were selected)

Protein Targets	Polypharmacological Bioactivity Profiles					
	A	B	C	D	E	F
D(2) dopamine receptor	1	1	1	0	0	0
Histamine H1 receptor	1	1	0	1	1	0
5-hydroxytryptamine receptor 2A	1	0	NA	NA	NA	NA
Transient receptor potential cation channel subfamily A member 1	NA	1	NA	NA	NA	NA
D(1B) dopamine receptor	NA	NA	1	NA	NA	NA
Muscarinic acetylcholine receptor M4	NA	NA	NA	1	1	NA
α-1A adrenergic receptor	NA	NA	NA	1	0	NA
Muscarinic acetylcholine receptor M1	NA	NA	NA	1	0	NA
D(4) dopamine receptor	NA	NA	NA	NA	NA	1

Prospective validation on both target and phenotypic level

- 7 marketed drugs/drug combinations were selected which are predicted to modulate sleep, are dissimilar to the training set, but were not annotated with this side effect

- *5 out of 7 marketed drugs (71%) tested increased sleep parameters (a sixth led to hyperactivity!)*

- 21 out of the 27 predicted *targets* (78%) were validated

- Overall 78% correct on target level, ~71% on phenotypic level (across 4 MoA classes)

Outline

- Why we need a chemical *and* biological view of modes of action (… and how little we sometimes understand of how drugs work)

‘Mode of action’ – what is this actually?

- Previous example: *Ligand* binding to a *target*
- Easy to understand by humans, very nice… *but!*

- Many years ago I looked at biological readouts (Western blots, gene expression data, …) of compounds with supposedly ‘similar’ pharmacology, and those were (vastly!) different

- *Hence, a protein-based view is often insufficient to describe the pharmacology of a compound on its own*
- Biased signalling, off-targets, binding kinetics, permeability/pharmacodynamics etc… many reasons

How little we sometimes know about how drugs work…

- Hypothesis: A CNS-active drug works by modulating neurotransmitters in the CNS (specific neurotransmitter, specific region) – basis of much current research

- We* compiled information from 15,777 research articles and neurotransmitter changes from experiments (comprising 110,674 rats)
- Drug class (ATC code - antipsychotic, stimulant, …), etc., neurotransmitter, region

***Neurochemical Fingerprints of Psychiatric Drugs.**
Hamid R. Noori, Lewis Mervin, Vahid Bokharaie, Özlem Durmus, Lisamon Egenrieder, Stefan Fritze, Britta Gruhlke, Hans-Hendrik Schabel, Sabine Staudenmaier, Nikos K. Logothetis, Andreas Bender, Rainer Spanagel (under preparation)
www.syphad.org (publicly, freely accessible)

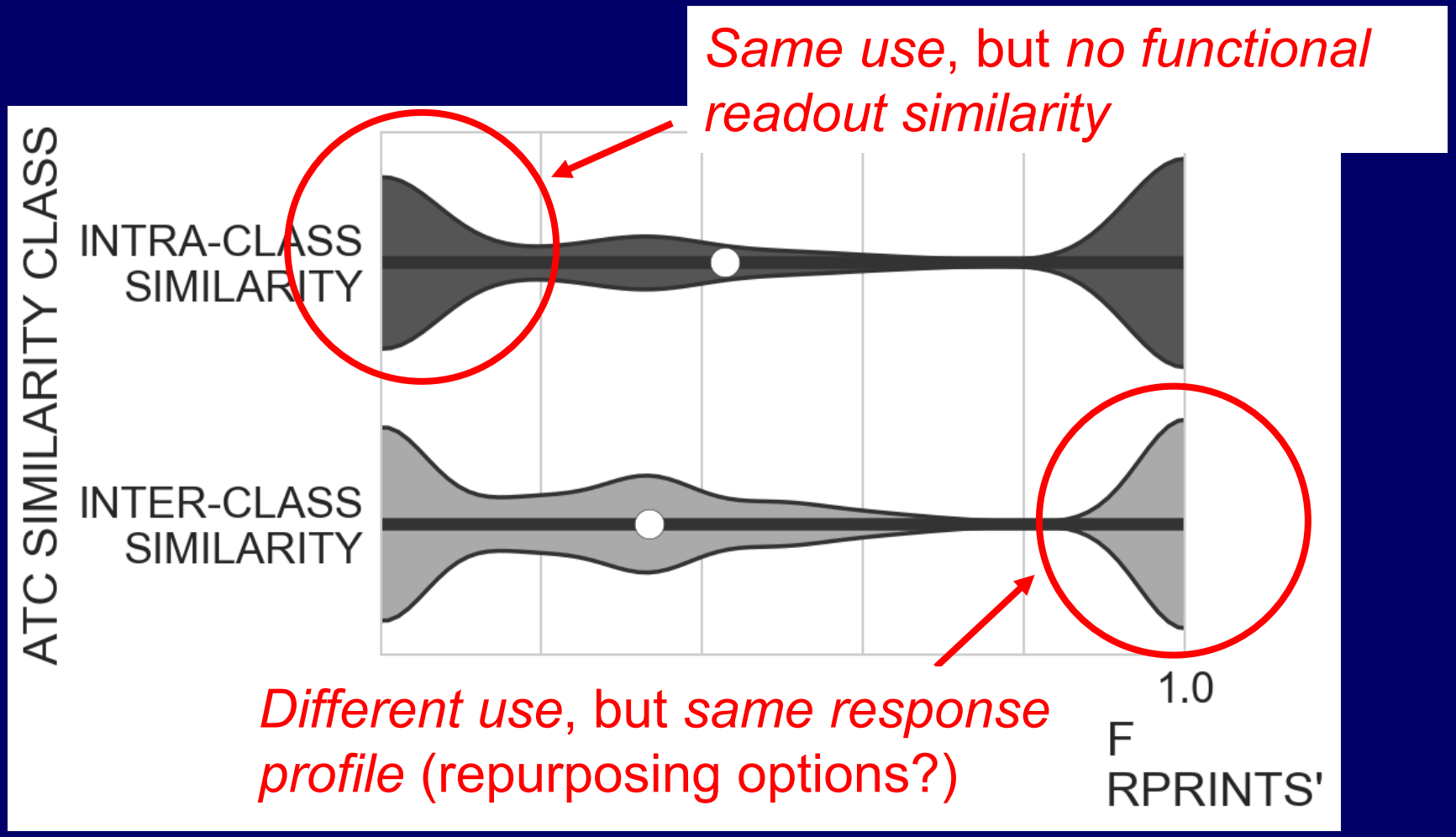
So what do sedatives, stimulants, antipsychotics, … have in common?

- You would assume that disease, and treatment (mode of action of drugs), are in some way ‘defined’

- According to working hypothesis, drugs are similar on the target level and/or the *functional level* (which we looked at here)

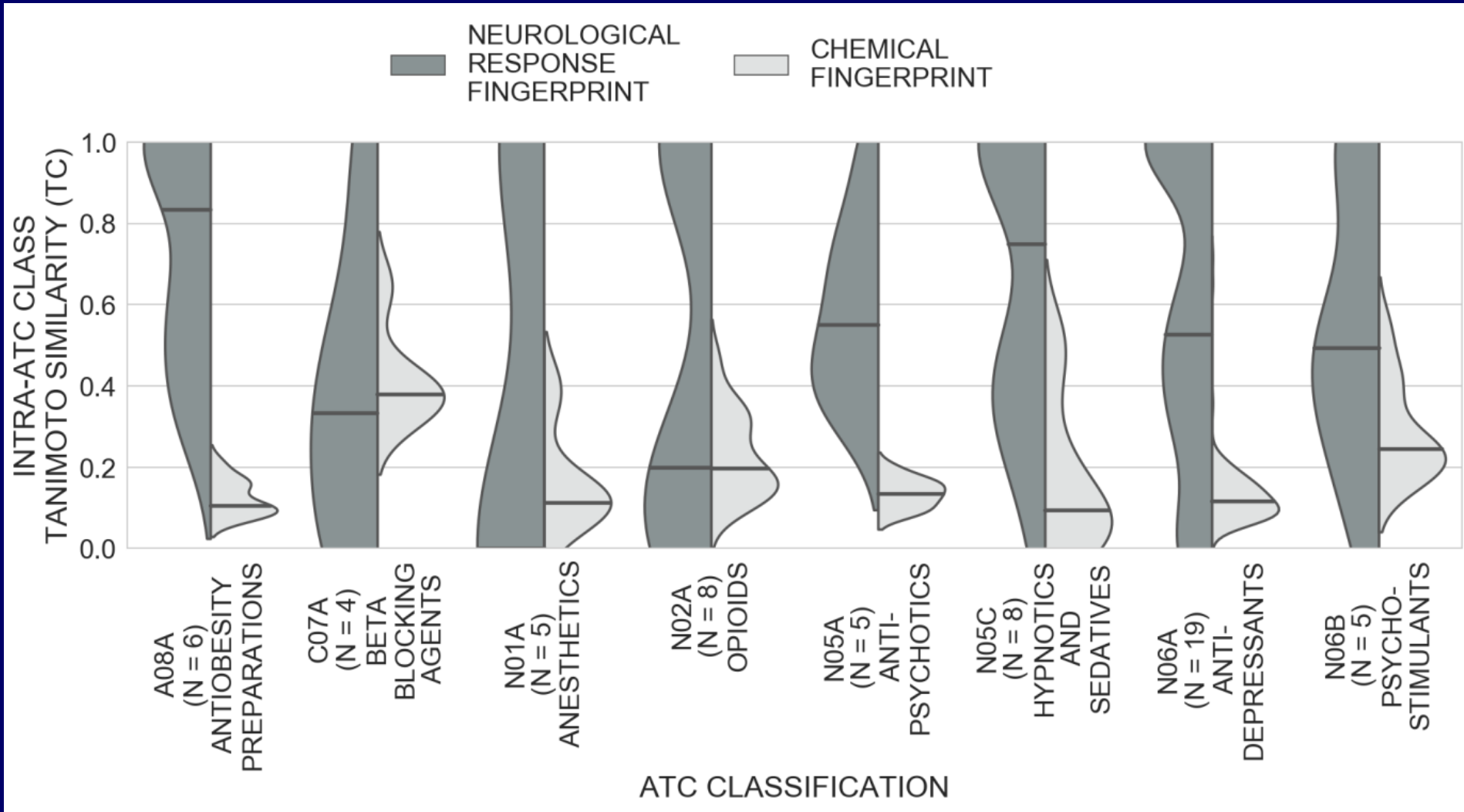
- So let’s look at the data…

Neurotransmitter (functional) similarity within and between ATC classes



Neurotransmitter changes are vaguely correlated with use (ATC codes) … but only *very* weakly. So what is their 'mode of action'???

Eg beta-blockers *high chemical similarity*, *low response similarity ('me too')*, antidepressants *assumed to hit* D2 (but profiles much more diverse)…



So… how should we define the mode of action of a compound?

- Using only *proteins* to explain mode of action (or design compounds) probably only works in narrowly defined cases (eg viral proteins involved in cellular entry; inhibiting blood clotting cascades, etc.)

- Using biological readouts is likely better, *but*…
- Even using 'biological readouts' is insufficient – they need to be disease-related (*hypothesis-driven*!)

- So… what is the 'mode of action' of (eg) many CNS-active compounds…? I don't think we understand this fully… (at least I don't!)

Outline

- Using chemical *and* gene expression data for MoA analysis and repurposing

Using gene expression and on-target activity to understand interactions in mesothelioma treatment

- Abo1 is a complex herbal mixture, based on Cynara scolymus (ie, artichoke), shows clinical efficacy in mesothelioma

- *How can we understand interactions (synergy?) based on chemical and biological information?*

- Complex mixtures (hundreds of different chemical species), which are characterized by (a) clinical studies, (b) chemically and (c) biologically

- Work of Nitin Sharma, with Jacopo Lucci (Aboca)

Using chemical *and* RNASeq data for mode-of-action analysis

- Project with Natural Bio-Medicine / Aboca (Italian Complex Natural Products company)

- Observation (eg): fraction of Cynara scolymus (ie, artichoke) induces apoptosis in mesothelioma (*Pulito et al., Oncotarget 2015)*

- Q: Can we provide MoA/biological evidence for admission as food supplement, medical device, food for medical purpose, … whatever the relevant category is that applies?

- Complex mixtures (hundreds of different chemical species), which are characterized by (a) clinical studies, (b) chemically and (c) biologically

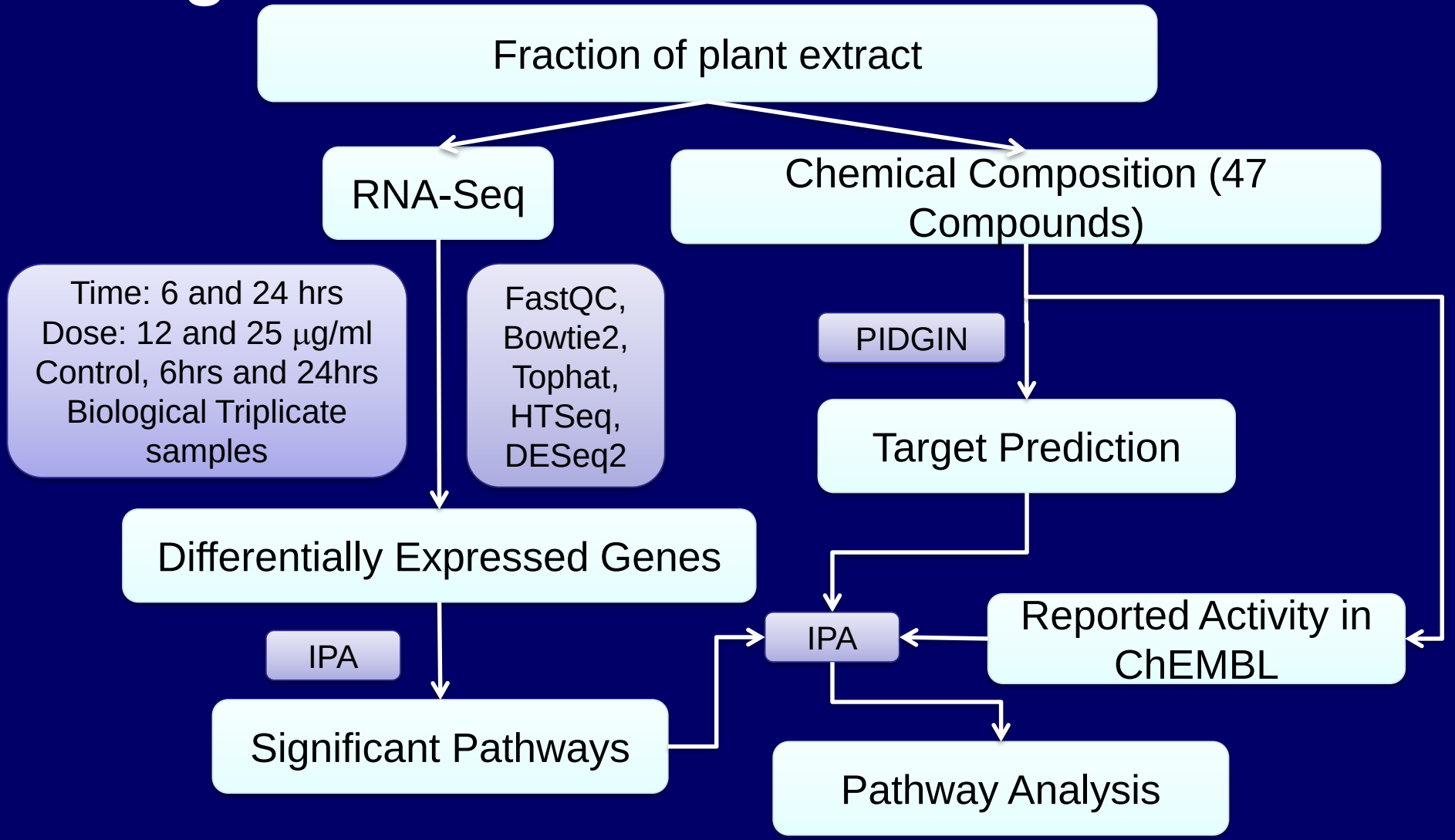
Using chemical *and* RNASeq data for mode-of-action analysis

- Target predictions are weak on quantitative aspects of predictions, as well as analyzing compound combinations (and interactions), *but* they are easily understandable

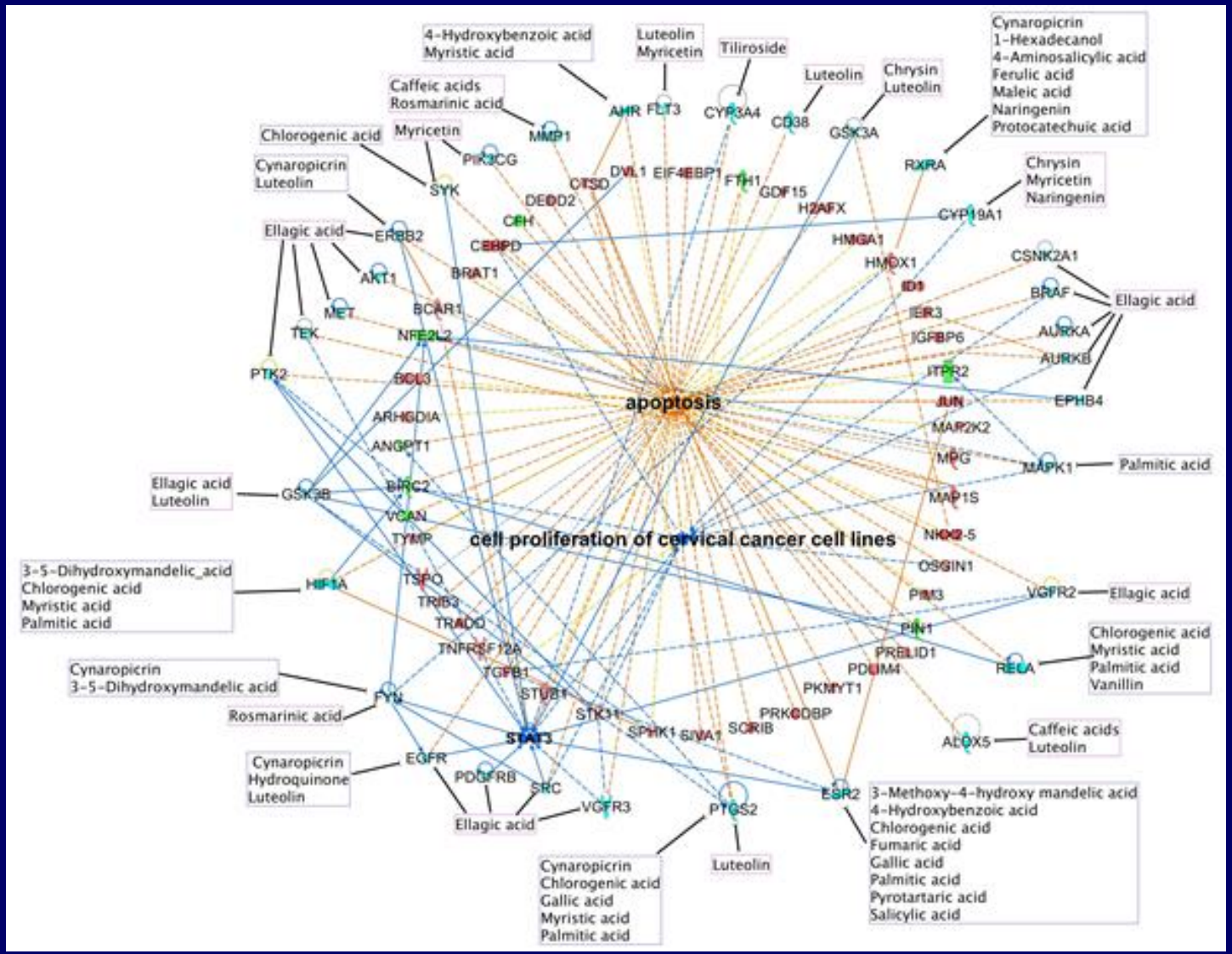
- Hence, we use an integrated bioinformatics (RNA-Seq after compound treatment) *and* cheminformatics approach to provide mode-of-action analysis

- Important: choice of model system, parameters (concentration, time, etc.) to ensure physiological relevance

RNA-Seq readouts (left) and on-target readouts to understand MoA



Target prediction *and* RNA-Seq data gives ‘integrated view on Mode of Action’…

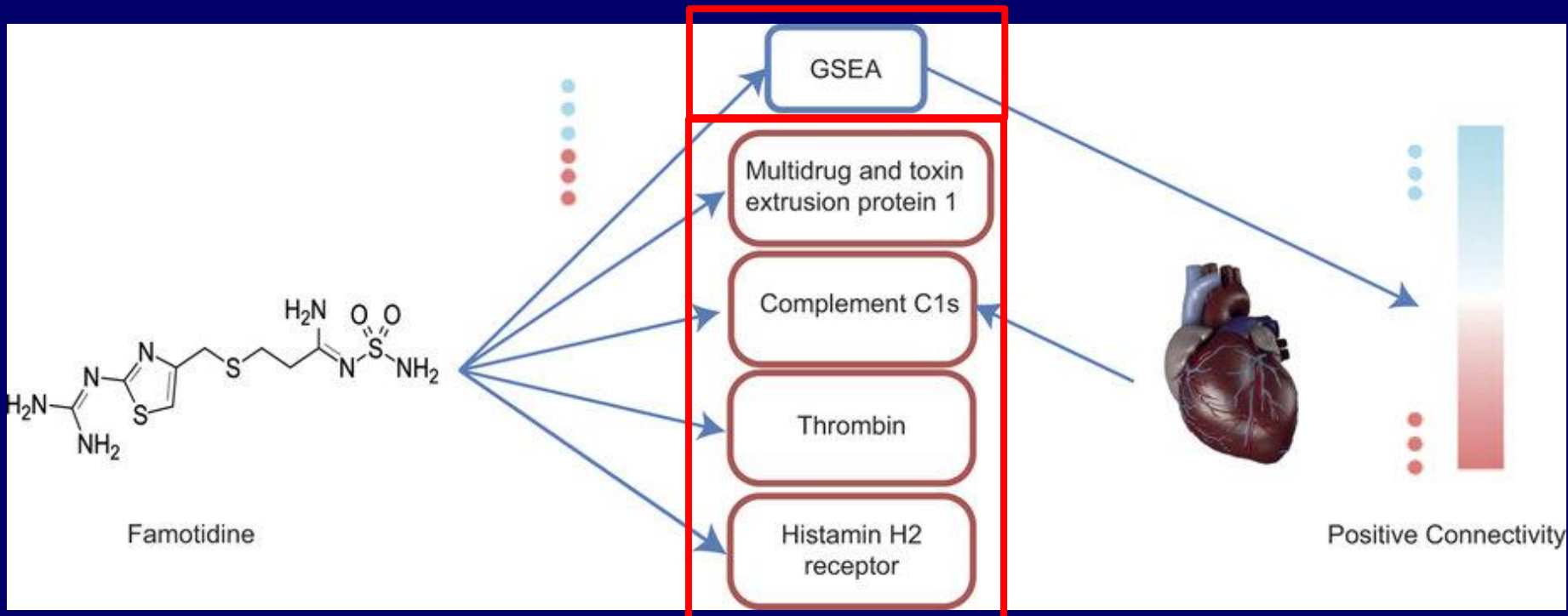


So what did we learn?

- Ligand-target activities are insufficient by themselves to understand MoA and synergy
- Biological readouts are needed, gene expression currently appears to have good signal-to-cost trade-off
- However, trying to understand the MoA of a complex mixture of compounds is still rather … complex

Combined gene expression / on-target activity analysis for compound selection

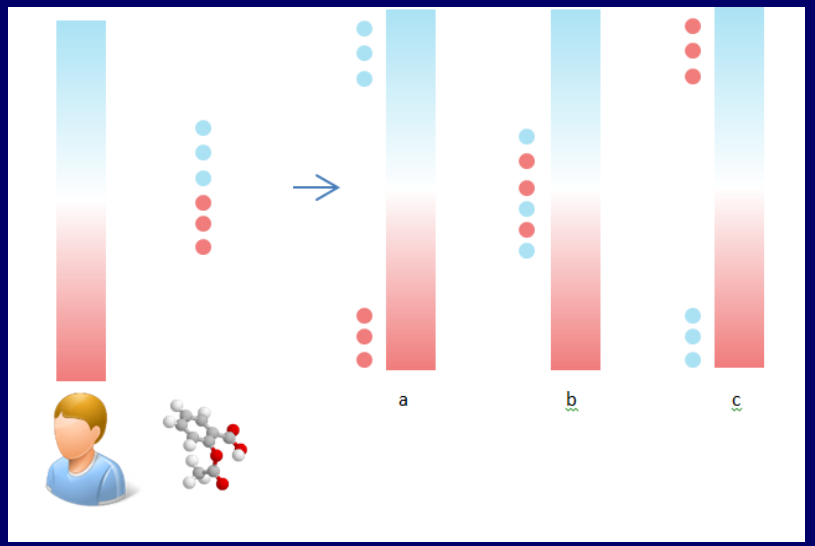
- Select compounds based *both* on gene expression and target prediction profiles



KalantarMotamedi *et al. Cell Death Discovery* **2016**

“BioStateConverter”
(work of Yasaman KalantarMotamedi)

- Compound-Disease mapping *via* gene expression data
- Drug should *invert* gene expression profile of disease
- This ‘returns the system to the healthy state’ (better seen as *signal*, not necessarily interpreted mechanistically)



Data Sources

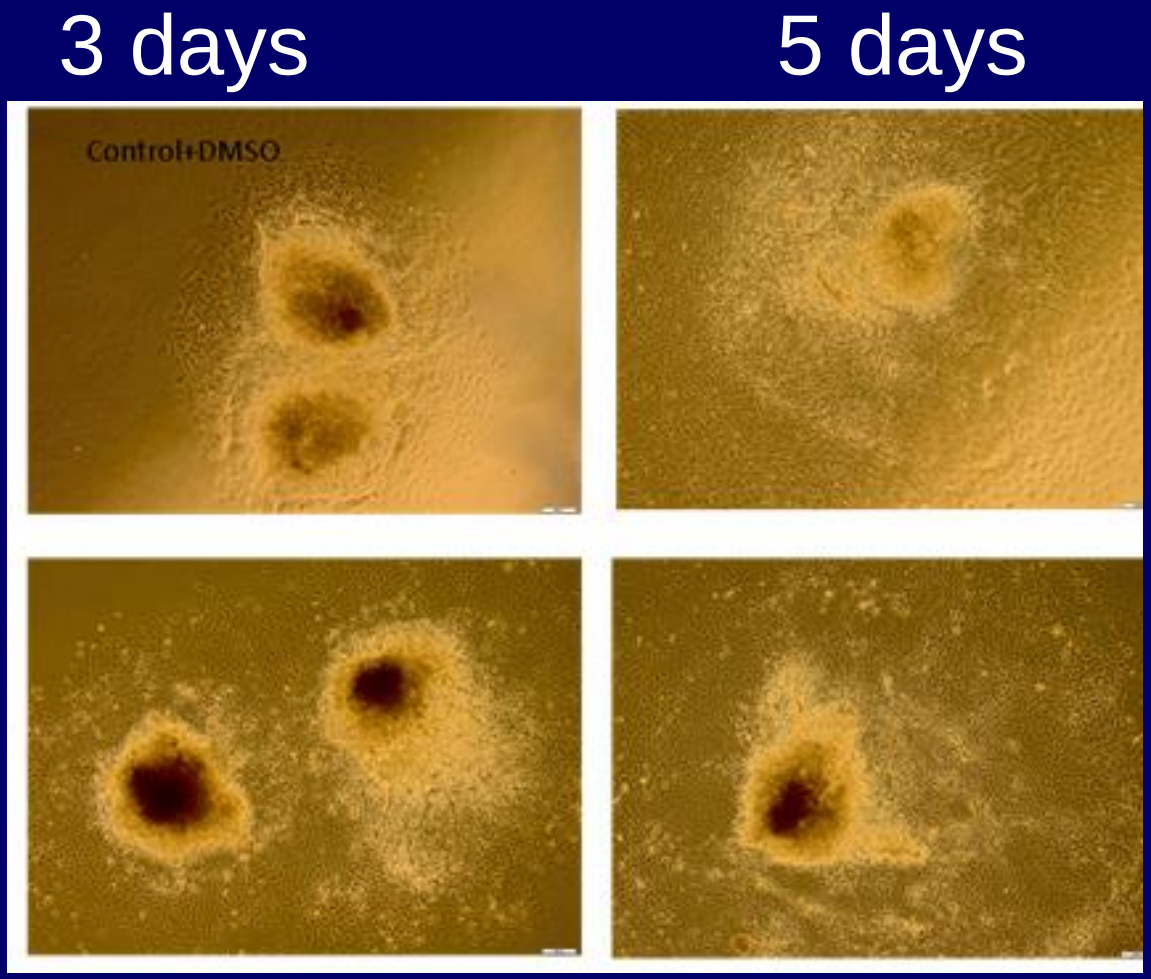
- ConnectivityMap (1,300 compounds to Affymetrix chips)
- LINCS (12,000 compounds to 1,000-gene expression signatures)

- *Many* issues with the data (dose/timepoint variability; reproducibility of controls, etc.)

- In our experience data contains sufficient signal for *signal detection* (but, possibly, less so for ‘modelling’)

- Gene expression data is still ‘difficult’ (regarding conditions, interpretability – less so its generation)

Selected compound induces differentiation of stem cells into cardiac myocytes (by RT-PCR; work with Dr Nasr, Royan Institute, Isfahan)

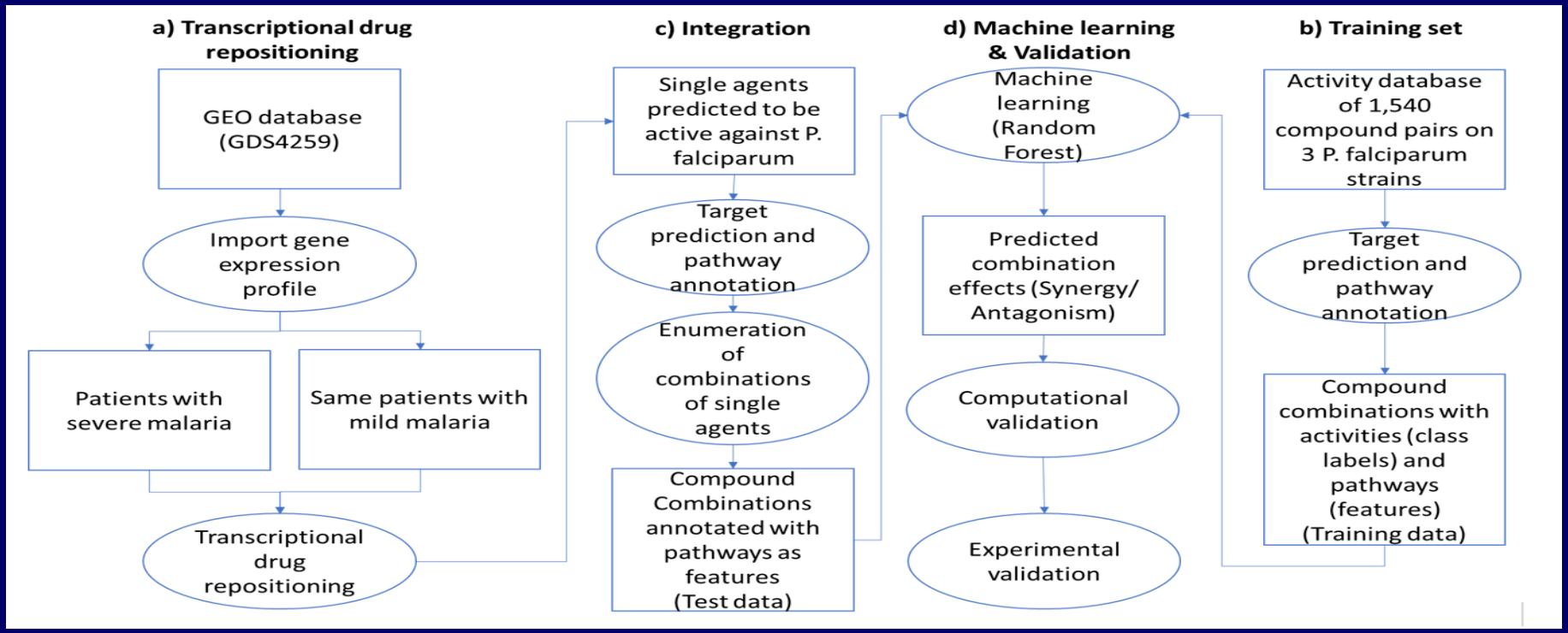


KalantarMotamedi *et al. Cell Death Discovery* **2016**

Selecting combinations active in malaria based on gene expression data

- Combining information from (a) ligand-target predictions and (b) combination screens, mapping both (c) on pathways, and (d) selecting novel synergistic combinations
- Work by Yasaman KalantarMotamedi (Cambridge), Raj Guha, Steve Eastman (NIH)
- KalantarMotamedi *et al.*, *Malaria Journal* **2018** (in press)

Combination of gene expression data and combination screens (here in malaria)



1. Select single active compounds based on GE data → 2. Enumerate target predictions, pathways of combinations → 4. Select compound combinations where synergistic pathway combinations are hit ← 3. Combination screen – predict targets, pathways of synergistic combinations

Overall we seem to enrich for synergistic combinations, based on selecting compounds that modulate targets/pathways *predicted* to be involved in synergistic effects

	Mild-to-Strong Synergy (gamma<=0.995)				Moderate-to-Strong Synergy (gamma<=0.975)				Strong Synergy (gamma<=0.95)			
Strain	**3D7**	**DD2**	**HB3**	**AVG**	**3D7**	**DD2**	**HB3**	**AVG**	**3D7**	**DD2**	**HB3**	**AVG**
True positives	18	18	19	18.333	7	13	12	10.667	2	5	1	2.667
False Negatives	11	7	12	10.000	3	4	3	3.333	1	2	1	1.333
False Positives	5	4	2	3.667	16	9	9	11.333	21	17	20	19.333
Precision	78.3%	81.8%	90.5%	83.5%	30.4%	59.1%	57.1%	48.9%	8.7%	22.7%	0.048	12.1%
Recall	62.1%	72.0%	61.3%	65.1	70.0%	76.5%	80.0%	75.5%	66.7%	71.4%	50.0%	62.7%
F measure	69.27%	76.59%	73.09%	164.89%	42.39%	66.68%	66.64%	59.36%	15.39%	34.45%	0.10%	20.29%
Training Set Synergies	42.8%	41.2%	40.0%	41.3%	25.7%	25.3%	23.0%	24.7%	15.8%	15.6%	13.4%	14.9%

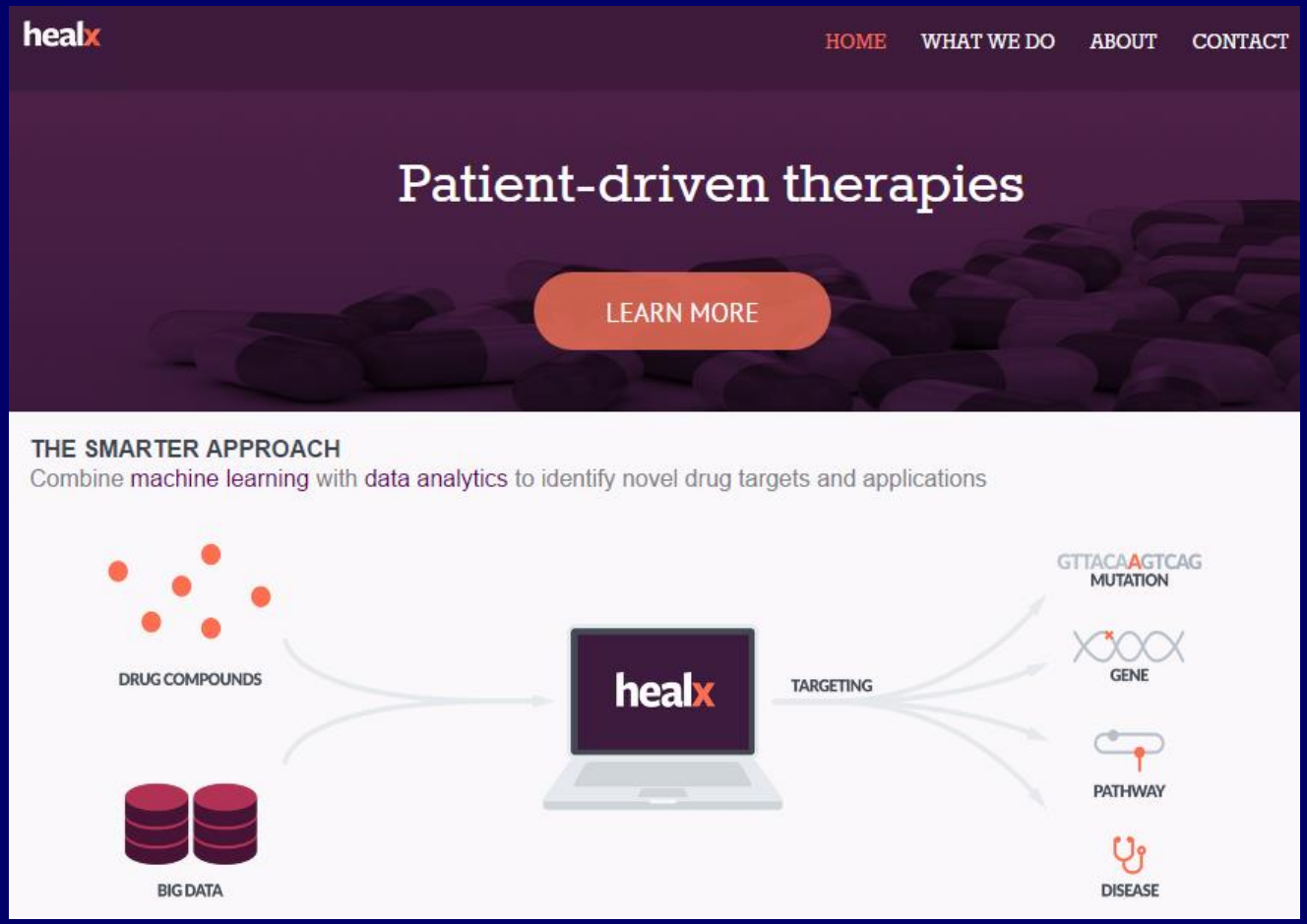
So what did we learn?

- We can apparently use information from many different sources (gene expression, ligand-target prediction, **pathway annotations**, …) to aim to understand and model synergy
- Pathway annotations are able to integrate ligand-target interactions with gene expression/other biological readouts
- How to put those parts together needs to be explored in more detail

Startup ‘Healx’ founded, for ‘data-driven drug repurposing in rare diseases’

- Emphasis on patient groups
- CEO Tim Guilliams, funded by Amadeus and others
- CUE ‘Life Science Startup of the Year’ 2015

www.healx.io; ~3yrs old; 15+ people



Positions available

PhD position with Bayer
“Using Deep Learning in Drug Discovery”
From October 2018 (3 years)

CAMS (Cambridge Alliance on Medicines Safety)
Junior Research Fellowship, Computational Toxicology, for PhD with postdoc experience
3 year position with research budget and co-supervision of several PhD students, working with GSK and AZ
Application deadline in June, start October 2018 - www.jobs.cam.ac.uk/job/17074

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Keerthy Kumar
Krishna Bulusu
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Lewis Mervin
Nitin Sharma
Ranjoo Choi
Richard Lewis
Samar Mahmoud
Salundi Basappa
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Sharif Siam
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