Update on the Pathway Project:
Feasibility of Using Pharmacoinformatics Methodology

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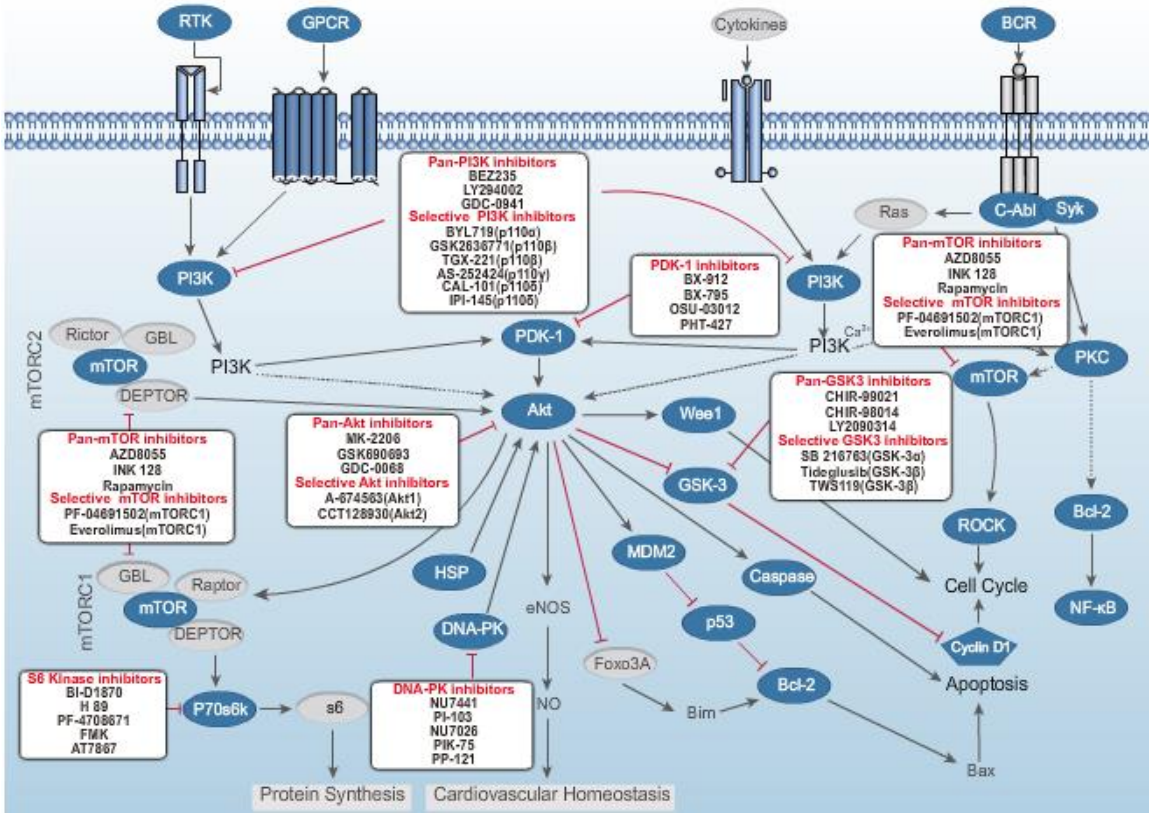
19 July 2017

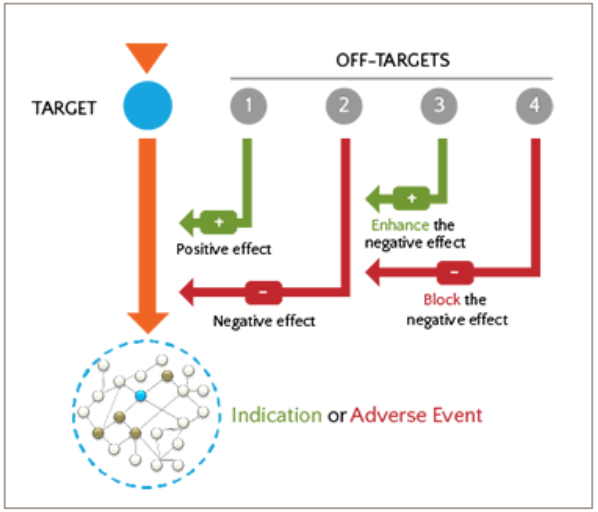
Lilly

Drug Target Efficacy & Safety Landscapes

Efficacy

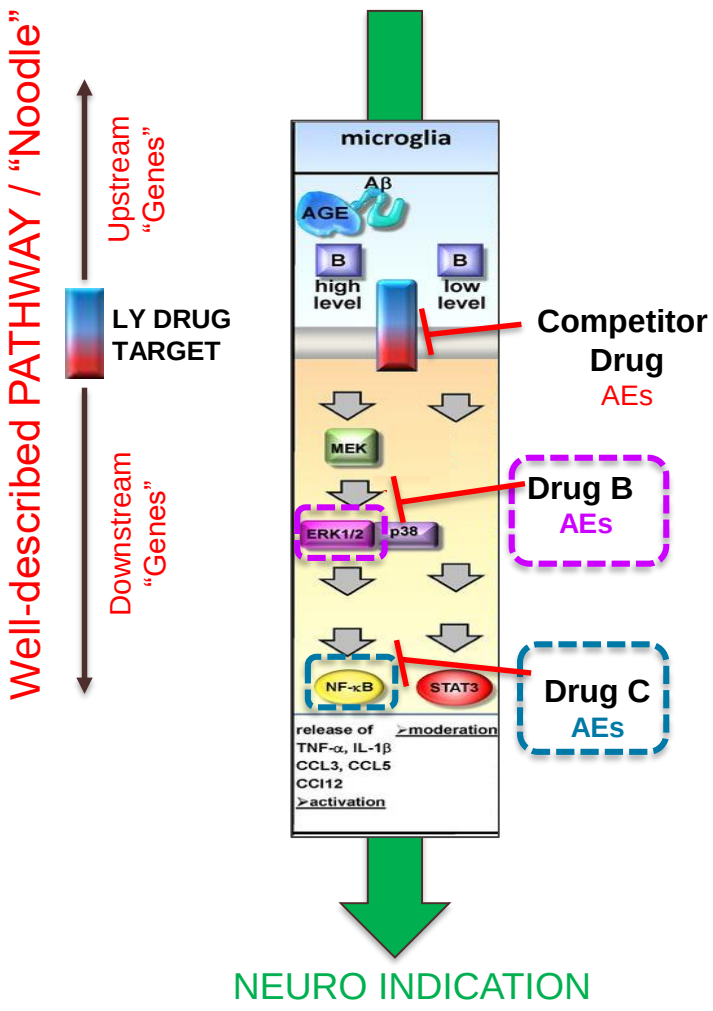


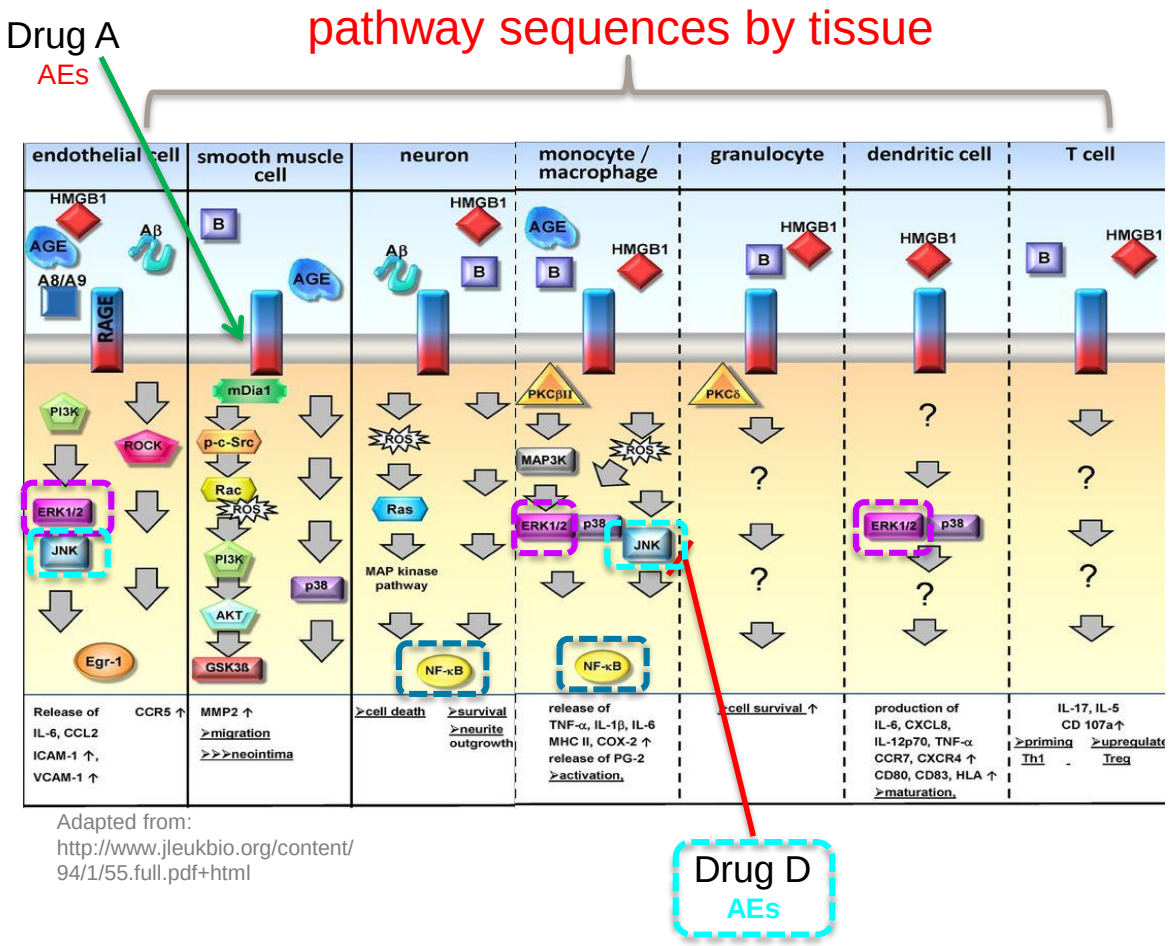
Safety



http://www.anaxomics.com/clinical-safety-and-efficacy-profile.php

Systems Pharmacology Approach

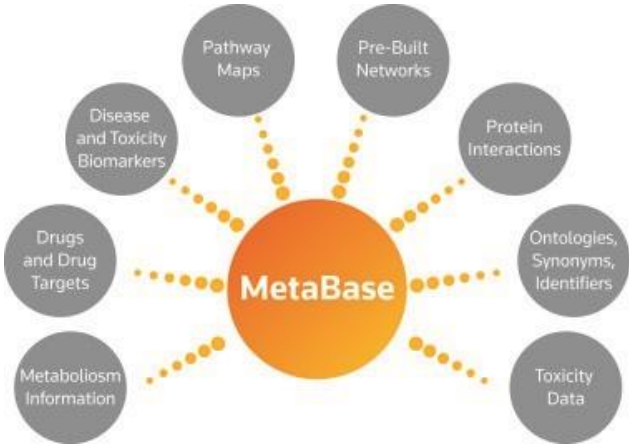




Adapted from:
http://www.jleukbio.org/content/94/1/55.full.pdf+html

Pharmacoinformatics Methodology

All Literature



MetaBase is a knowledge database manually curated/validated by scientists (Clarivate criteria)

CONTENT HIGHLIGHTS

- More than 1,600 interactive canonical pathway maps capturing nearly 200,000 human, mouse, and rat fine metabolic and signaling canonical pathways depicted based on consensus literature findings
- More than one million interactions of proteins with other proteins, DNA, RNA, metabolites, and xenobiotics
- Thousands of putative disease biomarkers
- Over 750,000 compounds with targets and bioactivity information
- More than 30,000 metabolic reactions
- Over 8,900 drugs
- Over 5,300 endogenous metabolites
- Millions of synonyms resolved for genes, proteins, and compounds
- Protein complexes and protein families resolved in human, mouse, and rat

R Interface

specific queries

- genes (exploratory)
- pathways (*noodles)
- drugs
- AEs
- tissues
- diseases

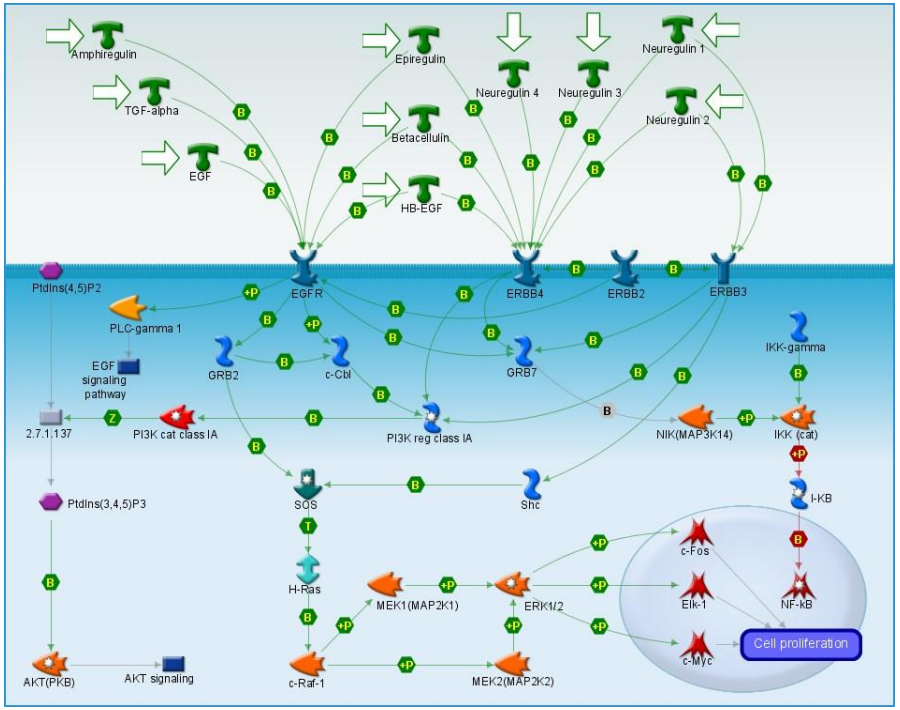
Tim's project

+

OpenVigil interface to FAERs (AEs)

MetaBase Background: Pathway maps

- Pathway maps are manually created on the basis of manually curated interactions.
- Comprise network objects, interactions between objects and references to other maps.
- Organized into the pathway map ontology.
- Graphical representation is available for each map
- A typical canonical pathway map contains 3-6 signaling pathways describing a particular biological mechanism, (e.g. ERBB-family signaling) (1)



1. THOMSON REUTERS METHODOLOGY, METABASE SCRIPTS LIBRARY 4.2.3 (Tutorial)

Metabase Background: Noodles

- Noodles are automatically generated linear sequences of highly curated interactions. They are derived either from pathway maps or curated networks (1).
- Noodles represent canonical signaling paths, typically originating from a ligand or another important signaling molecule and ending with transcription regulation event or another downstream molecular response (1).
- Noodles are highly redundant;

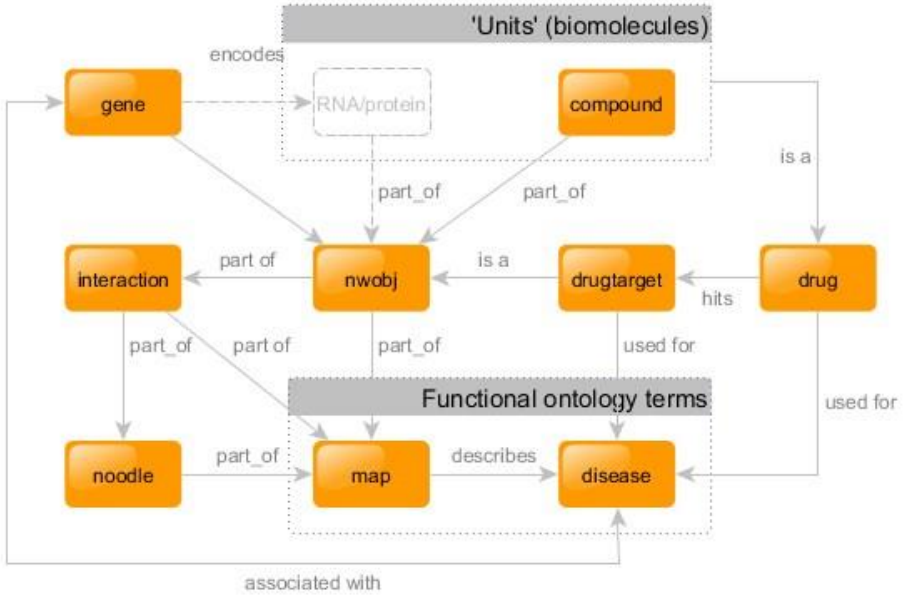
  e.g. A -> B -> C -> X and

  A ->B->D->X

  are 2 different noodles.

1. THOMSON REUTERS METHODOLOGY, METABASE SCRIPTS LIBRARY 4.2.3 (Tutorial)

MetaBase terminology accessed through R script



1. THOMSON REUTERS METHODOLOGY, METABASE SCRIPTS LIBRARY 4.2.3 (Tutorial)

Interaction Screenshot

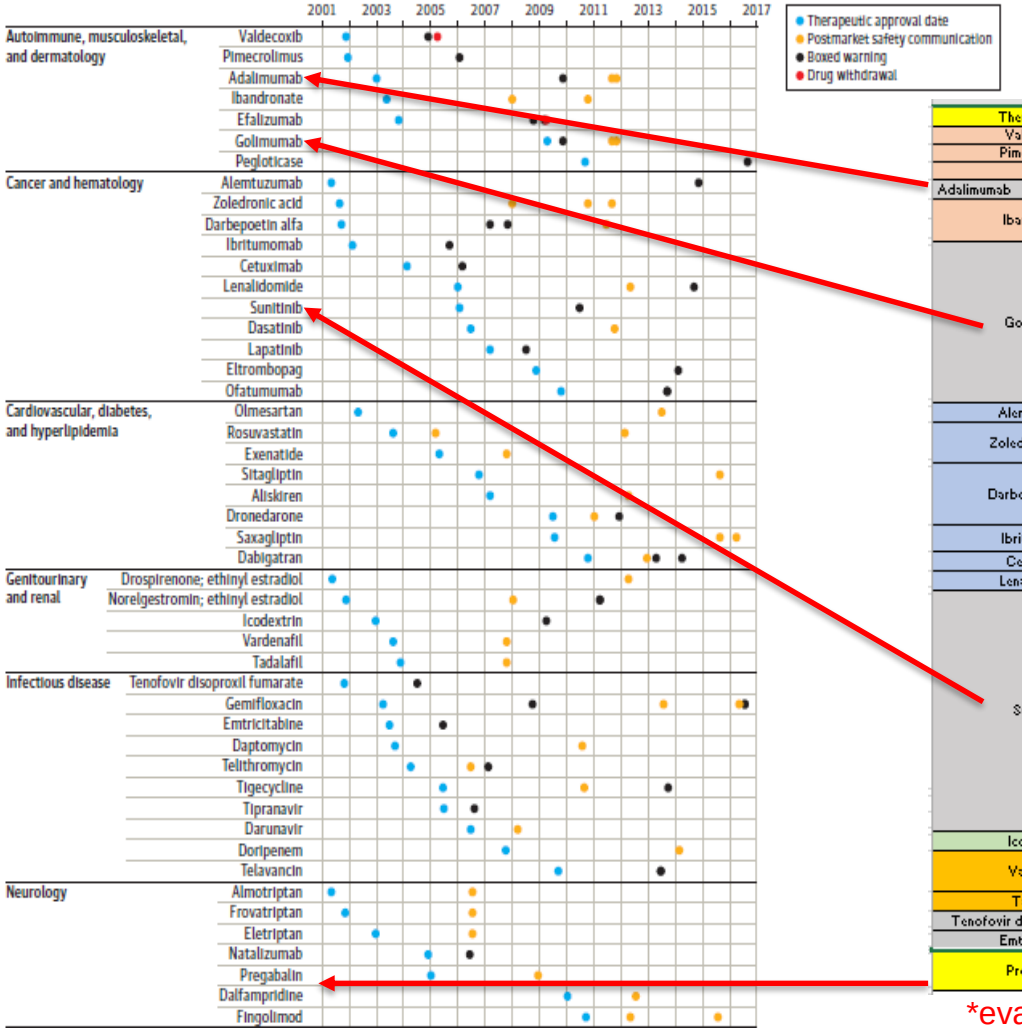
```
> A.interactions <- get.gene.interactions(A, filter = list(effect = c("Activation", "Inhibition"), has.compounds = FALSE))
>  A.interactions
```

	link.id	id1	id2	effect	mechanism	trust
9	1051	CD45	Lck	Activation	Dephosphorylation	Present
11	21417	Lck	CD84	Activation	Phosphorylation	Probably present (possible domain interaction)
25	-599529310	SHP-1	Lck	Inhibition	Dephosphorylation	Present
28	-166510129	PPAR-alpha	Lck	Inhibition	Influence on expression	Probably present (animal model)
38	-1802424070	CD81	Lck	Activation	Binding	Probably present (possible domain interaction)
39	-1513987210	LAT	Lck	Inhibition	Binding	Probably present (animal model)
41	-1382794544	Lck	LAT	Activation	Phosphorylation	Present
45	-428039891	Lck	c-Abl	Activation	Binding	Present
48	10222	CD2	Lck	Activation	Binding	Present
58	-1147072241	Lck	VAV-3	Activation	Phosphorylation	Probably present (possible domain interaction)
74	-1638776954	Lck	Rap1GAP1	Activation	Unspecified	Probably present (putative interaction for signaling pathway)
90	17529	PTPRF (LAR)	Lck	Activation	Dephosphorylation	Probably present (possible domain interaction)
92	-1815220718	CXCR4	Lck	Activation	Unspecified	Probably present (putative interaction for signaling pathway)
93	-1627237950	SHP-2	Lck	Inhibition	Dephosphorylation	Present
101	-329987222	BOB1	Lck	Activation	co-regulation of transcription	Probably present (possible domain interaction)
106	-1725139602	DNMT1	Lck	Inhibition	co-regulation of transcription	Present
116	-477182747	Lck	PI3K reg class IA (p85-alpha)	Activation	Phosphorylation	Present
118	3609	Lck	GIT1	Activation	Phosphorylation	Probably present (possible domain interaction)
143	29663	Lck	MUC1	Activation	Phosphorylation	Probably present (possible domain interaction)
155	5942	Lck	Filamin A	Activation	Phosphorylation	Probably present (possible domain interaction)
168	-406533293	Lck	NF-kB p65/c-Rel	Activation	Binding	Probably present (possible domain interaction)
171	2864	Lck	ITK	Activation	Phosphorylation	Present
185	-752485842	Oct-2	Lck	Activation	Transcription regulation	Probably present (possible domain interaction)
193	2836	Lck	TRIM	Activation	Phosphorylation	Probably present (possible domain interaction)
194	377	Lck	CD3 zeta	Activation	Phosphorylation	Present

Identification of a Literature-Based Pathway

Postmarket Safety Events Among Novel Therapeutics Approved by the US Food and Drug Administration Between 2001 and 2010," Downing et al. (2017)

Figure 1. Timeline of Novel Therapeutics Approved by the US FDA, 2001-2010, That Experienced Postmarket Safety Events, Grouped by Therapeutic Area

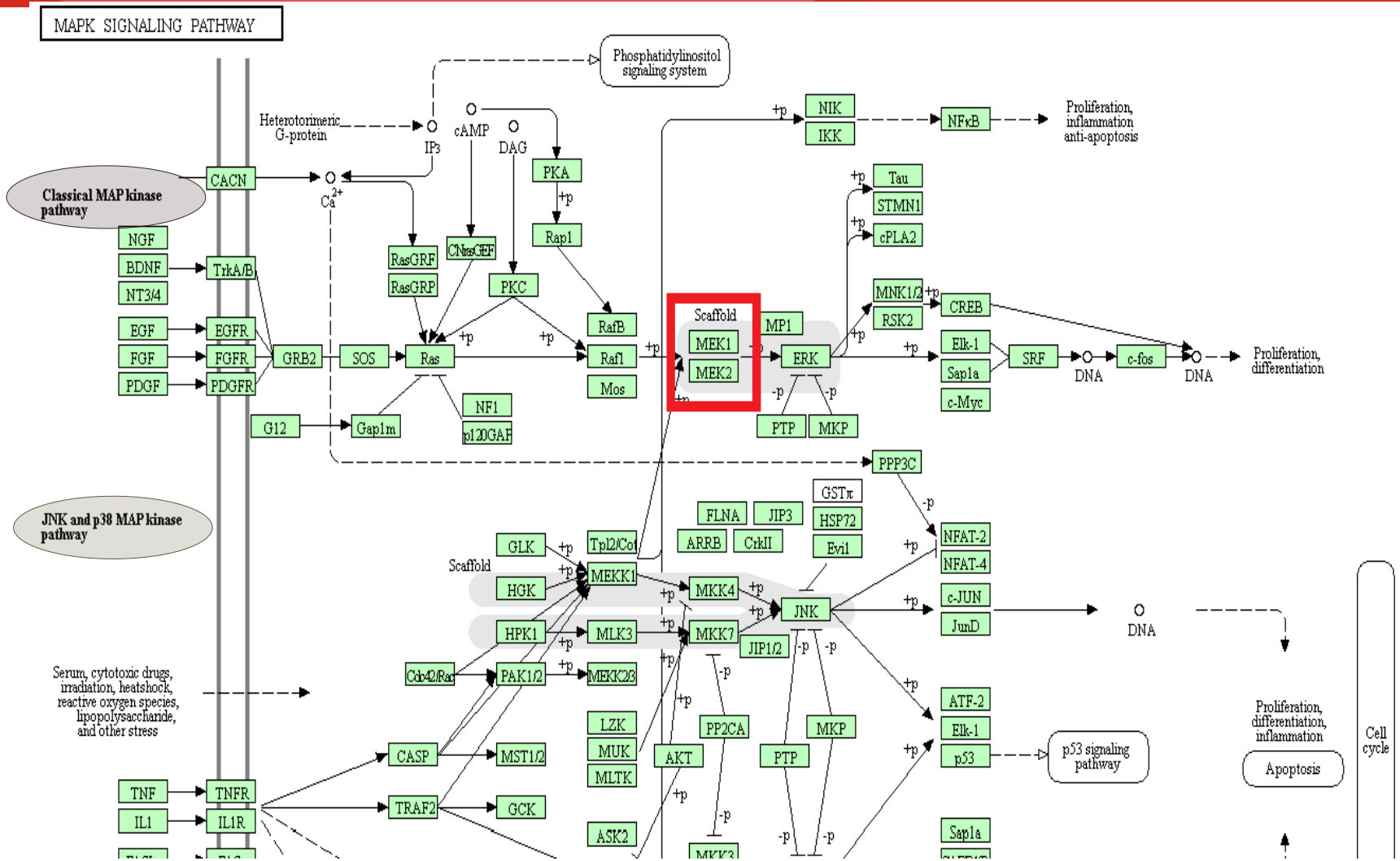


KEGG, DrugBank

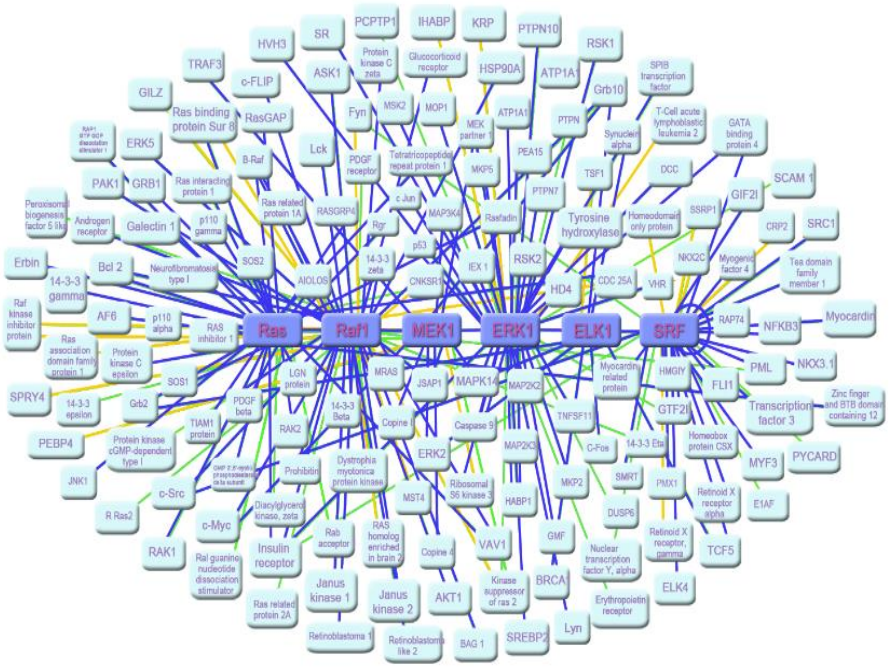
Therapeutics	Molecular Targets	Pathway
Valdecoxib	Prostaglandin G/H synthase 2	Arachidonic acid metabolism
Pimecrolimus	Serine/threonine-protein kinase mTOR	Antigen processing and presentation
	Peptidyl-prolyl cis-trans isomerase FKBP1A	TGF-beta signaling pathway
Adalimumab	Tumor necrosis factor	MAPK signaling pathway
Ibandronate	Farnesyl pyrophosphate synthase	Terpenoid backbone biosynthesis
	Hydroxylapatite	
Golimumab	Tumor necrosis factor	MAPK signaling pathway Cytokine-cytokine receptor interaction TGF-beta signaling pathway Fc epsilon RI signaling pathway Adipocytokine signaling pathway Asthma Rheumatoid arthritis
Alemtuzumab	CAMPATH-1 antigen	
Zoledronic Acid	Farnesyl pyrophosphate synthase	Terpenoid backbone biosynthesis
Darbepoetin alfa	Erythropoietin receptor	Cytokine-cytokine receptor interaction Jak-STAT signaling pathway Hematopoietic cell lineage
Ibritumomab	B-lymphocyte antigen CD20	
Cetuximab	Epidermal growth factor receptor	Cetuximab Action Pathway
Lenalidomide	Protein cereblon	
Sunitinib	Platelet-derived growth factor receptor beta	hsa04010 MAPK signaling pathway hsa04020 Calcium signaling pathway hsa04060 Cytokine-cytokine receptor interaction hsa04370 VEGF signaling pathway hsa05200 Pathways in cancer
	Macrophage colony-stimulating factor 1 receptor	
	Platelet-derived growth factor receptor alpha	
Icodextrin		peritoneal lymphatic pathways
Vardenafil	cGMP-specific 3',5'-cyclic phosphodiesterase	Purine metabolism
Tadalafil	cGMP-specific 3',5'-cyclic phosphodiesterase	Purine metabolism
Tenofovir disproxil fumarate	Reverse transcriptase/RNaseH	Tenofovir Metabolism Pathway
Emtricitabine	Reverse transcriptase/RNaseH	Emtricitabine Action Pathway
Pregabalin	voltage-dependent calcium channel alpha-2/delta-1	MAPK signaling pathway

*evaluated 48 drugs

KEGG: MAPK Pathway



Identifying Genes Related to MEK: An Exploratory Approach



Pros	Cons
Comprehensive	Long Run Time (Using R, but other possibilities exist)
May identify new interactions (possible advantage when categorizing by tissue type)	Requires more user inputs (e.g. definition for parameterized distances)

INTERACTIONS HAVE A TRUST LEVEL!
METABASE CONTENT

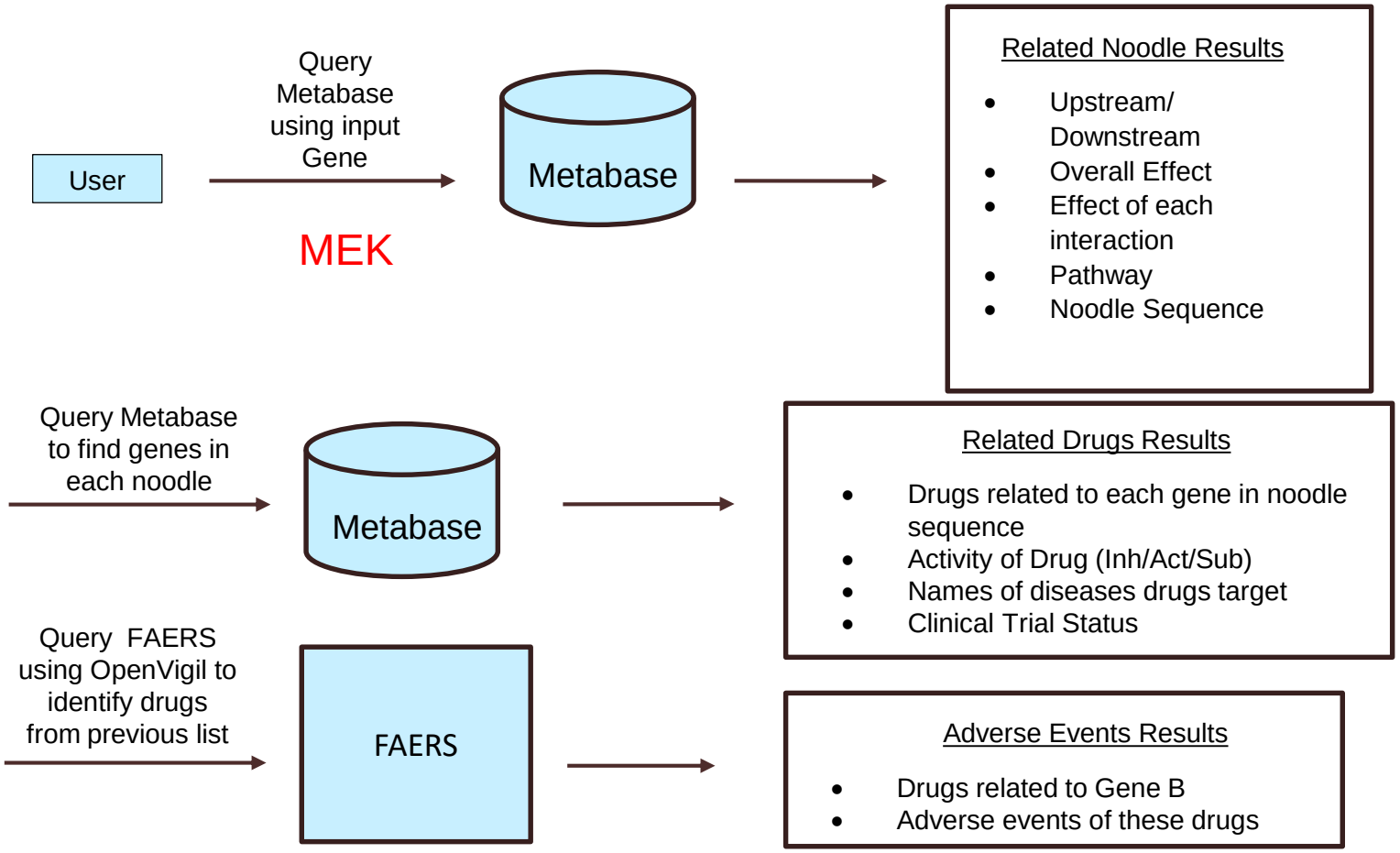
- **REGULATION_RELS_ORG** has TRUST field that specifies trust level for a specific interaction-species combination
- **REGULATION_RELS.TRUST** corresponds to Human species
- *When **TRUST == -1** it means that this interaction doesn't exist and there is a literature evidence confirming this fact

You can exclusively filter for species specific interaction data!

- TRUST LEVELS (FROM HIGH TO LOW)

ID	Value	Meaning	Trust level
0	Present	Interaction is proven by trusted methods on this organism	High
8	Approved	Interaction is proven for all protein group members (with Present trust)	High
9	Conflicting data	Proven interaction, but different effects in different papers	High
3	Animal model	Proven on animal model	High
7	Possible common	Proven for some protein group members, but not all	Medium
6	Mix	Proven for the protein group as a whole, but not for individual members	Medium
2	Domain interaction	Interaction derived using unreliable methods (yeast2hybrid), only binding site for trans. Factors	Low
10	Signaling pathway	Interaction is made specially for signaling pathway map, may be indirect	Low
1	NLP	Result of data mining, or paper with high-throughput screen (chip on chip, prediction)	Low
-1	No link	Means that this interaction is absent for the particular species	N/A

Flowchart of the Pharmacoinformatics Approach

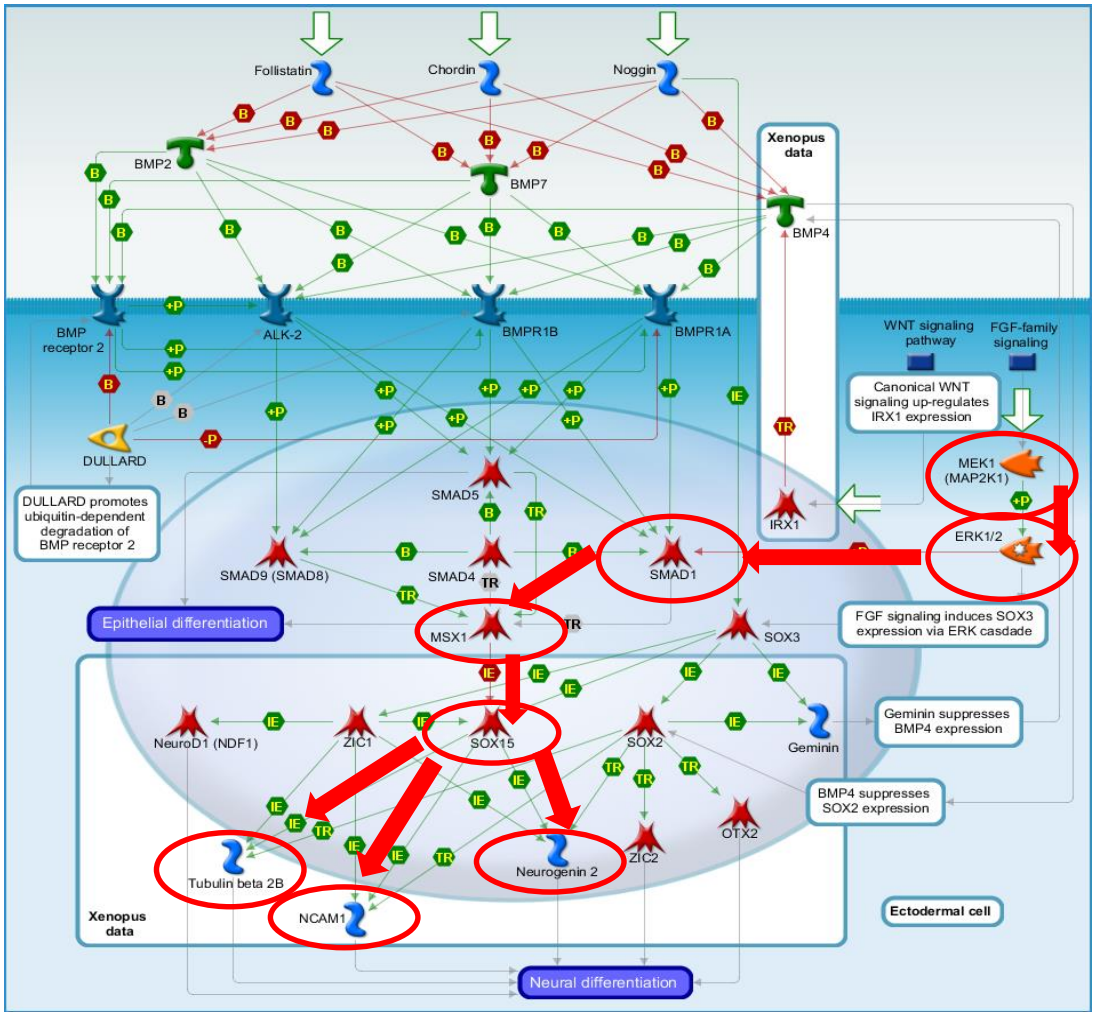


Identification of Noodles

- To identify upstream or downstream proteins of MEK in various pathways, the MEK gene was identified as either the **start (head)** or **end (tail)** of a sequence of proteins in well-described (high trust) canonical signaling pathways

Gene A	Gene B	Noodle	Pathway Name
MEK1	Tubulin beta 2B	**MEK1**--MSX1--Tubulin beta 2B	BMP signaling in embryonic stem cell neural differentiation
MEK1	Neurogenin 2	**MEK1**--MSX1--Neurogenin 2	BMP signaling in embryonic stem cell neural differentiation
MEK1	NCAM1	**MEK1**--MSX1--NCAM1	BMP signaling in embryonic stem cell neural differentiation
IL-6	MEK1	IL-6--IL-6 receptor--**MEK1**	IL-6 signaling in breast cancer cells
GnRH1	MEK1	GnRH1--GnRH receptor--**MEK1**	Gonadotropin-releasing hormone (GnRH) signaling
Urease B (H. pylori)	MEK1	Urease B (H. pylori)--CD74--**MEK1**	Effect of H. pylori infection on inflammation in gastric epithelial cells

Metabase BMP Signaling Pathway Depicting R-selected Noodles



Related Drugs

- An R script was written to select distinctive genes in the BMP signaling pathway and any FDA approved drugs targeting these genes from Metabase:
  - Nine distinctive genes SOX15, TUBB2B, MAP2K1, MAPK1, MAPK3, MSX1, SMAD1, NEUROG2, NCAM1 were identified.
  - Six FDA approved drugs were found to target these nine genes.

Drug Name	Target	Indication	Effect
Eribulin	Tubulin beta-1 chain (in microtubules)	Breast neoplasms	Inhibitor
Estramustine	Tubulin beta-1 chain (in microtubules)	Prostatic neoplasms	Inhibitor
Ixabepilone	Tubulin beta-3 chain (in microtubules)	Breast neoplasms	Inhibitor
Paclitaxel	Tubulin beta-1 chain (in microtubules)	Breast neoplasms	Inhibitor
Vindesine	Tubulin beta-1 chain (in microtubules)	Lung neoplasms	Inhibitor
Vinorelbine	Tubulin beta chain (in microtubules)	Carcinoma, Non-Small-Cell Lung	Inhibitor

Selected Adverse Events Results: Drugs targeting the BMP pathway

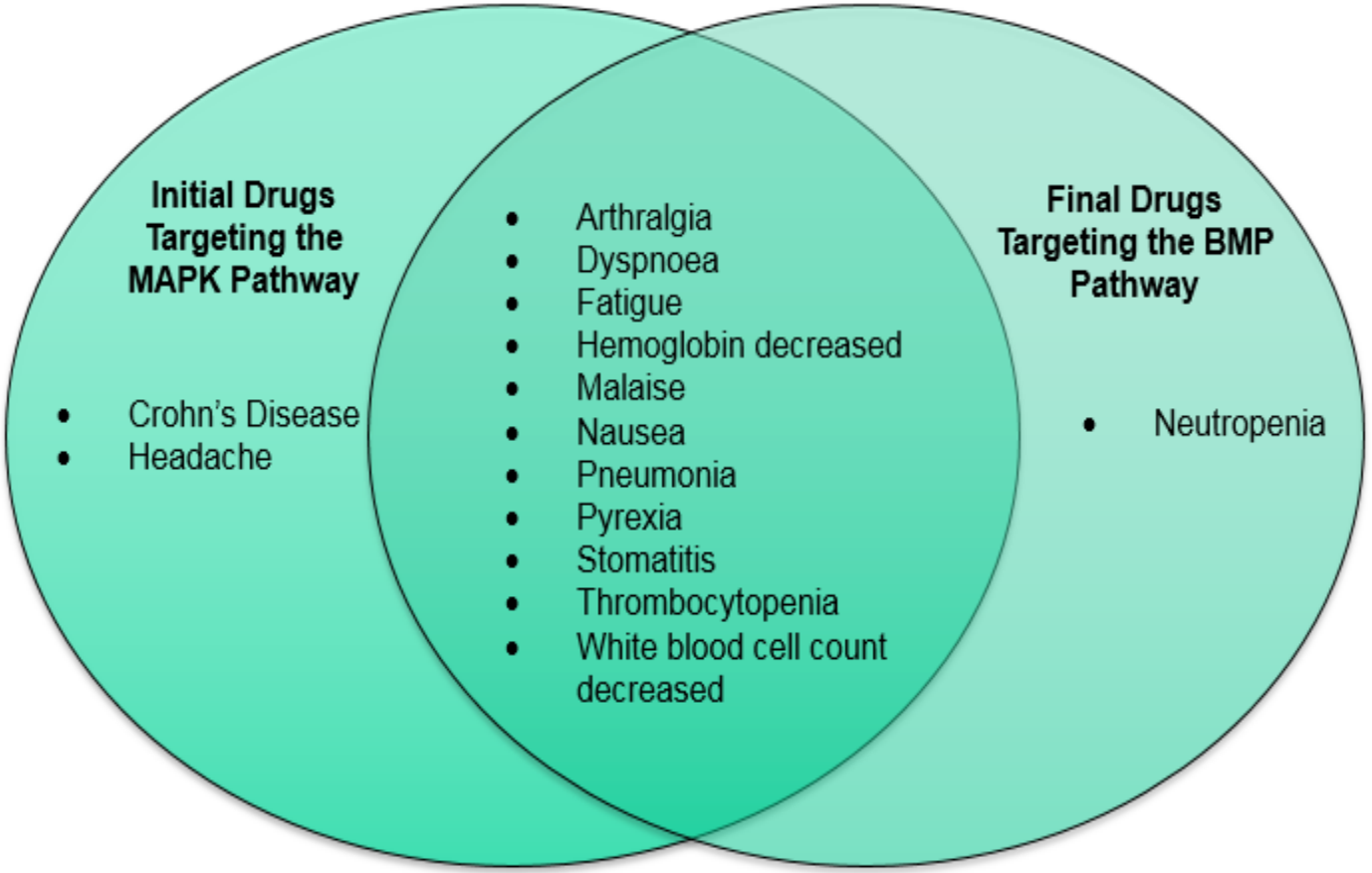
Drug Name	Adverse Events (count of reports)
Eribulin	Neutropenia (56) Pyrexia (29) Pneumonia (27) Thrombocytopenia (12)
Estramustine	Dyspnoea (25) Thrombocytopenia (14) Pulmonary embolism (14) Malaise(10)
Ixabepilone	Stomatitis (51) Thrombocytopenia (37) Arthralgia (37) White blood cell count decreased (35)
Paclitaxel	Dyspnoea (1461) Pyrexia (1075) Pneumonia (591) White blood cell count decreased (589)
Vindesine	Thrombocytopenia (15) Pyrexia (14) Pneumonia (12) Hemoglobin decreased (9)
Vinorelbine	Pyrexia (258) Nausea (159) Pneumonia (137) Fatigue (135)

Selected Adverse Events Results: Drugs targeting the MAPK pathway

Drug Name	Molecular Target	Adverse Events (count of reports)
Adalimumab	Tumor necrosis factor	Arthralgia (4781) Pyrexia (3765) Nausea (3119) Crohn's Disease (2800)
Golimumab	Tumor necrosis factor	Pneumonia (277) Arthralgia (92) Headache (89) Malaise (60)
Sunitinib	Platelet-derived growth factor receptor beta	Thrombocytopenia (472) Stomatitis (373) White blood cell count decreased (252) Hemoglobin decreased (193)
Pregabalin	Voltage-dependent calcium channel alpha-2/delta-1	Nausea (2177) Malaise (2062) Fatigue (1947) Dyspnoea (1600)

Comparison of Adverse Events of Drugs Targeting Pathways

Adalimumab
Golimumab
Sunitinib
Pregabalin



Eribulin
Estramustine
Ixabepilone
Paclitaxel
Vindesine
Vinorelbine

Conclusions and Next Steps

- ✓ Multiple adverse events were identified in common for drugs targeting upstream and downstream proteins in the MAPK and BMP pathways.
- ➢ Differentiating pathways by tissue type may further help to predict the adverse events of a new drug target.
- ➢ Visual-analytic descriptive techniques can be used to map AEs to a pathway by tissue type

Schematic of Next Steps

User

Query Metabase using input Gene A

Metabase

Related Gene B Results

- Cell Types
- Upstream/ Downstream
- Distance from Input Gene A to Gene B
- Effect
- Pathway
- A to B sequence

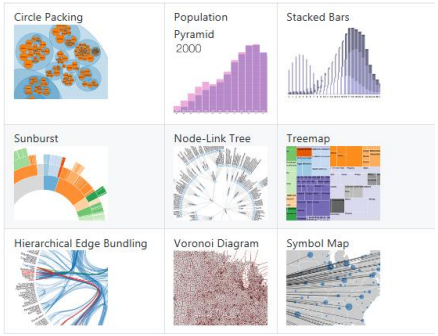
Query I2E using Gene B list

I2E

Results

- Drugs related to Gene B
- Activity of Drug (Inh/Act/Sub)
- Adverse Events of Drugs
- Names of diseases drugs target
- Clinical Trial Status

Visualize Results



I2E

FDA Labels

Clinical Trials

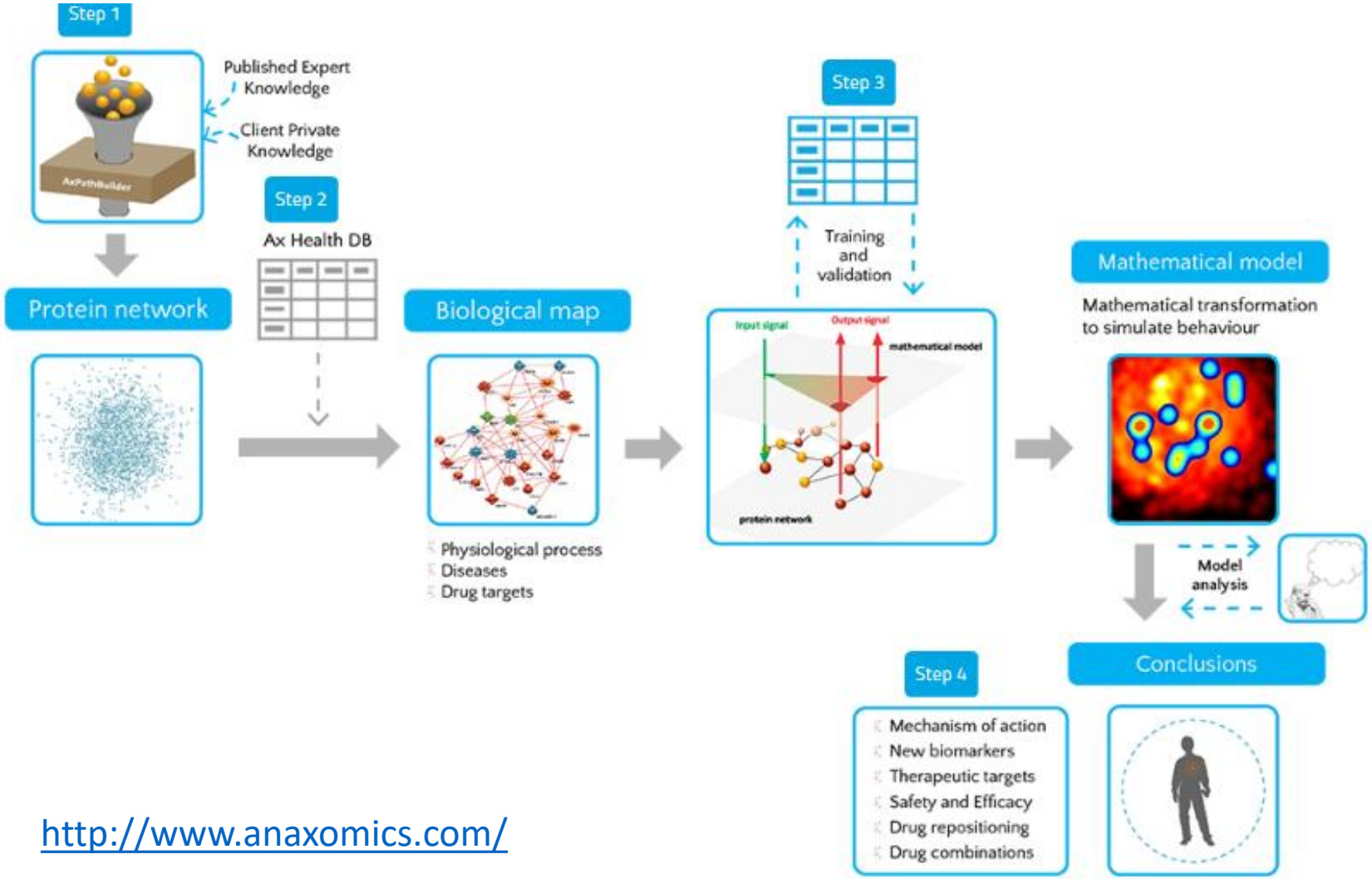
FAERS

Drug Bank

Two Examples of Similar Approaches

1. Anaxomics
2. ASCPT

Therapeutic Performance Mapping System (TPMS)



http://www.anaxomics.com/

Therapeutic Performance Mapping System (TPMS)

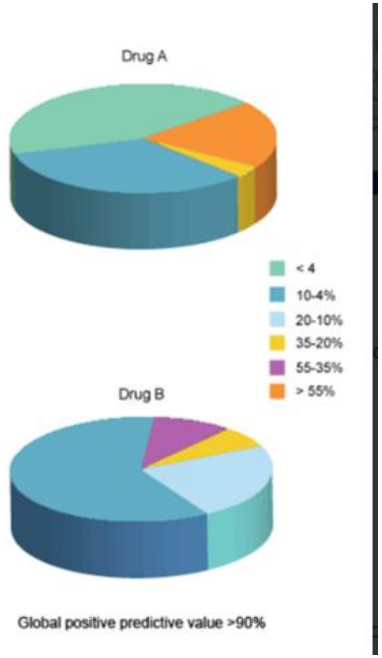
STEP 4

Extraction of biological and clinical conclusions

The analysis of the mathematical model reveals functional properties and mechanistic insights that are otherwise inaccessible. When the models are asked with the client's demands, they suggest new hypotheses that can be readily tested *in vitro* or *in vivo* for validation:

- What is the mechanism of action of my drug? → *Mechanism of action*
- Will my drug be effective? Will it be safe? → *Clinical safety and efficacy profile*
- What other uses can I find for my drug? → *Drug repositioning*
- Which compounds of my pipeline should I prioritize? → *Early Product Development Strategy*
- How can I avoid or control drug safety issues? How can I enhance drug efficacy? → *Strategies and solutions for clinical trials*

ADVERSE EVENTS	Drug A	Drug B
Anemia	78%	8%
Anorexia	91%	14%
Constipation	0%	10%
Cramps	3%	6%
Depression	5%	8%
Diarrhea	90%	45%
Dyspepsia	3%	8%
Dyspnea	4%	5%
Emesis	76%	33%
Fatigue	88%	18%
Fever	5%	5%
Flatulence	6%	6%
Flushing	3%	6%
Geadache	1%	5%
Heartburn	8%	6%
Kalemia-Hypo	3%	45%
Leukopenia	3%	5%
Lymphopenia	3%	5%
Natremia-Hypo	1%	6%
Nausea	95%	12%
Neutropenia	5%	5%
Pain	3%	5%
QTC Prolongation	0%	10%
Somnolence	6%	16%
Tachycardia	4%	38%
Taste-disorders	5%	8%
Tension-Hypo	5%	16%
Thrombocytopenia	21%	5%
Uricemia-Hyper	3%	5%
Weight-Loss	5%	22%



An exemplar of a systems pharmacology approach for a detailed investigation of an adverse drug event as a result of drug-drug interactions

Poster #: PT-012

ASCPT Annual Meeting 2017
Washington, DC

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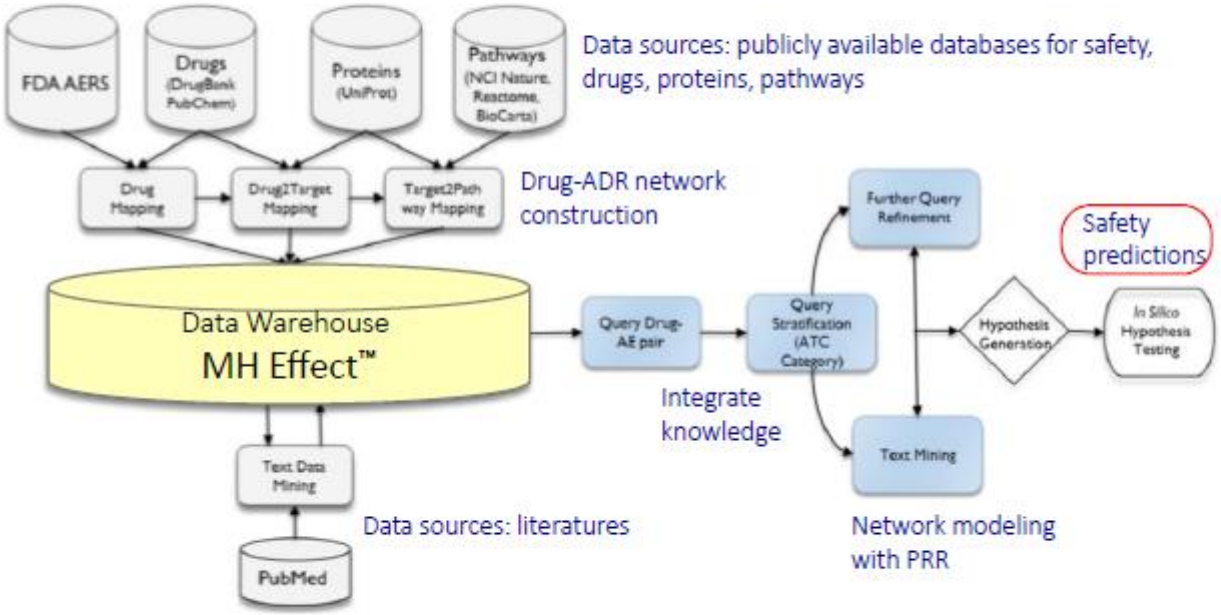
[*]Corresponding author: mtrame@cop.ufl.edu

Systems Approach to Drug Safety utilizing Adverse Event Databanks



The objective of the following analysis was to select a prototype ADR to develop a systems pharmacology platform for drug safety

Poster #: PT-012

Discussion and Conclusions

Hypothesis: The combination therapy of trastuzumab and doxorubicin may induce a synergistic effect of mitochondrial dysfunction in cardiomyocytes through different molecular pathways of the BCL-2 family, PPARα and PPARβ proteins, leading to an increased risk of developing cardiotoxicity.

What did this Systems-based approach provide versus a non-mechanistic approach?

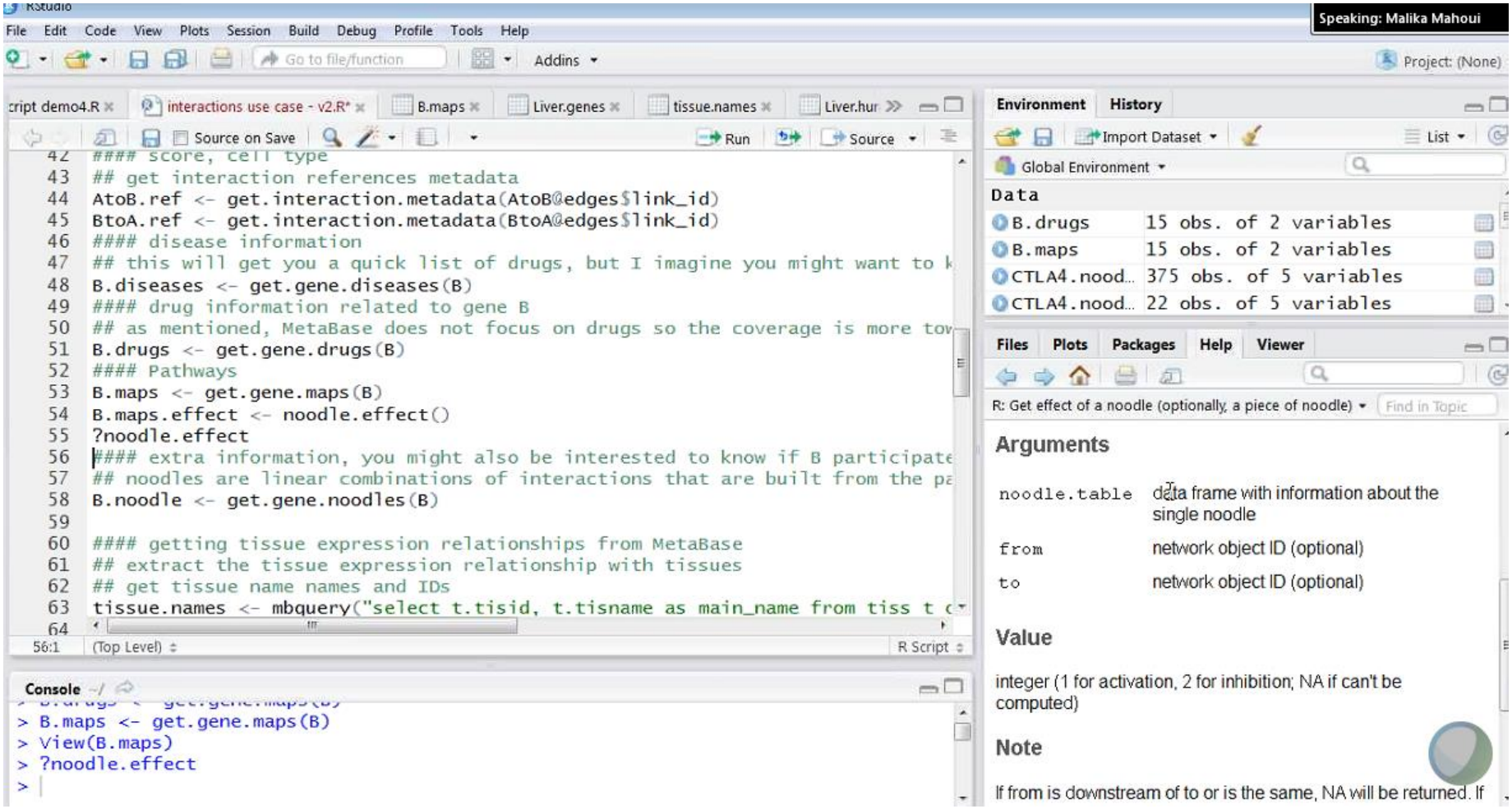
- This **systems-based approach** provides a process to better map the mechanism of **drug-drug interactions** to targets and pathways

Inform Drug Development Pipelines

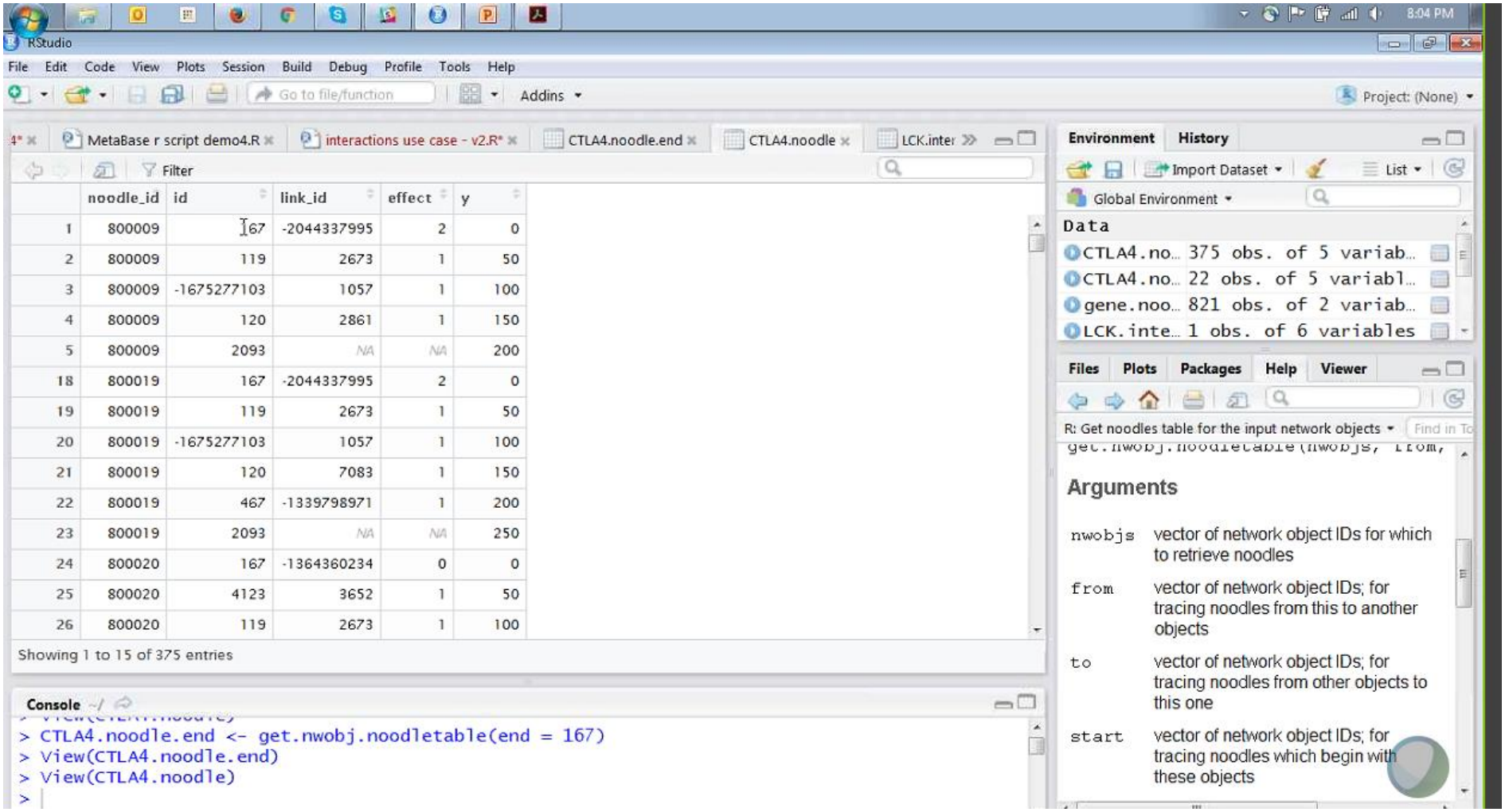
Acknowledgements

- Indiana Project STEM and Indiana Clinical and Translational Sciences Institute (CTSI)
- Mr. Elmer Sanders, Ms. Charity Scott, Mrs. Shari Harrison, and Mr. Jacob Olsen
- Deirdre Kelley and Grace LeFevre
- Dr. Paul Ardayfio

An Example


RStudio
File Edit Code View Plots Session Build Debug Profile Tools Help
Speaking: Malika Mahoui
Addins
Project: (None)
cript demo4.R
interactions use case - v2.R*
B.maps
Liver.genes
tissue.names
Liver.hur
Source on Save
Run
Source
score, cell type
get interaction references metadata
AtoB.ref <- get.interaction.metadata(AtoB@edges$link_id)
BtoA.ref <- get.interaction.metadata(BtoA@edges$link_id)
disease information
this will get you a quick list of drugs, but I imagine you might want to k
B.diseases <- get.gene.diseases(B)
drug information related to gene B
as mentioned, MetaBase does not focus on drugs so the coverage is more tov
B.drugs <- get.gene.drugs(B)
Pathways
B.maps <- get.gene.maps(B)
B.maps.effect <- noodle.effect()
?noodle.effect
extra information, you might also be interested to know if B participate
noodles are linear combinations of interactions that are built from the pa
B.noodle <- get.gene.noodles(B)
getting tissue expression relationships from MetaBase
extract the tissue expression relationship with tissues
get tissue name names and IDs
tissue.names <- mbquery("select t.tisid, t.tisname as main_name from tiss t
56:1
(Top Level)
R Script
Console
> B.maps <- get.gene.maps(B)
> View(B.maps)
> ?noodle.effect
Environment
History
Import Dataset
List
Global Environment
Data
B.drugs 15 obs. of 2 variables
B.maps 15 obs. of 2 variables
CTLA4.nood... 375 obs. of 5 variables
CTLA4.nood... 22 obs. of 5 variables
Files
Plots
Packages
Help
Viewer
R: Get effect of a noodle (optionally, a piece of noodle)
Find in Topic
Arguments
noodle.table data frame with information about the single noodle
from network object ID (optional)
to network object ID (optional)
Value
integer (1 for activation, 2 for inhibition; NA if can't be computed)
Note
If from is downstream of to or is the same, NA will be returned. If

An Example


RStudio
File Edit Code View Plots Session Build Debug Profile Tools Help
Addins
Project: (None)
MetaBase r script demo4.R
interactions use case - v2.R*
CTLA4.noodle.end
CTLA4.noodle
LCK.inter
Filter
noodle_id id link_id effect y
1 800009 167 -2044337995 2 0
2 800009 119 2673 1 50
3 800009 -1675277103 1057 1 100
4 800009 120 2861 1 150
5 800009 2093 NA NA 200
18 800019 167 -2044337995 2 0
19 800019 119 2673 1 50
20 800019 -1675277103 1057 1 100
21 800019 120 7083 1 150
22 800019 467 -1339798971 1 200
23 800019 2093 NA NA 250
24 800020 167 -1364360234 0 0
25 800020 4123 3652 1 50
26 800020 119 2673 1 100
Showing 1 to 15 of 375 entries
Console
> CTLA4.noodle.end <- get.nwobj.noodletable(end = 167)
> View(CTLA4.noodle.end)
> View(CTLA4.noodle)
Environment History
Import Dataset
List
Global Environment
Data
CTLA4.no... 375 obs. of 5 variab...
CTLA4.no... 22 obs. of 5 variabl...
gene.noo... 821 obs. of 2 variab...
LCK.inte... 1 obs. of 6 variables
Files Plots Packages Help Viewer
R: Get noodles table for the input network objects
Arguments
nwobjs vector of network object IDs for which to retrieve noodles
from vector of network object IDs; for tracing noodles from this to another objects
to vector of network object IDs; for tracing noodles from other objects to this one
start vector of network object IDs; for tracing noodles which begin with these objects