Mode of Action Analysis Using Chemical and Biological Data

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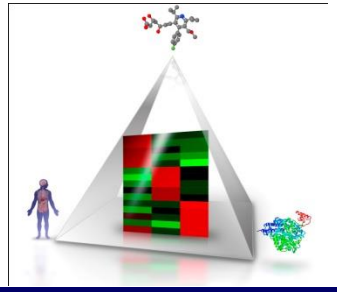
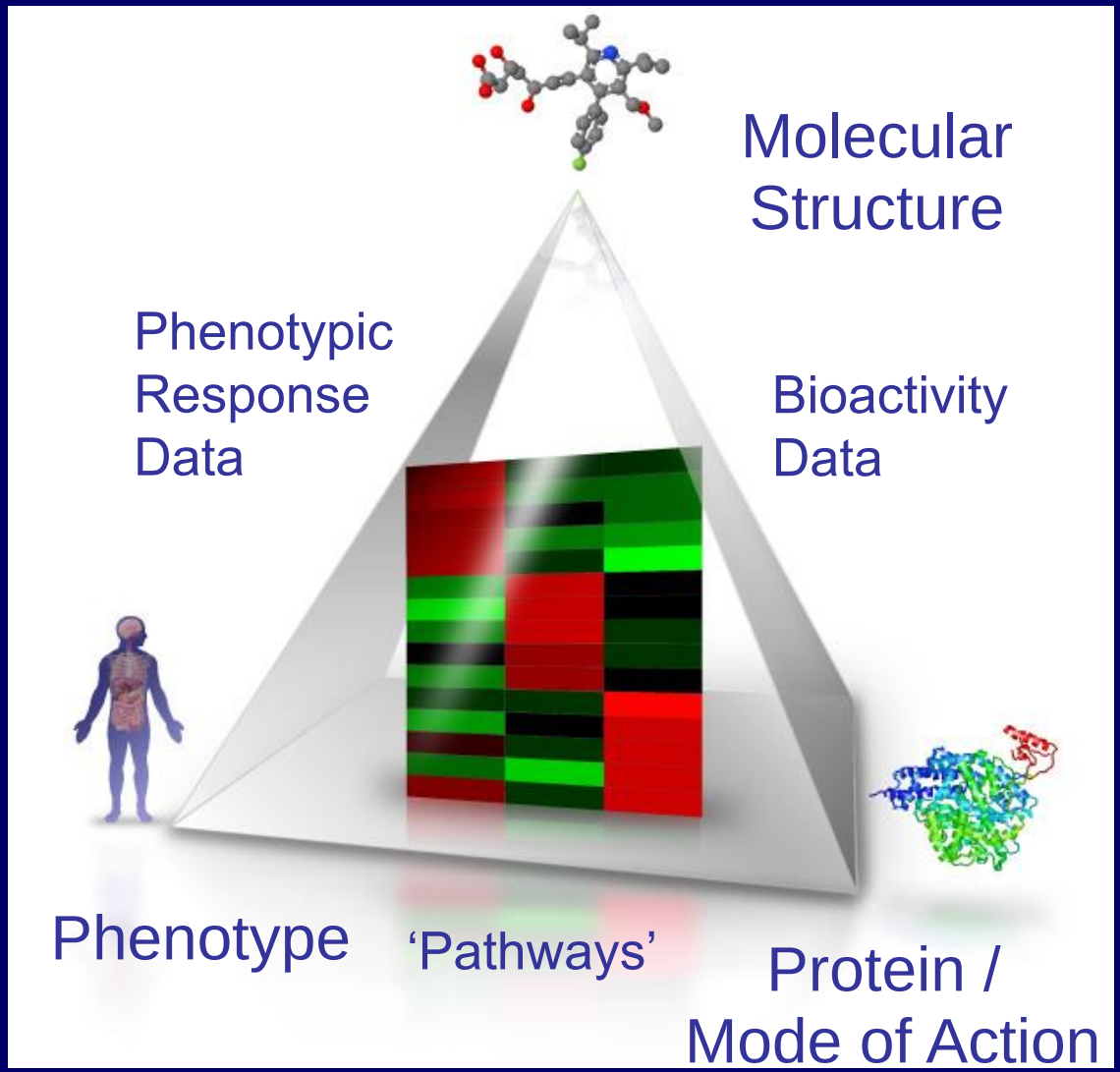


Outline

- Chemical and biological data
- Using *in* silico methods to understand modes of action, case studies
- The problem with ‘modes of action’
- Using understanding of MoA to go forward – synergistic compound selection

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



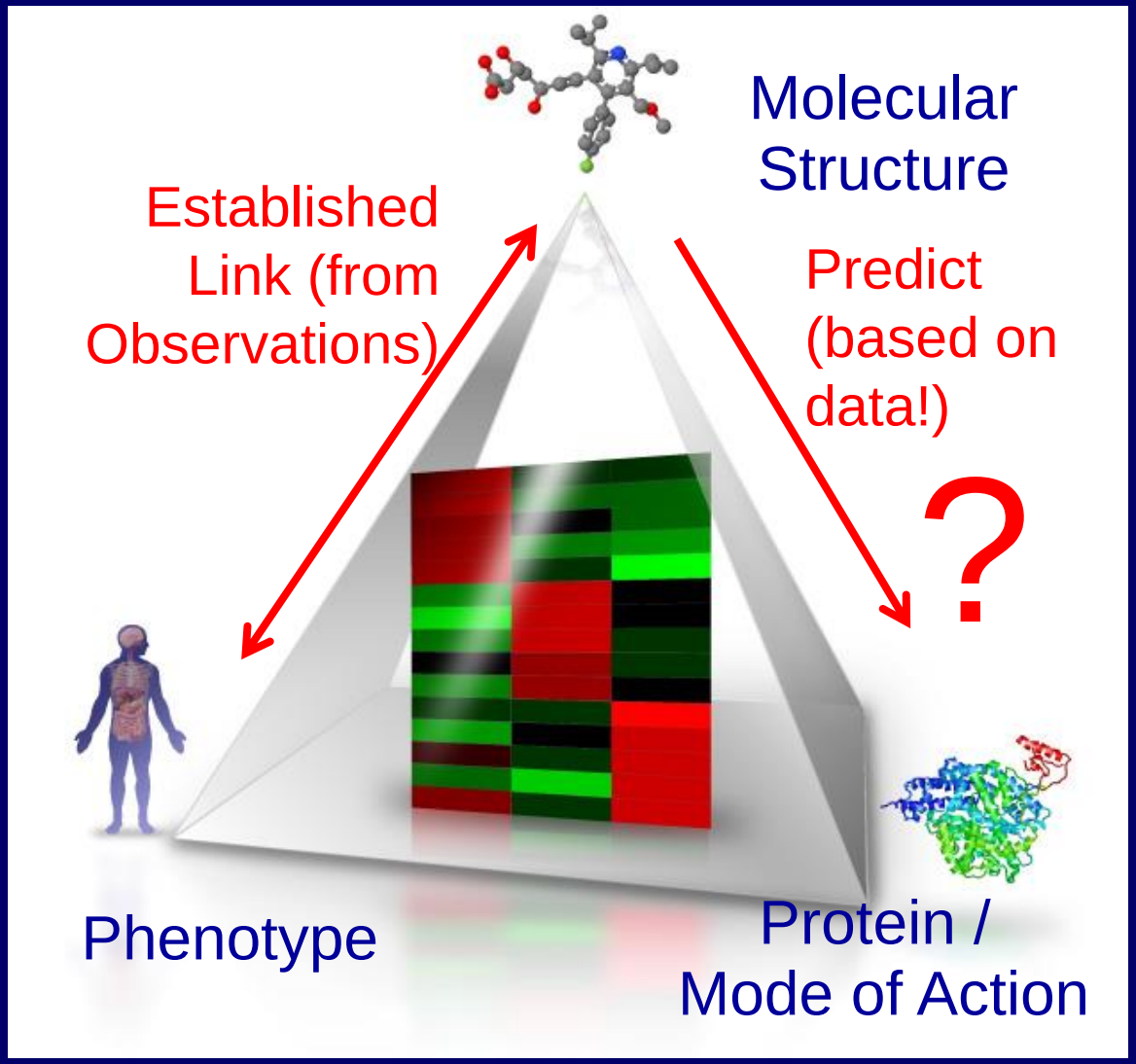
So what's the point of it all?
We would like to answer questions!

- "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*

BUT…This is a very simplified view…

- Links between drugs/targets/diseases are quantitative (and incompletely characterized)
- There are subtle differences in eg compound effects (partial agonists vs full agonists, off-targets, residence times, etc.)
- Effects are state-dependent (variation between individuals, … depends on even what you have eaten in the morning/absorption…), not captured in the data
- Data quality is often not sufficient (biology is inherently noisy; noise+species variation)
- …
- All of this makes assigning labels such as ‘active’, ‘toxic’ etc to compounds *very difficult!*

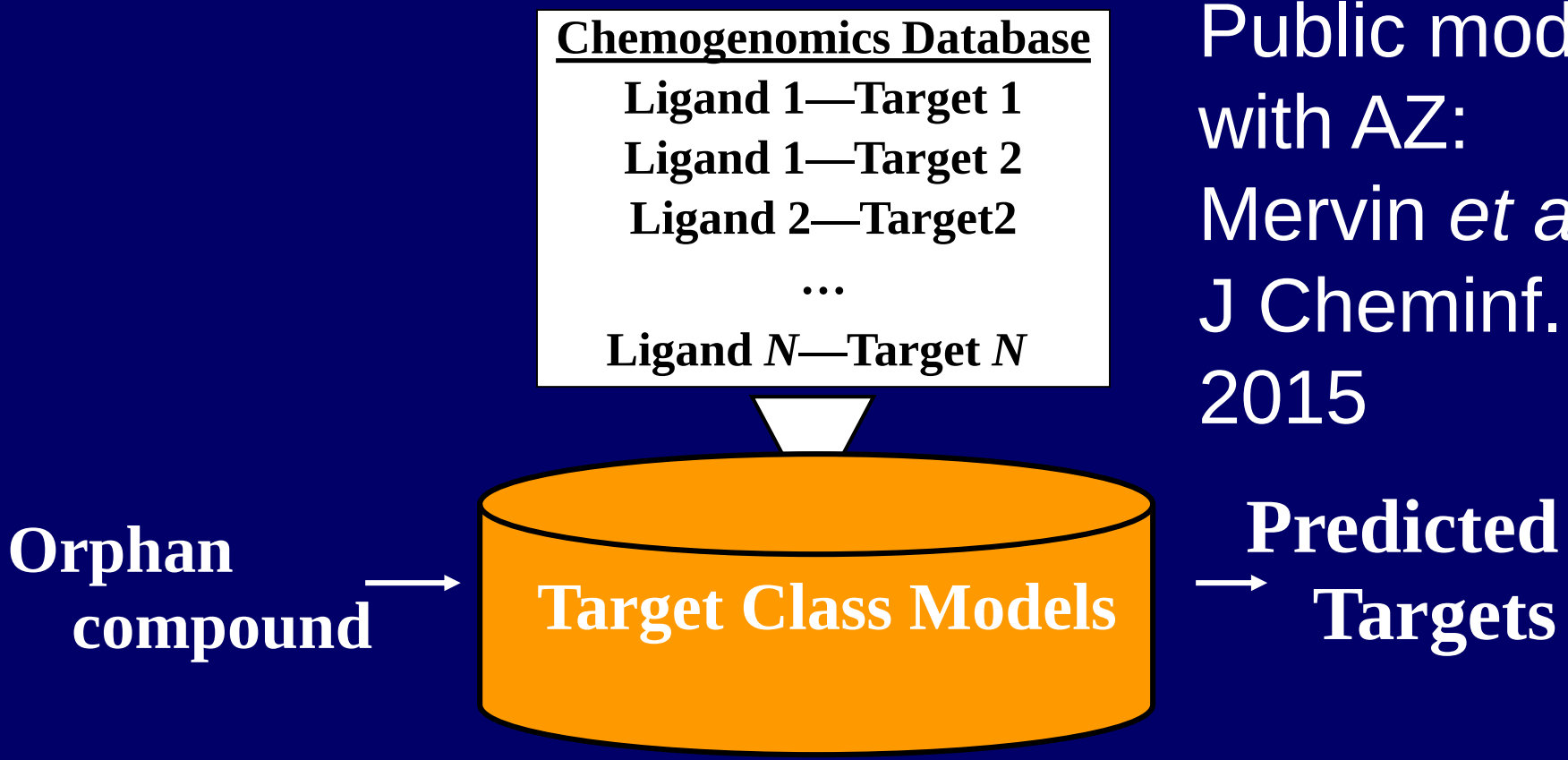
Starting from *in vivo* efficacy we can predict the MoA, based on ligand chemistry



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.



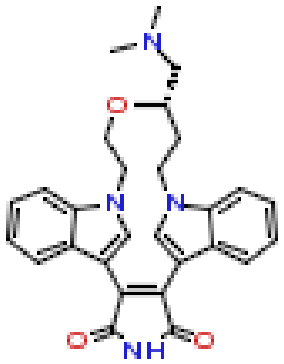
Public model with AZ: Mervin *et al.*. J Cheminf. 2015

Prediction Examples: Gleevec, Ruboxistaurin

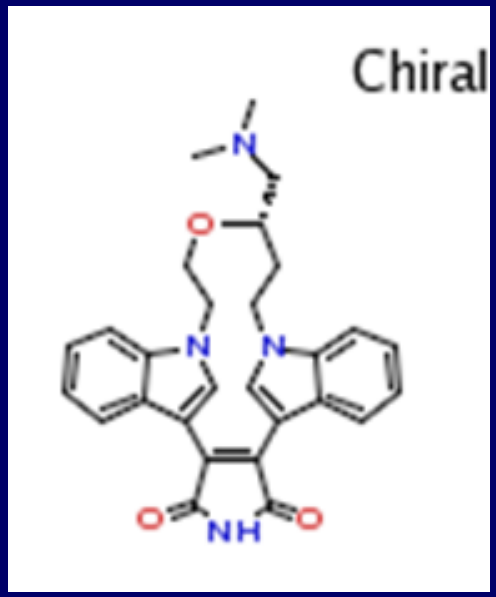
- Gleevec (Novartis),
  - Launched
  - Targets Bcr-Abl, c-kit, PDGFRb

- 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

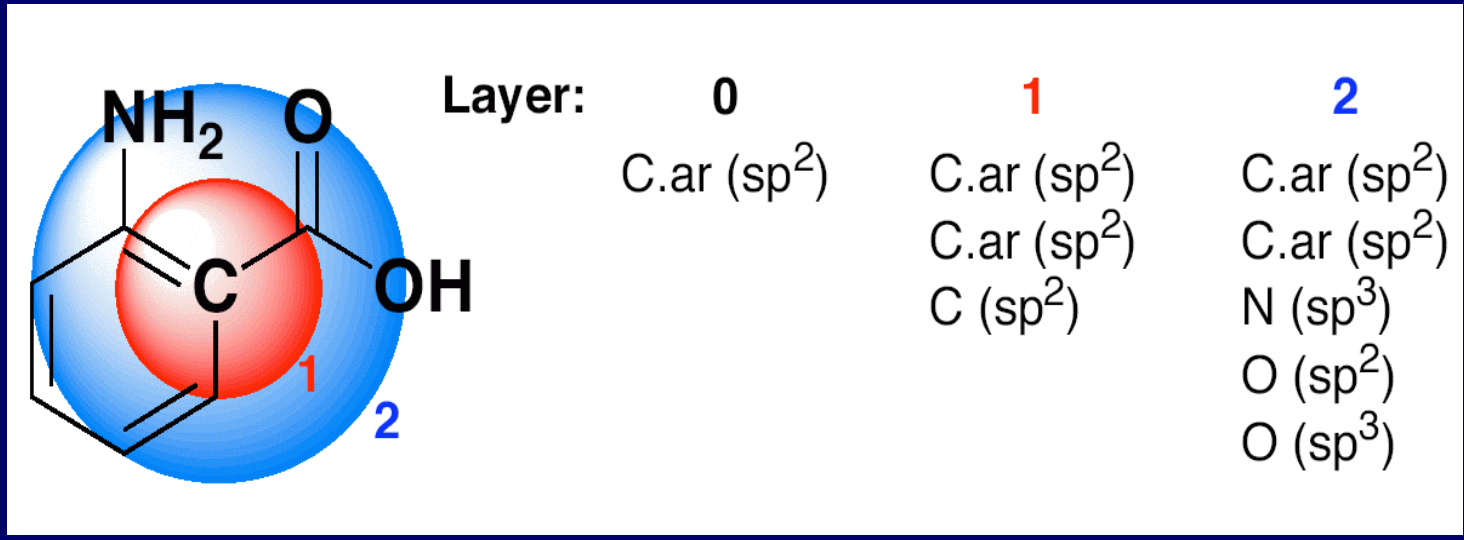
Problem of representation of chemical structure



- No ‘natural’ way of encoding molecules
- Graph-based descriptors are information-rich; however binding is mediated ‘via the *surface*’ of the molecule

- Too close to the connectivity matrix doesn’t generalize; too abstract not specific enough
- ‘Middle ground’ is needed
- In (many) retrospective studies circular fingerprints gave best performance

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



- Each fingerprint represents a *central atom and its neighbors*
- For each molecule, there are as many fingerprints as heavy atoms in the molecule

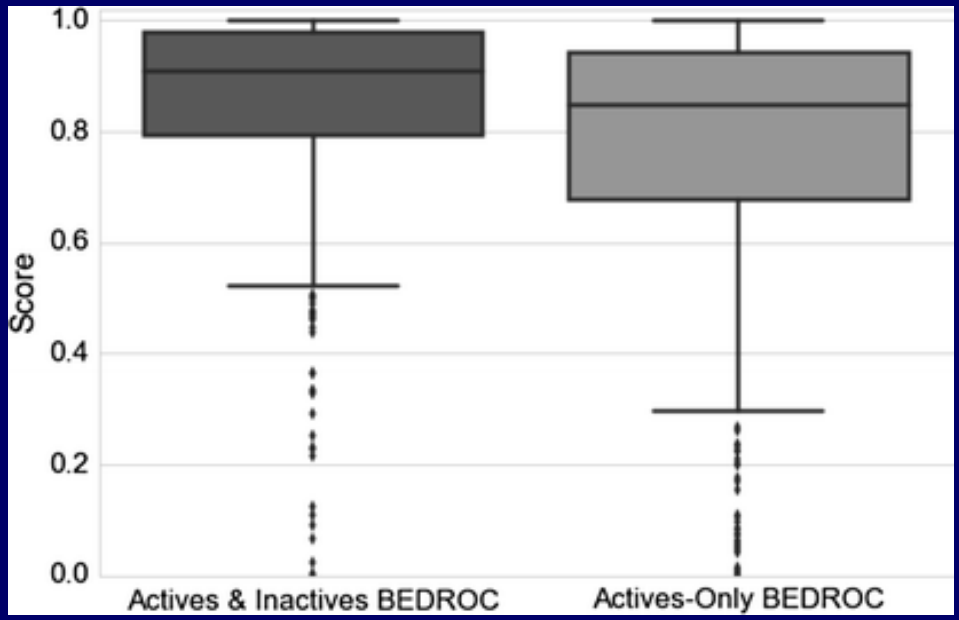
RC Glen, A Bender, CH Arnby, L Carlsson, S Boyer, J Smith
IDrugs 2006, **9**:199-206

Public target prediction model, based on ~200 mio data points

- Work of Lewis Mervin, with AstraZeneca
- 2015, *J. Cheminformatics* (7) 51
- ChEMBL actives (~300k), PubChem inactives (~200m)
- Can be retrained on in-house data
- 1,080 targets

- https://github.com/lhm30/PIDGIN

Also data is available to everyone!



Training MoA models using in-house SAR data

ChemConnect

- Orthologs with 85% sequence similarity from Homologene
- Retain targets with 10 or more active data points
- **9,570,000** actives
- **2,882** Targets

AZ HTS Datamart

- 420 HTS screens
- 343 Targets
- **189,500,000** inactives

PubChem

- 300,000+ screens
- 2,116 Targets
- Annotated inactives from HTS screens
- **420,000,000 +** inactives

AZ Data and PubChem data combined:

- **603,000,000 inactive data points**
- **2,161 Targets**

Functional target prediction

- Compounds do not only have a ‘class label’ against a protein
- Modulating a protein can have multiple effects (say, in the simplest case, activating and inactivating/inhibiting effects)
- Needed to map activity types to binary activating/inhibiting labels
- Complicates classification even further – now we have 500-5,000 classes, and two subtypes each!

Problem: Biased data

Typical data looks as follows:

- ~ 500-5,000 classes
- ~ 20-10,000 *actives* per class
- ~ 1,000-1,000,000 *inactives* per class
- ~ 1-100 classes per compound (instance)
- Some classes are diverse, some are not

- No reliable way to estimate underlying distributions ('background chemical space'), or priors for classes ('how much' of chemical space belongs to one class)

- Problem: Estimating class-membership across this type of biased data

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

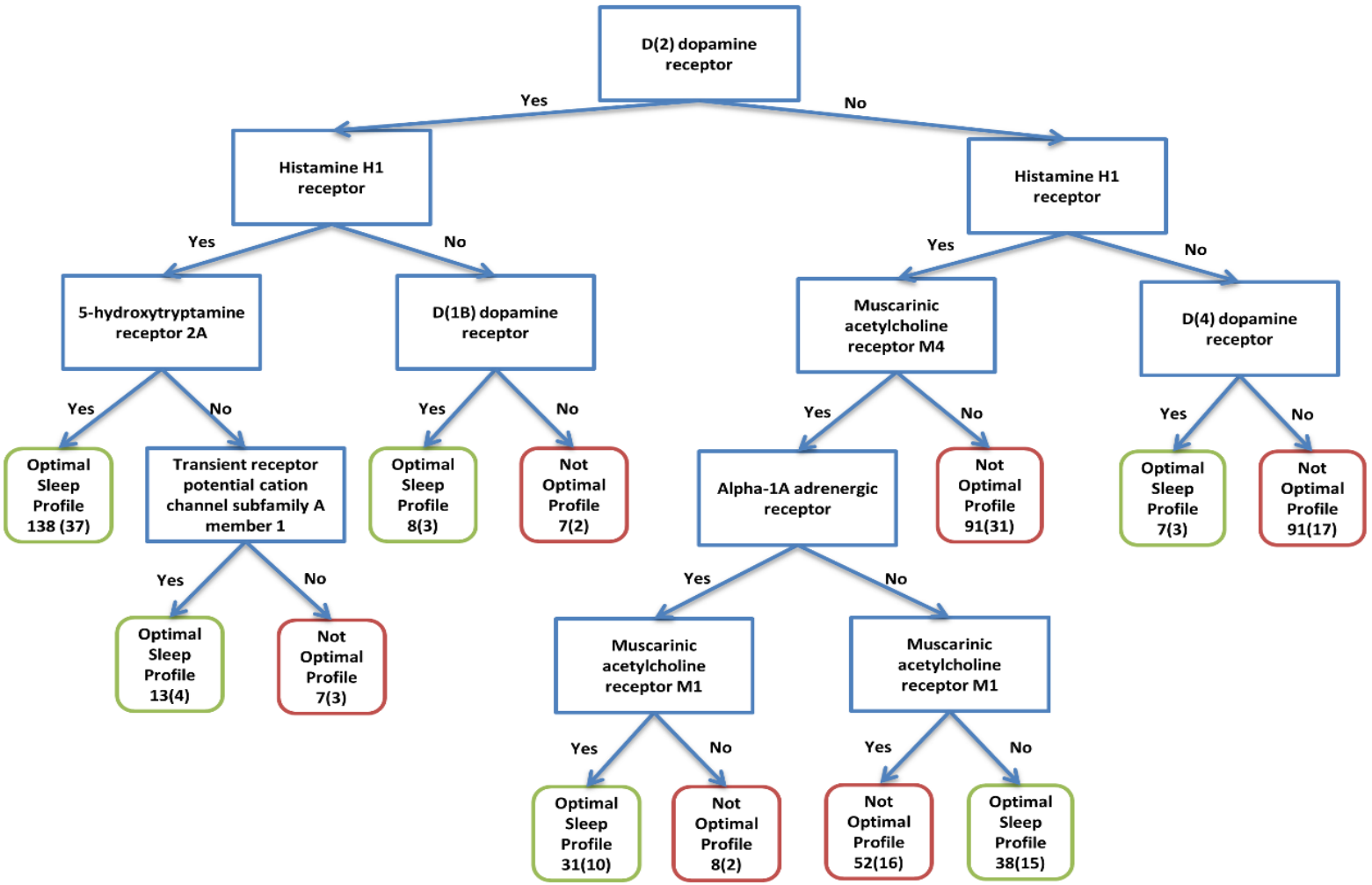
Compounds classified, followed by pattern discovery in target space

- Efficacy and side-effect readouts used to define ‘good’ and ‘bad’ compound class

- Target prediction, say:
- ‘Good’ compound targets: ABC, ABD, ABE
- ‘Bad’ compound targets: ACE, BCD, BCE

- Decision trees for pattern discovery: Here targets ‘AB’ are associated with efficacy, and tolerable side effects

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

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

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

- Overall 78% correct on target level, ~71% on phenotypic level (‘positive predictive value’)

What did we learn?

- We went *in silico* from single targets to multiple targets, and multiple hypotheses, in mode of action analysis

- Able to *understand* (hypothesize) modes of action, *and select* new compounds

- Missing: Functional effects, quantitative activities (to some extent in new versions of *in silico* models), any *in vivo* (PK/PD) properties, etc.

Application: Understanding and predicting cytotoxicity in screening HTS collections

Work of Lewis Mervin, with AstraZeneca (MoIndal/Cambridge)

ACS chemical biology

Articles

pubs.acs.org/acschemicalbiology

Understanding Cytotoxicity and Cytostaticity in a High-Throughput Screening Collection

Lewis H. Mervin,[†] Qing Cao,[‡] Ian P. Barrett,[§] Mike A. Firth,[§] David Murray,[∥] Lisa McWilliams,[∥] Malcolm Haddrick,[∥] Mark Wigglesworth,[∥] Ola Engkvist,[⊥] and Andreas Bender*[,†]

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[‡]Discovery Sciences, AstraZeneca R&D, Waltham, United States

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[⊥]Discovery Sciences, AstraZeneca R&D, Mölndal, Sweden

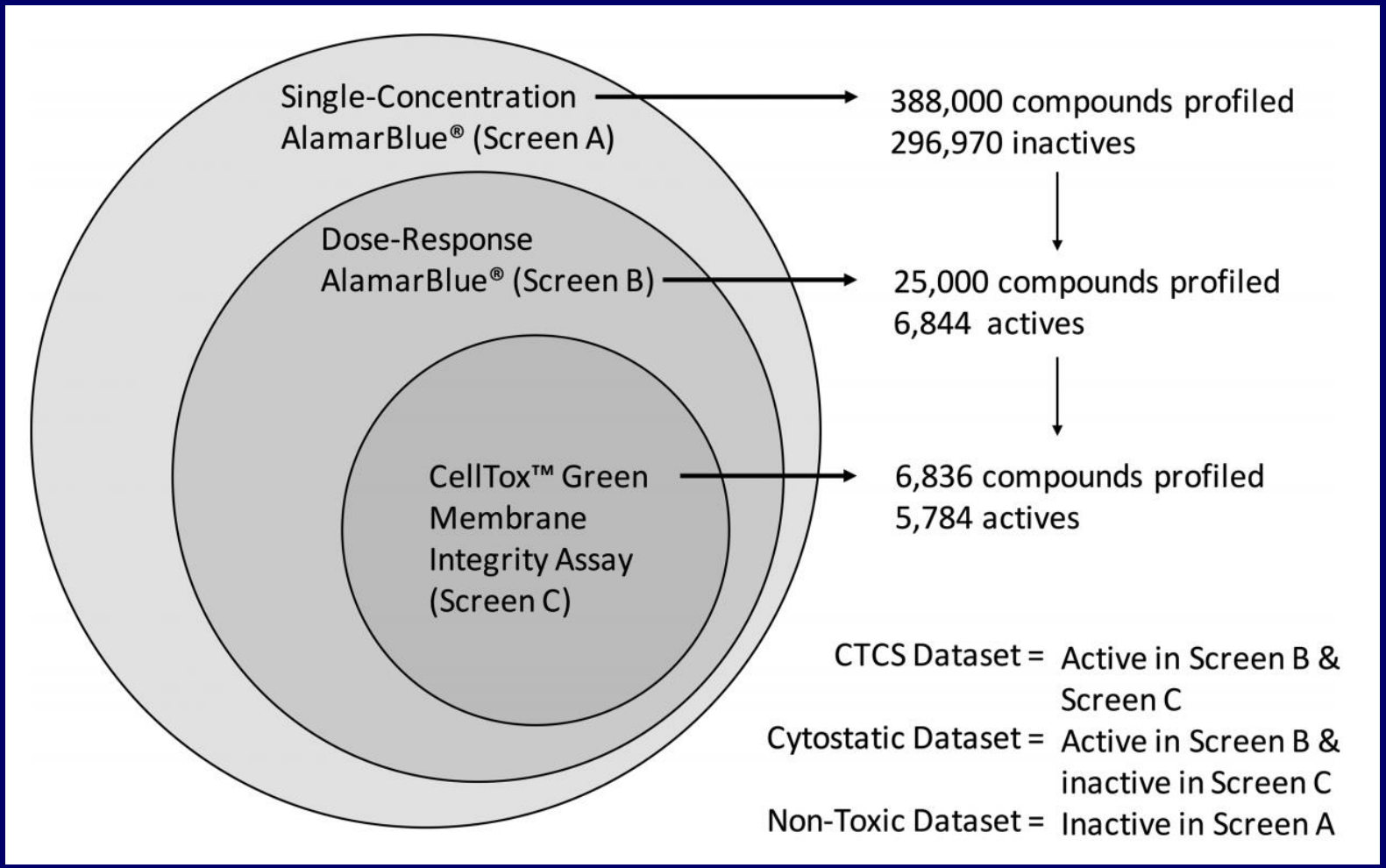
Cytotoxicity in compound sets

- Even low level cytotoxicity is linked to adverse events in man, and is hence often undesired (…where not explicitly desired)

- Aims of project three-fold:
  - Predict cytotoxicity of new compounds
  - Gain *chemical* insight into cytotoxic substructures
  - Gain *biological* insight into cytotoxicity-related mechanisms activated by small molecules

Predicting and understanding cytotoxicity of compound libraries

300k compounds profiled with AstraZeneca for cytotoxicity (in dose response)



Chemical fragments, targets can be used for predictions, interpretation of cytotoxicity

Table 7. The Top 10 Enriched Cytotoxic Fragments with Low Kinase Prediction Rates.

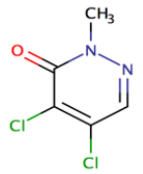
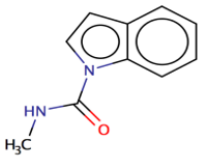
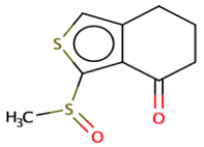
Fragment	CTCS Count	CTCS %	Non-Toxic Count	Non-Toxic %	Frag. Ratio	Binomial P-Value	1st Predicted Class
	16	0.28	1	0.00	0.00	5.00E-27	Other : 15
	7	0.12					
	6	0.10					

Table 2. Top Enriched Targets for Cytotoxic Compounds versus Non-Toxic Compounds

Prediction Ratio and accompanying Fisher Test p-values. Results illustrate a high frequency of h
demonstrating the importance of normalizing predictions to reduce biases in the chemical ar
sampling bias). The top enriched targets show links to cell-death in literature, show a mix of ap
targets in Table 1. Fisher test p-values of "0.00E+00" indicate scores that are less than the sma

EGID	Name	Classification	CTCS Hit Rate (%)	Prediction Ratio	Fisher Exact Test P-Value	
SNRK	SNF related kinase	Kinase	2.44	0.01	2.58E-176	Up-regulated i apoptosis. Re
CDK13	Cyclin-Dependent Kinase 13	Kinase	1.43	0.02	6.13E-95	Expressio
DSTYK	Dual Serine/Threonine And Tyrosine Protein Kinase	Kinase	6.29	0.02	0.00E+00	Overexpres
MAK	Male Germ Cell-Associated Kinase	Kinase	6.14	0.02	0.00E+00	Role in mitotic c
MAP3K6	Mitogen-Activated Protein Kinase Kinase Kinase 6	Kinase	7.12	0.02	0.00E+00	Involved in p arrest, t
STK32A	Serine/Threonine Kinase 32A	Kinase	1.69	0.02	3.99E-109	Implicated i
CDKL3	Cyclin-dependent kinase-like 3	Kinase	4.91	0.02	4.48e-314	RNAi shows
IFNG	Interferon, Gamma	Cytokine	1.95	0.02	3.53E-124	Indu

- The problem with ‘modes of action’

“Mode of action”… words easily said, not so easily verified

- Need to show achievement of effect, via proposed ‘mechanism’
- Involves e.g. target engagement *in vivo*; ruling out other ‘routes’ of activity

- MoA has different levels – target, gene level, protein level, protein activity level, …
- Operating on eg target level ‘simplifies’ problem, but possibly also oversimplifies it

- Q: *What is the desired activity of a small molecule that inverts the disease state (to ‘healthy’)?*

Investigating links between indications and neurotransmitter level changes

- Frequent working hypotheses of CNS active drugs: We aim for particular activity on the *target level* and/or the *biomarker level* (eg here neurotransmitter/brain area level)
- Hoped to be linked to efficacy *in vivo*

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

- So let’s look at the data…

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

- Hypothesis: “A CNS-active drug of a certain type works by modulating neurotransmitters (specific neurotransmitter(s), specific region(s))”

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

***Neurochemical Fingerprints of Psychiatric Drugs.**
Hamid R. Noori (MPI Tuebingen), 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 revision) **www.syphad.org (publicly, freely accessible)**

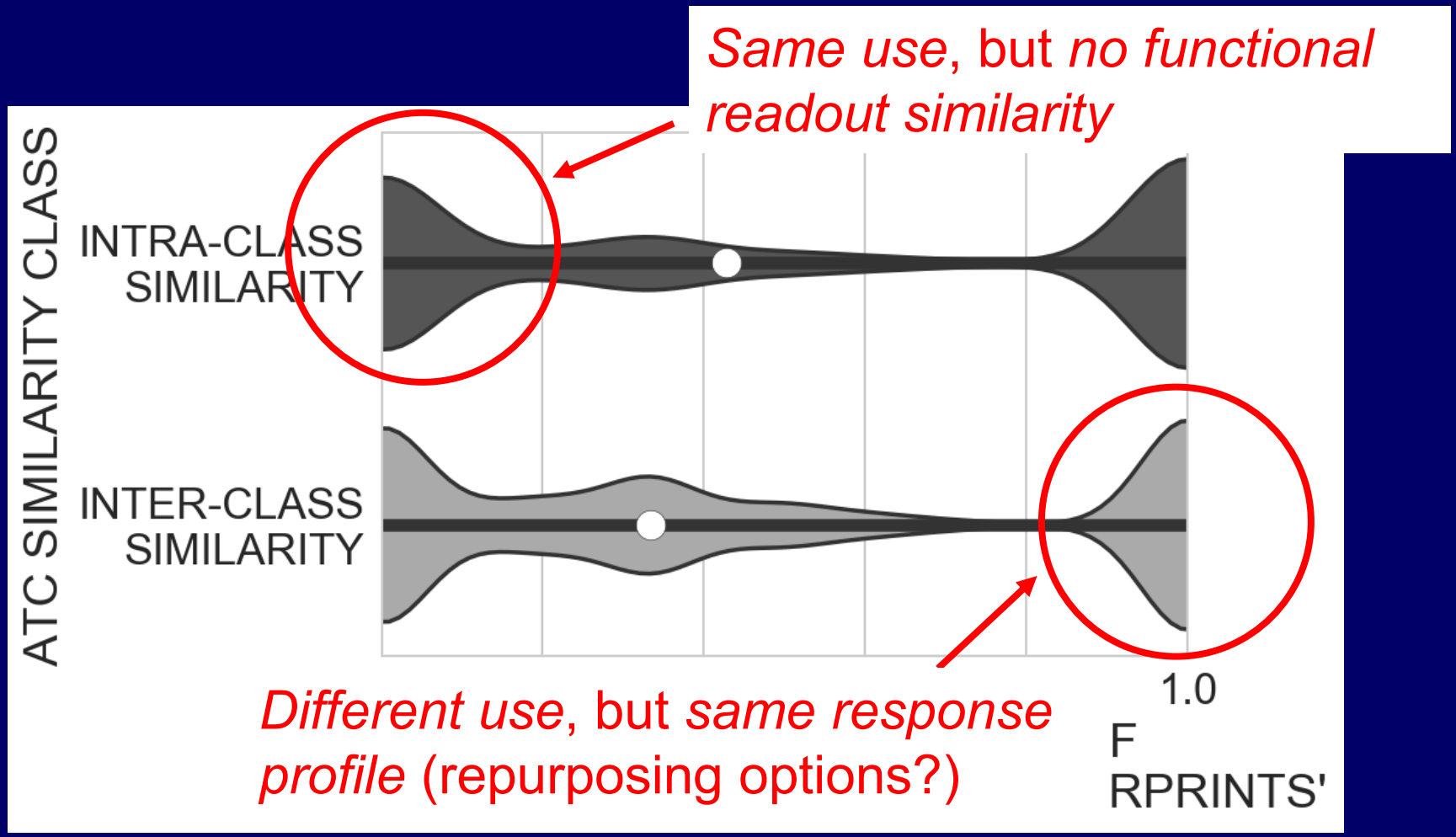
So what do antidepressants, antipsychotics,… have in common?

- You would *assume* that diseases, and hence treatments (via their ‘mode of action’), are in some way ‘defined’

- How consistent are changes to neurotransmitter levels, *within* and *between* drug classes?

- 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… how should we define the mode of action of a CNS-active compound?

- Not really defined on neurotransmitter (so likely also not protein) level

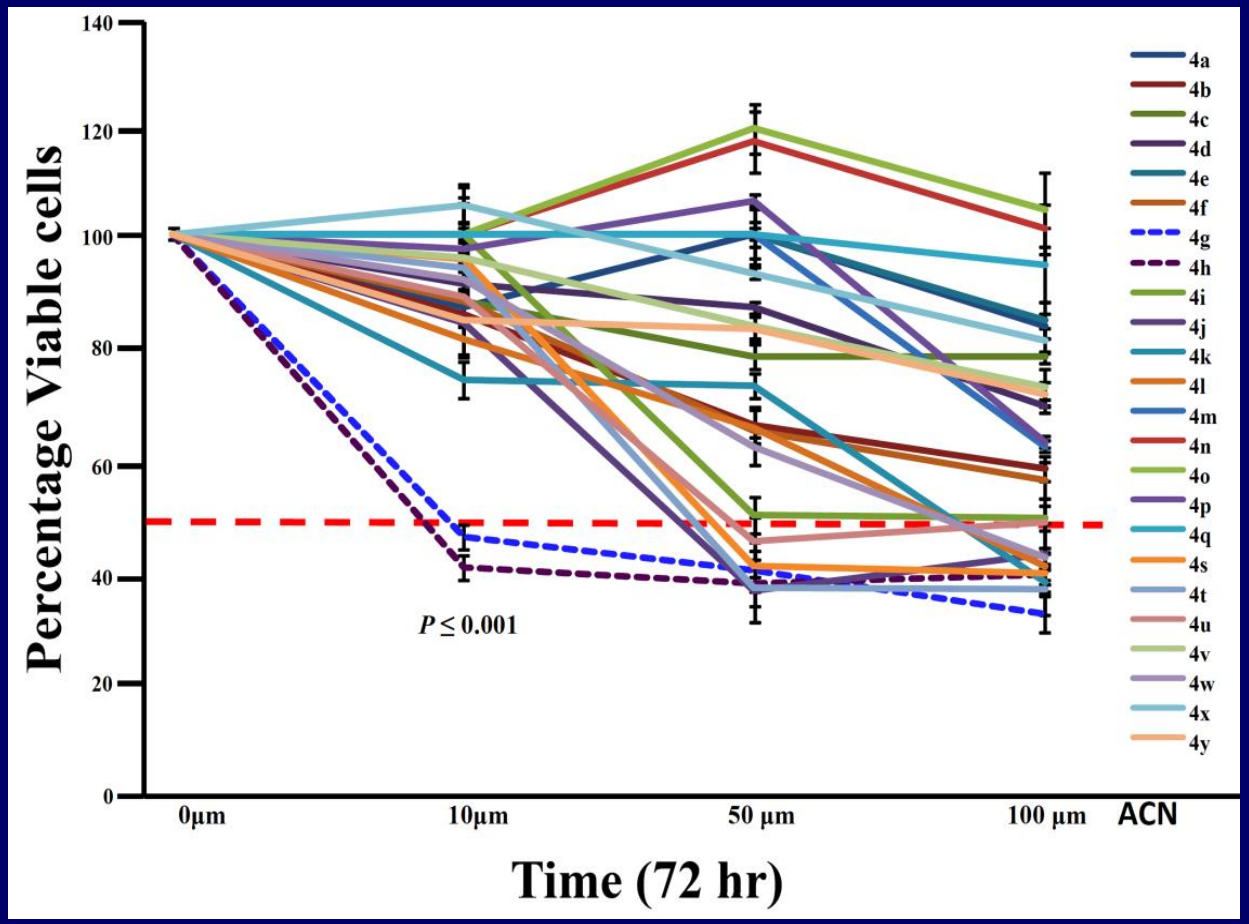
- Using *protein targets* to explain mode of action/ design compounds probably only ‘really’ works in narrowly defined cases (eg infectious diseases, activation of kinases/enzyms, …)

- Using biological readouts is likely better, *but*… they need to be mechanistically related to disease
- Poses problems when developing a design MoA hypothesis – what do we need to target, and how?

- Time and spatially resolved data *might* help

Novel 2-Amino-Chromene-Nitrile that Targets Bcl-2 in Acute Myeloid Leukemia (AML)

Work with Dr Basappa's and Prof Rangappa's Groups and Philip Koeffler; first authors are Keerthy, Manoj Garg



Screening of active compounds affecting the proliferation of HL-60 cells from a library of chromene derivatives

In Silico Target Predictions Suggest Bcl-2 as a Protein Targeted by this Compound

2-Amino-Chromene-Nitrile

Chemogenomics Database

Ligand 1—Target 1

Ligand 1—Target 2

Ligand 2—Target2

…

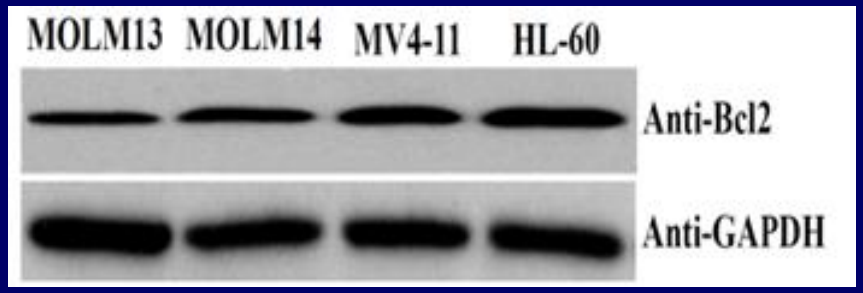
Ligand *N*—Target *N*

Target Class Models

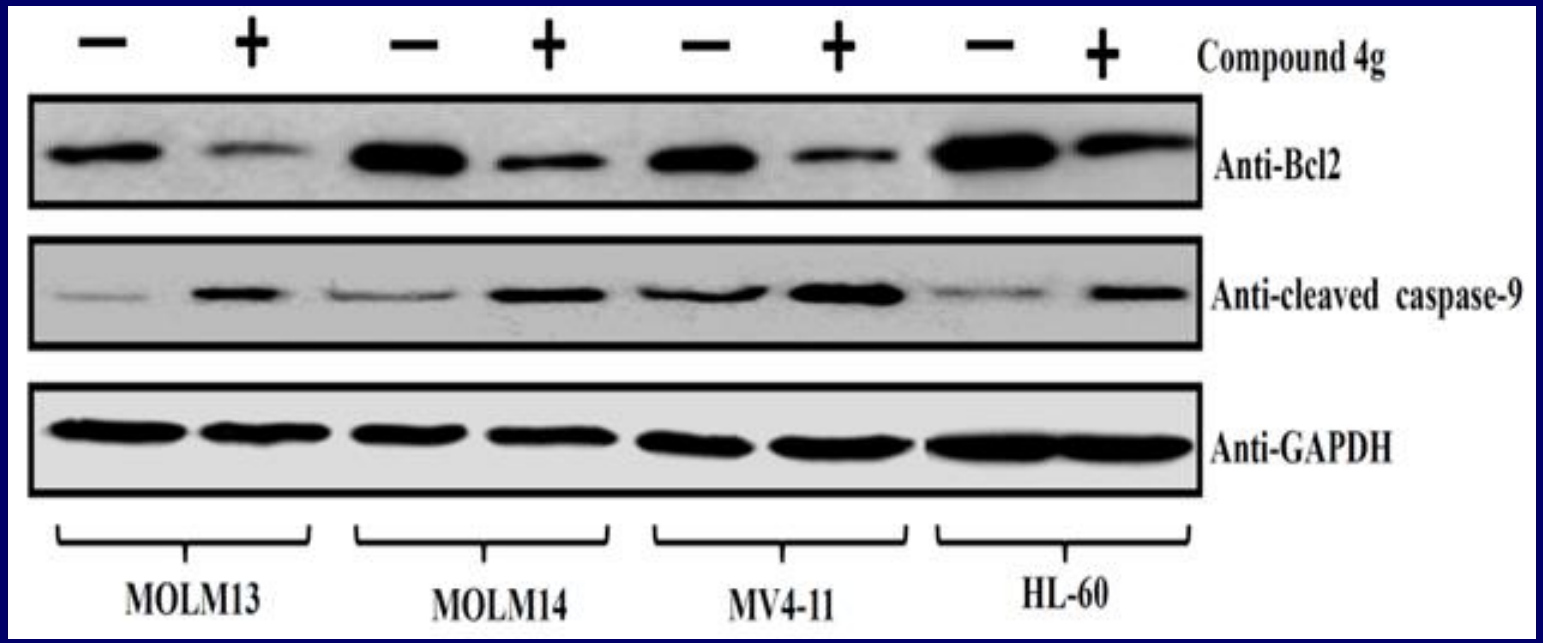
Predicted Targets: Bcl-2 as top target

Note: In some cases – such as here – the predicted target is not necessarily the direct target, often they turn out to be indirectly targeted!

Compound 4g Decreases Expression of Bcl-2 And Increases Levels of Activated, Cleaved Caspase-9 in Human AML Cell Lines

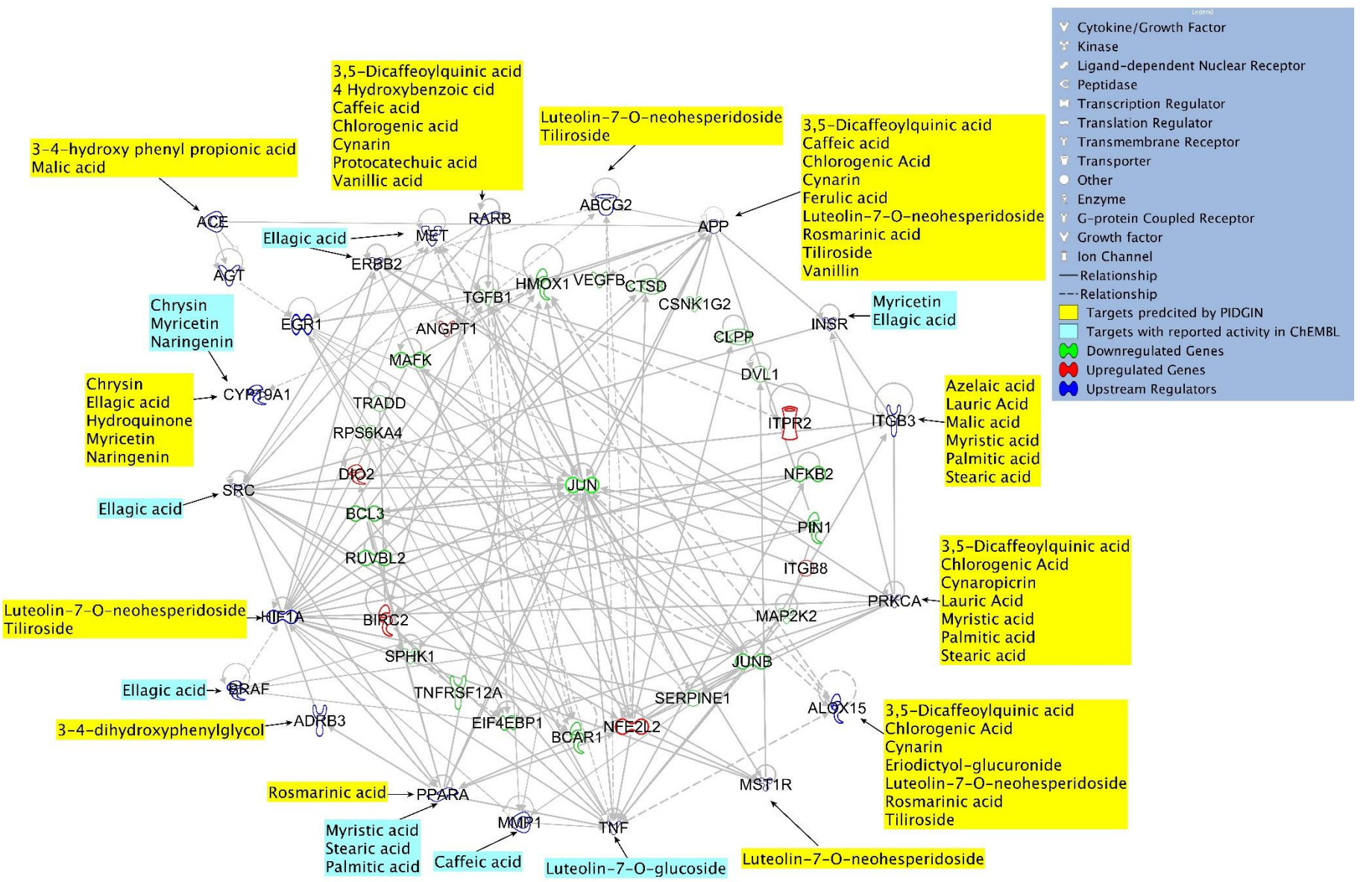


MOLM13, MOLM14, MV4-11 and HL-60 all expressed anti-apoptotic Bcl-2 as determined by Western Blotting



Treatment with compound 4g decreased bcl-2 expression and increased levels of activated, cleaved Caspase-9

Integrated chemical and biological view on compound action..??



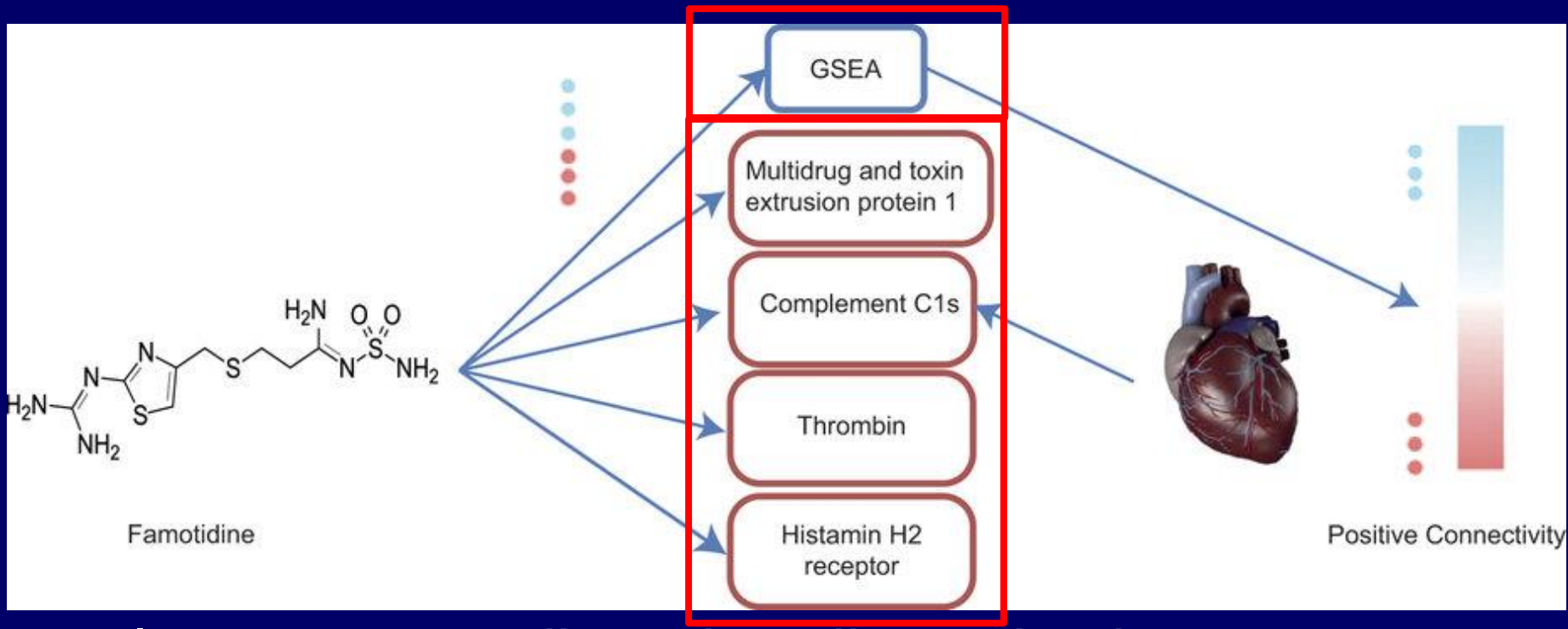
- Using gene expression data to understand modes of action, and explain/select synergistic compound combinations

Note on chemical and biological data

- In *chemistry*
  - We can (generally) characterize the system (compound) reasonably well
  - Chemical space is large (say, 10^63 molecules?)
  - Compounds exist in different forms (conformations, etc.).
- *Biology*
  - Operates on ‘different levels’ (spatial, time, context such as cell type and state, etc.)
  - Space is smaller (say 200k proteins?) but highly connected, conditional (different cells, states of a cell/protein, etc.)
  - We (generally) don’t know what the readouts (genes, imaging readouts, ..) mean, where the signal is
  - Technology development & relevance of data don’t always go hand in hand (‘technology push’ not always helpful…)

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

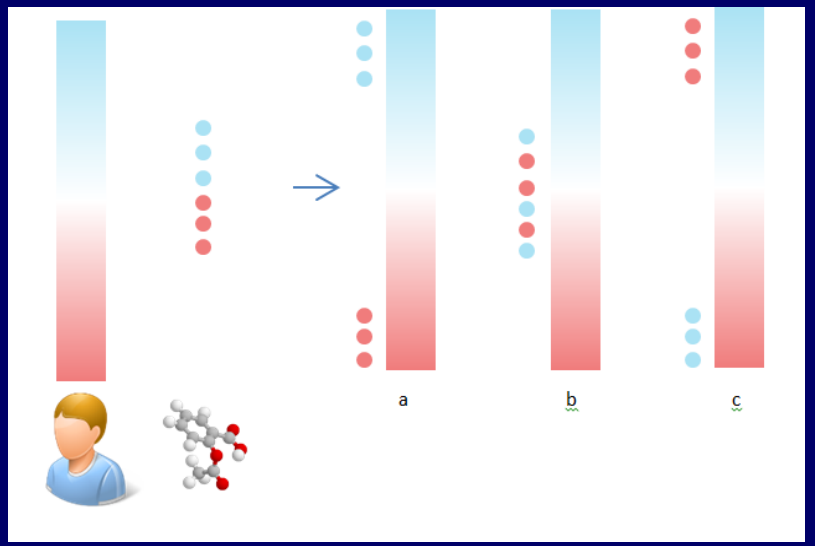
- Select compounds based *both* on gene expression and target prediction *profiles*



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

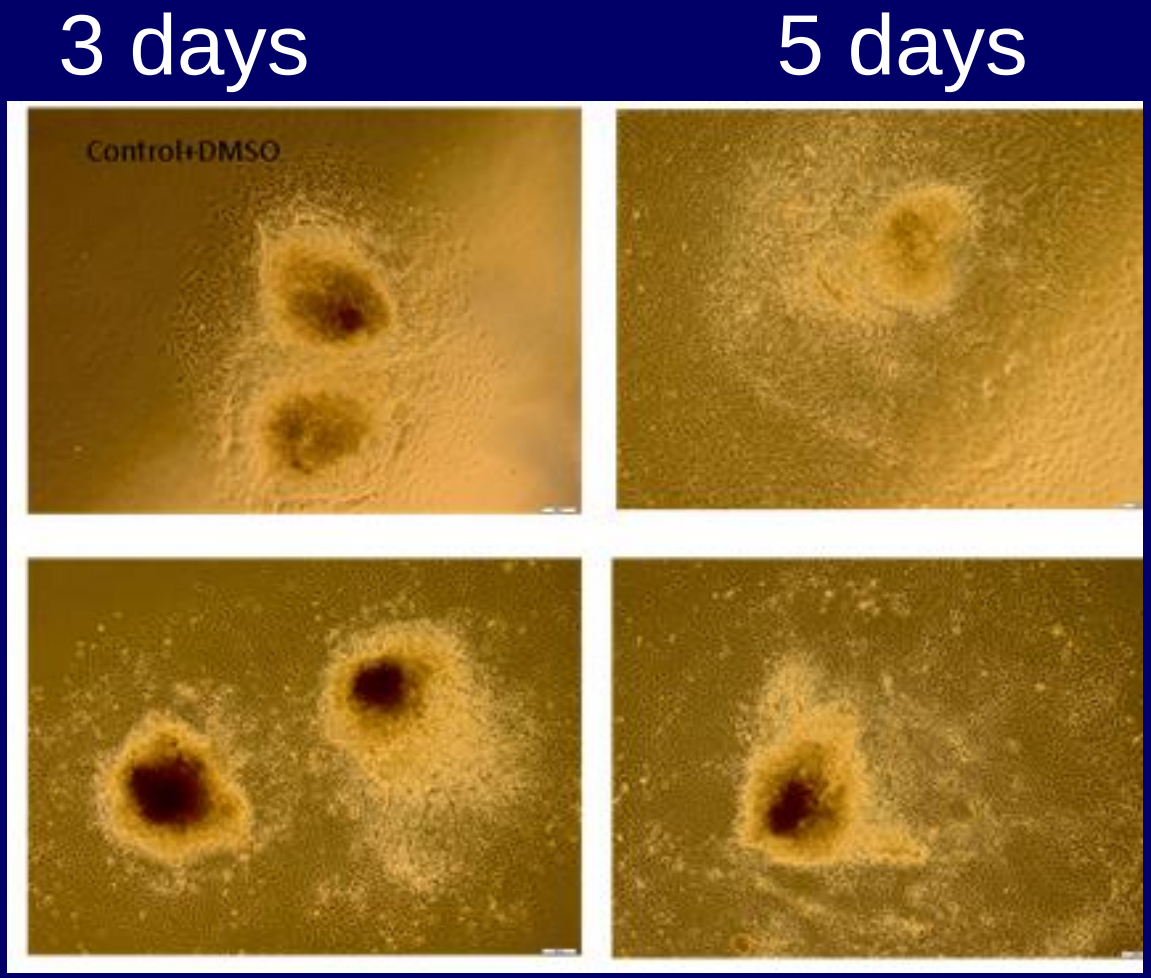
- ConnectivityMap (1,300 compounds to Affymetrix chips)
- LINCS (12,000 compounds to 1,000-gene expression signatures)

- *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)



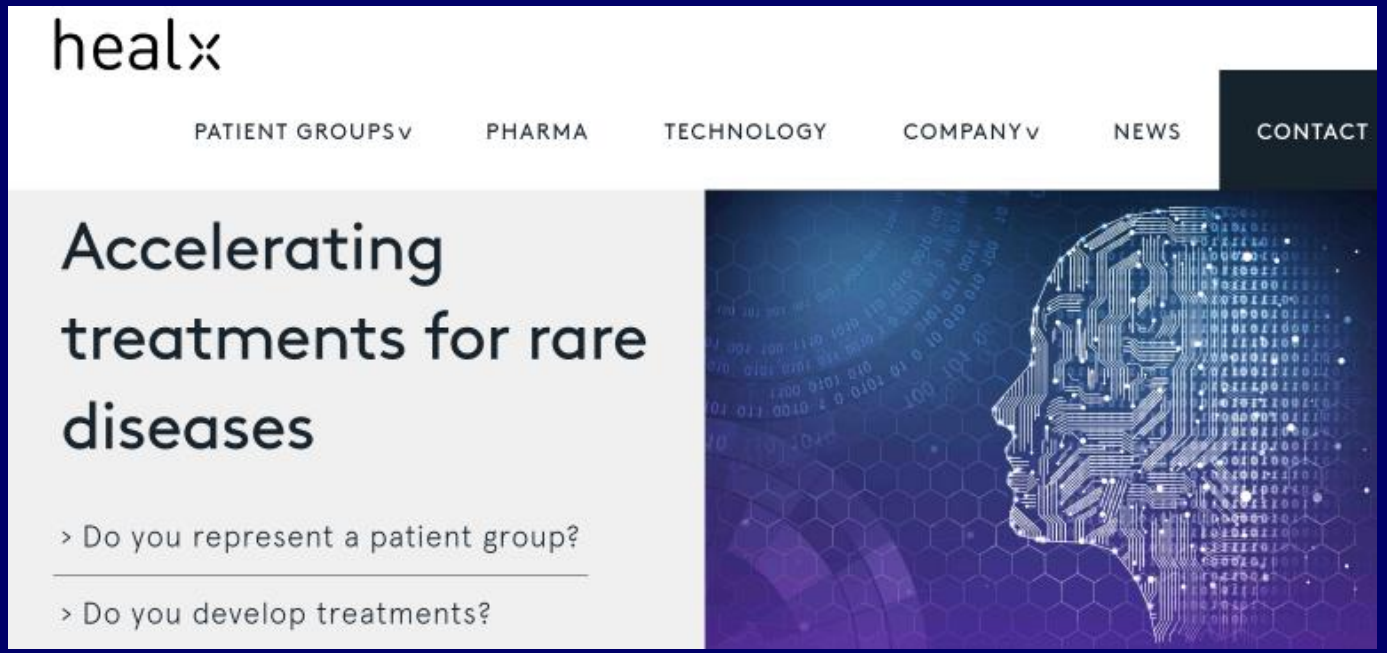
KalantarMotamedi *et al. Cell Death Discovery* **2016**

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; 4yrs old; ~35 people

July 2018 Series A funding
$10m, led by Balderton Capital



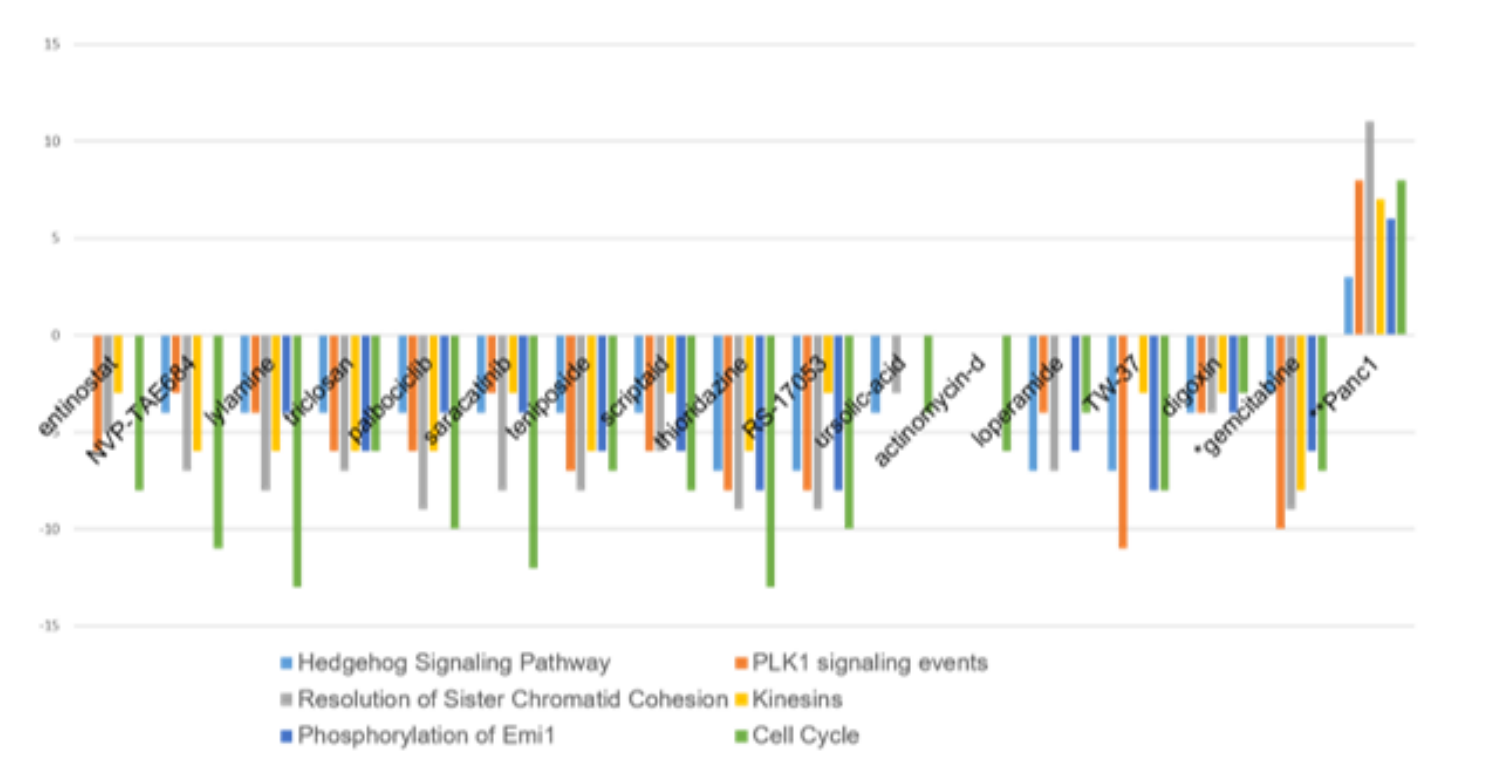
Identifying synergistic combinations with Gemcitabine in Pancreatic cancer

- Pancreatic cancer difficult to treat (chemotherapy; targeted treatments erlotinib, larotrectinib, not many other options)
- Gemcitabine frequently used, but efficacy relatively low
- Looking for synergistic combinations

- How? Correlation, anticorrelation, particular pathways, …
- “Desired combination on pathways level – keeping desired anticorrelation part of activity, finding second drug that increases overall anticorrelation with disease signature”

Criteria for selecting combinations

- Score for (a) reversing *undesired* anticorrelation with disease signature, and (b) taking (resistant) Panc-1-specific differentially expressed genes into account (Panc1 vs BXPC3, Mia Paca-2, HPAFII and HS766T)

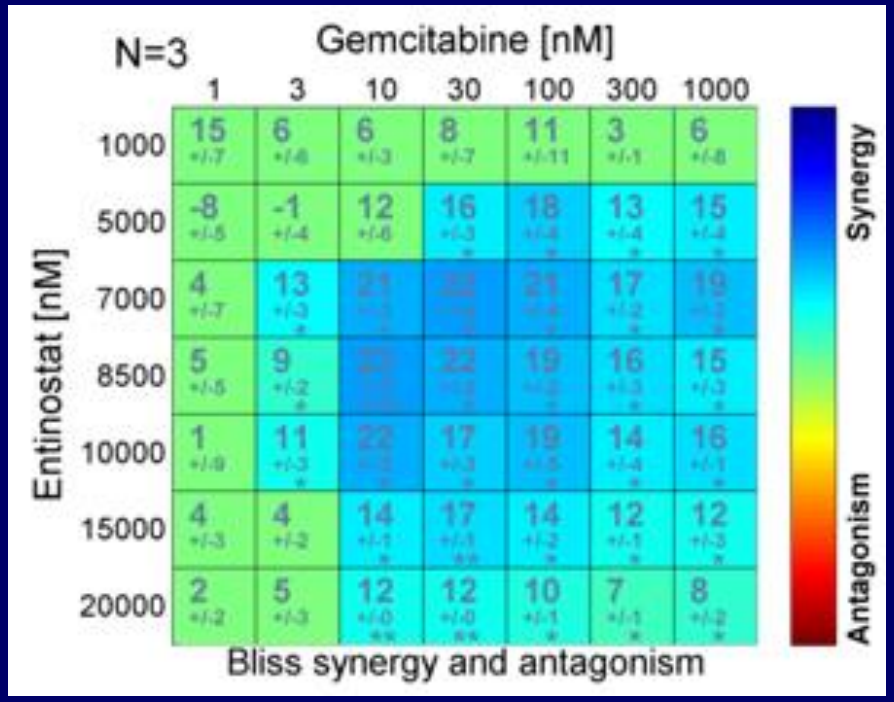


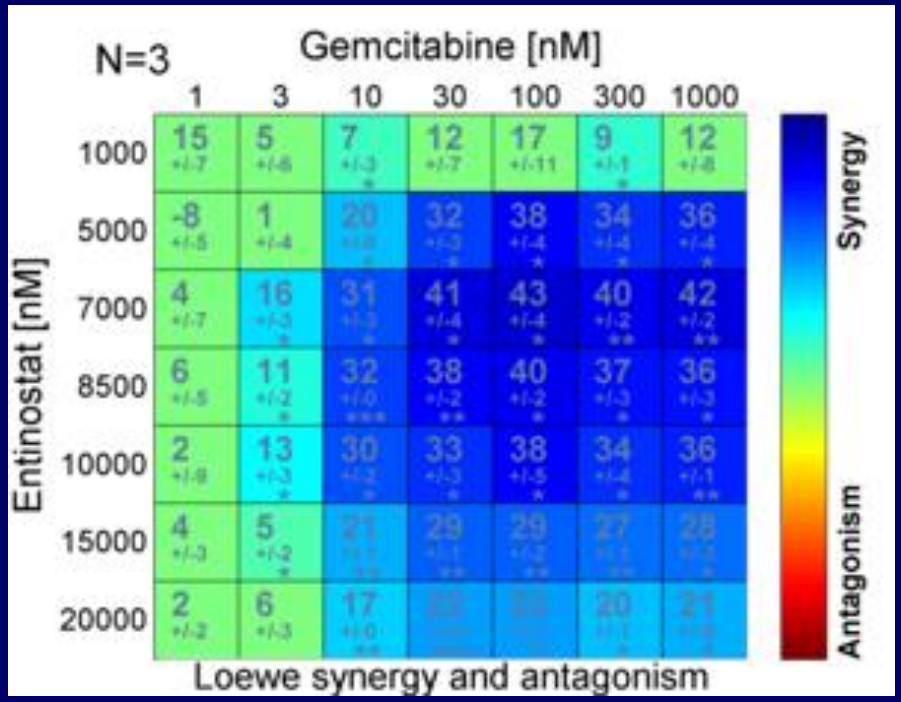
LINCS dataset for selection of compounds selective for Panc1 vs epithelial cells

- Gene expression from Panc-1, BXPC3, Mia Paca-2, HPAFII and HS766T cells as signal, selective over human pancreatic ductal epithelial cells
- 20,413 compounds applied to 77 different cell lines including 59 cancer and 10 primary cell lines with eight other cell lines compared to gene expression
- No Panc-1 in LINCS, assumed/hoped MCF-7 differential gene expression extrapolates to Panc-1
- Pathway-based signature matching of disease and compound space

Prospective validation – 9/30 combinations synergistic

- 30 compound combination prospectively tested
- 9 out of 30 compounds showed synergy (according to SUM_SYN_WEIGHTED metric in the Combenefit software using Bliss and Loewe synergy definitions)





Conclusions from pancreatic cancer part

- Gemcitabine+entinostat dose reduction index/DRI_{50} = 43, compared to gemcitabine+trichostatin-A DRI_{50}=3
- Despite Trichostatine HDAC1 IC_{50} of 20nM, entinostat IC_{50} of 510nM, so other factors in addition to HDAC inhibition possible relevant

- **LINCS-derived Hypothesis (untested!): "Entinostat transcriptional profile in LINCS reverses undesired effect of gemcitabine on chromosome maintenance pathway by down-regulating BRCA1, RFC5, LIG1, POLE2 and PCNA. Only PCNA and POLE2 are down regulated in gene signature profile of Trichostatine-A as well"**

- Combination changes mechanism over gemcitabine treatment alone

Understanding synergy in Shexiang Baoxin Pill (SBP)

- SBP is treatment for cardiovascular diseases from Traditional Chinese Medicine; 7 Materia Medica, 22 compounds detected in blood plasma – *how do they interact pairwise?*
- Modelled based on predicted targets, *network information*

- Work of Siti Zobir, Ranjoo Choi, Tai-Ping Fan, Dezso Modos (Cambridge)

Shexiang Baoxin Pill (SBP)

- SheXiang BaoXin Pill (SBP) is a widely-used Chinese prescription medicine for the treatment of cardiovascular diseases in China.
- Comprises seven materia medica, with “aromatic herbs activating yang, benefiting vital energy and strengthening the heart for treating angina and myocardial infarction caused by ischemia.”
- **MOAs of SBP involves neovascularization through promoting angiogenesis in the heart**

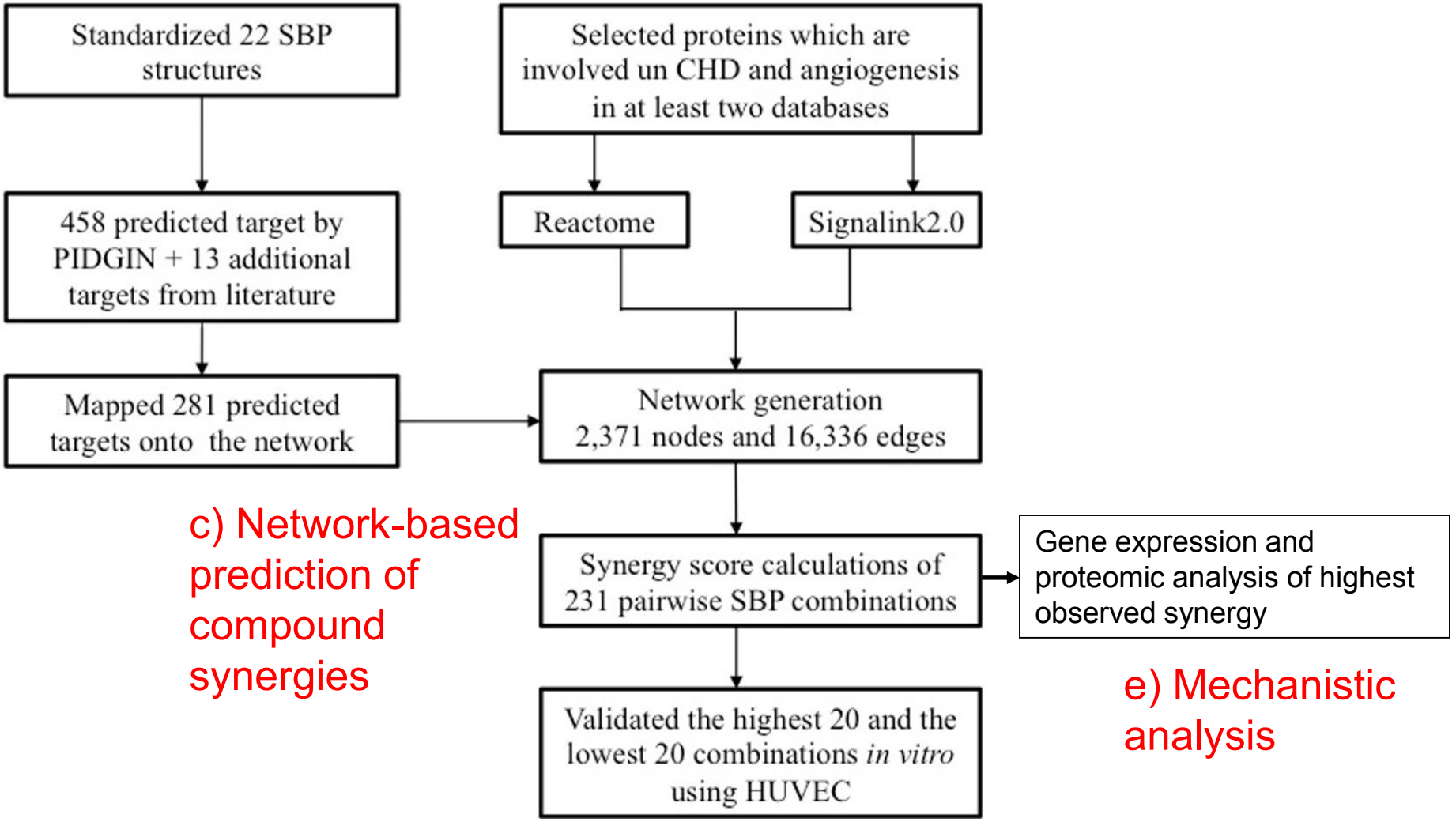
AIM: To elucidate mechanism of action of the synergistic pairwise combination in promoting angiogenesis by using in silico and RNA-seq analysis

SBP’s plasma absorbed compounds

1	gamabufotalin
2	bufalin
3	cinobufagin
4	ginsenoside Re
5	ginsenoside Rb1
6	ginsenoside Rb2
7	ginsenoside Rb3
8	ginsenoside Rc
9	ginsenoside Rd
10	cholic acid
11	hyodeoxycholic acid
12	chenodeoxycholic acid
13	deoxycholic acid
14	borneol
15	cinnamaldehyde
16	cinnamic acid
17	muscone
18	benzyl benzoate
19	17-hydroxyprogesterone
20	11- hydroxyprogesterone
21	ginsenoside Rg1
22	ginsenoside Rg3

a) Compounds mapped to targets

b) Generation of disease network

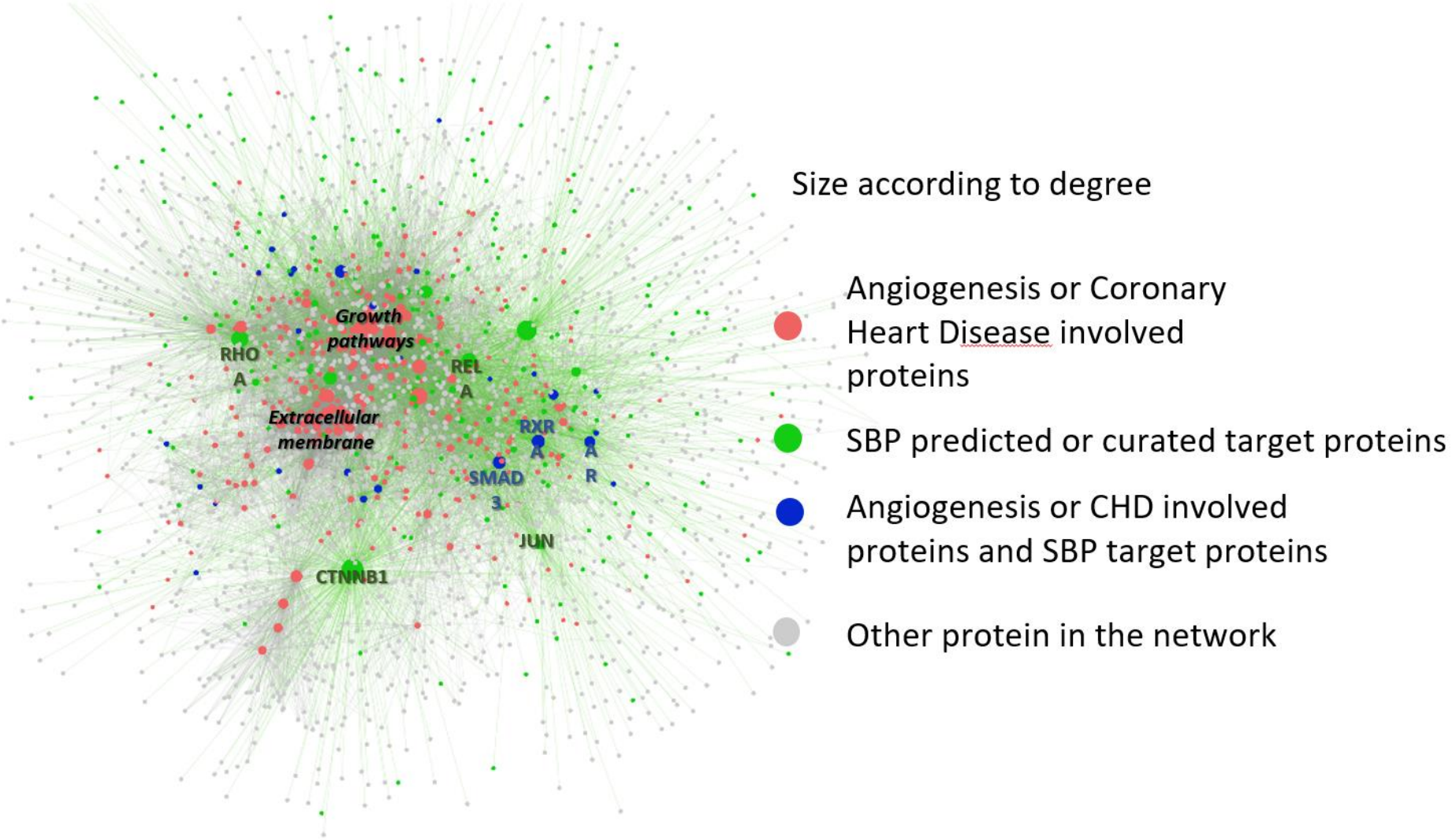


c) Network-based prediction of compound synergies

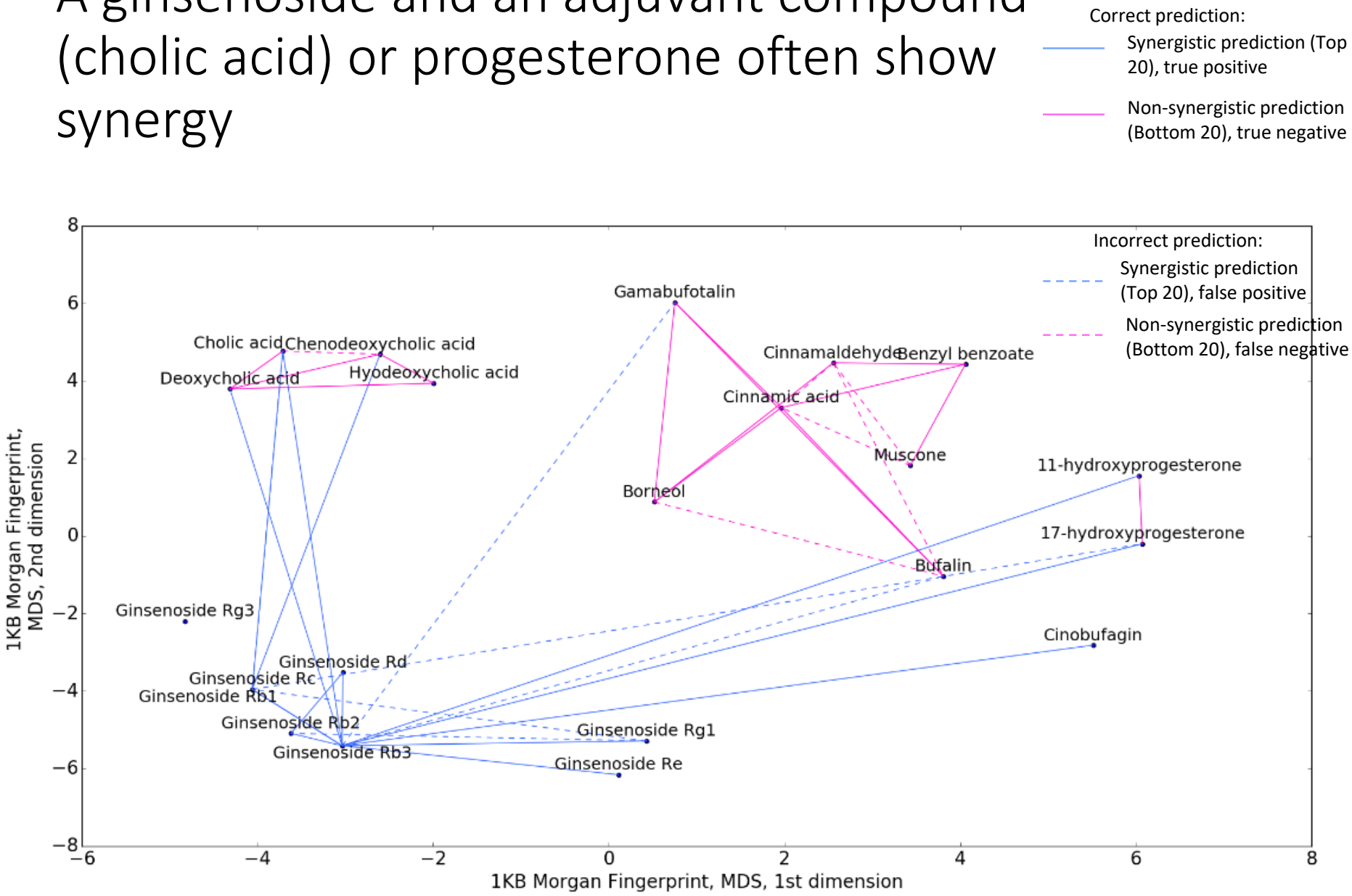
d) Quantitative validation of synergy predictions

e) Mechanistic analysis

SBP targets the central nodes of the angiogenesis and coronary hearth disease network



A ginsenoside and an adjuvant compound (cholic acid) or progesterone often show synergy



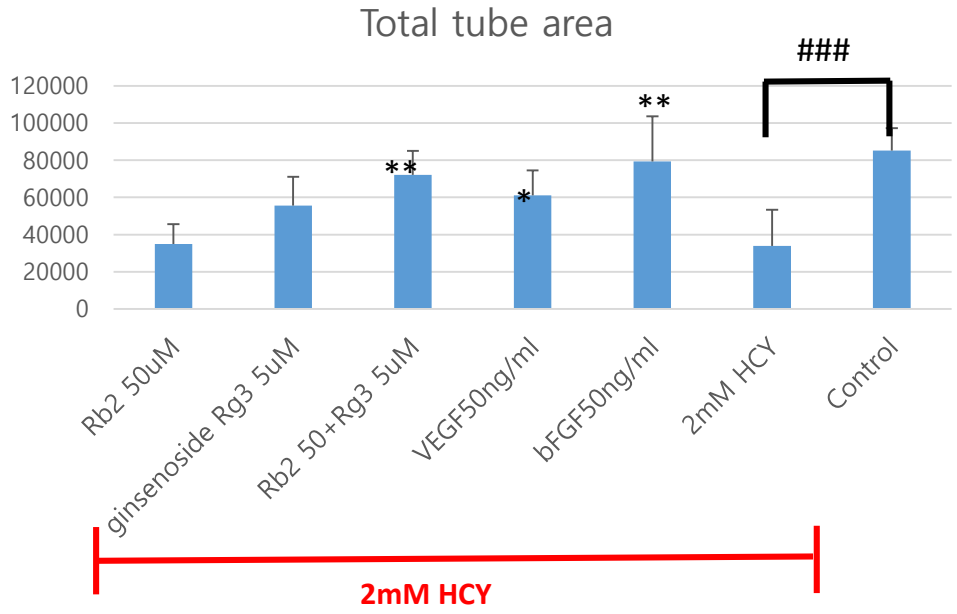
Rg3/Rb2 combination synergistic in cell proliferation, tube formation assay

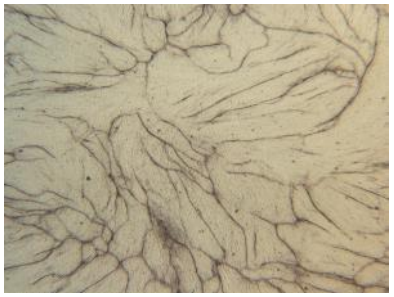
Biological readouts

1. Endothelial cell proliferation
2. **Rescue of Homocysteine-induced tube damage**
3. RNA-seq analysis
4. Validation of key genes and pathways

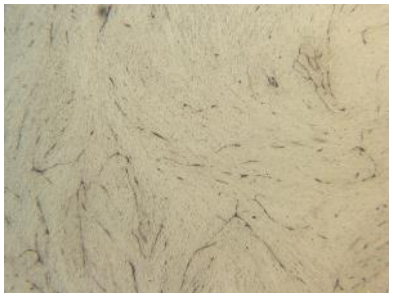
Elucidate MOAs

< Total tube area >

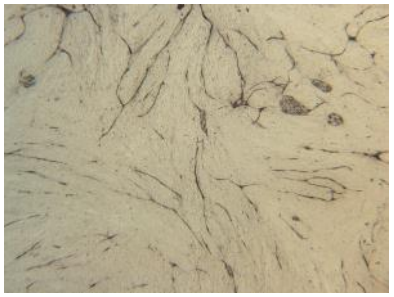




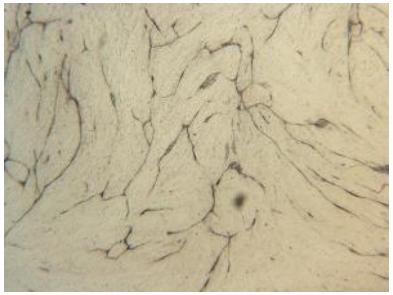
Control, no HCY



2mM HCY

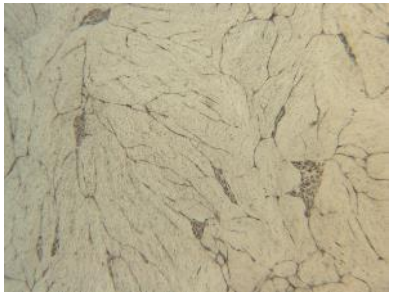


Rb2 50μM+2mM HCY



Rg3 5μM+2mM HCY

(Rb2+Rg3)+2mM HCY



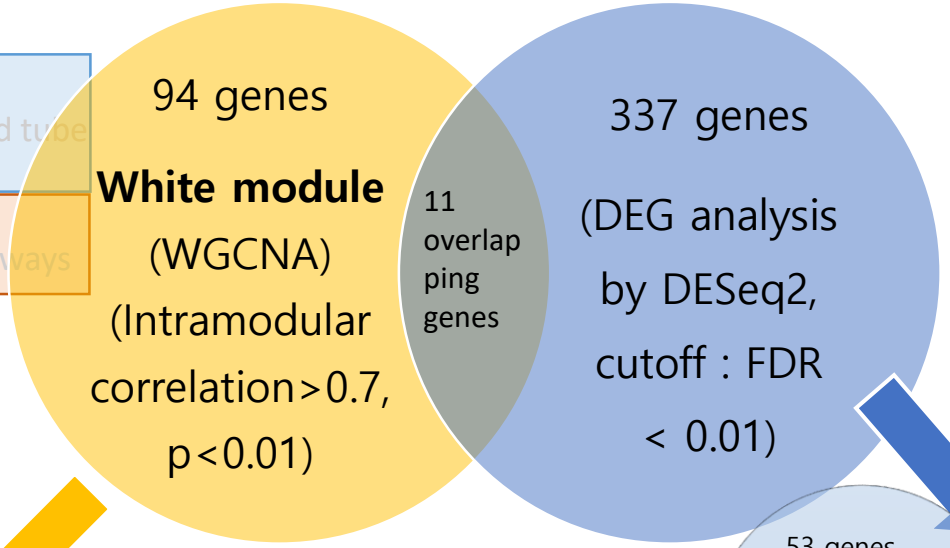
bFGF 50ng/ml+2mM HCY

Using gene expression data for mechanistic insight (2)

Biological readouts

1. Endothelial cell proliferation
2. Rescue of Homocysteine-induced tube damage
3. **RNA-seq analysis**
4. Validation of key genes and pathways

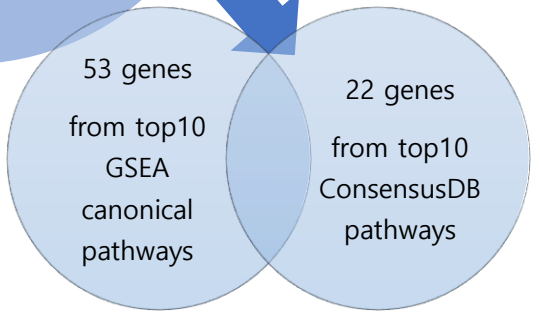
Elucidate MOAs



GO analysis of the white module : angiogenesis

GO Shortlisted 7 genes

Category	Term		PValue
GOTERM_BP_DIRECT	GO:0097191	extrinsic apoptotic signaling pathway	0.014722
GOTERM_BP_DIRECT	GO:0001525	angiogenesis	0.016897
GOTERM_BP_DIRECT	GO:0090050	positive regulation of cell migration involved in sprouting angiogenesis	0.030449
GOTERM_BP_DIRECT	GO:0008152	metabolic process	0.038043
GOTERM_BP_DIRECT	GO:0097105	presynaptic membrane assembly	0.038979
GOTERM_BP_DIRECT	GO:0070886	positive regulation of calcineurin-NFAT signaling cascade	0.038979
GOTERM_BP_DIRECT	GO:0043085	positive regulation of catalytic activity	0.049651



frequently occurring genes (more than twice)

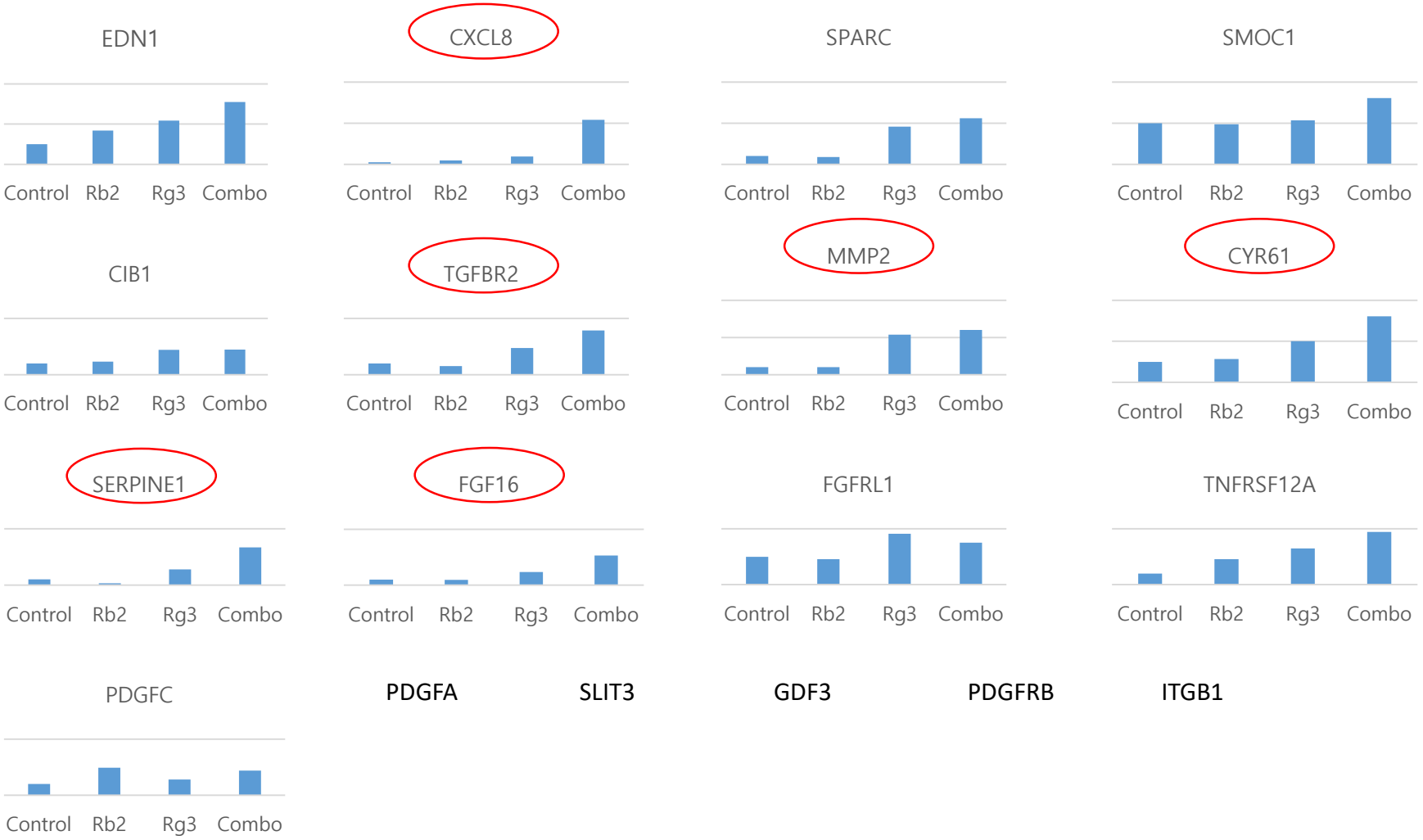
WGCNA

Highly correlated genes

12 genes from GSEA

6 genes from ConsensusDB

Validation by RT-PCR – eg CXCL8 is synergistically upregulated (etc)



So what did we learn?

- Predicting targets, using disease networks, connects formulation, chemistry, protein targets and disease biology
- We can use network topology to generally understand and predict synergy, as demonstrated for SBP
- Experimental analysis provides hypothesis for mechanism of synergy

Summary

- Chemical and biological data tell us something different about the ‘mode of action’ of a molecule

- We *can* use target prediction, gene expression data to understand parts of the mode of action of a compound

- … but MoA is not uniquely defined, different data sources provide difference parts of the puzzle

- Gene expression data helps understand MoA, repurposing, help select synergistic compound combinations

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