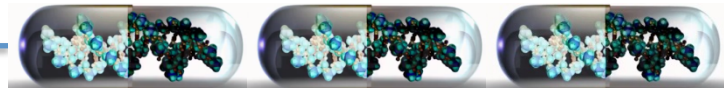
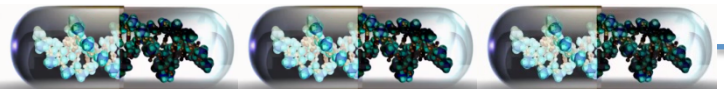
The University of Texas at Austin
Waggoner Center for
Alcohol & Addiction Research



Using LINCS to Identify Novel Drugs for Alcohol Dependence Treatment

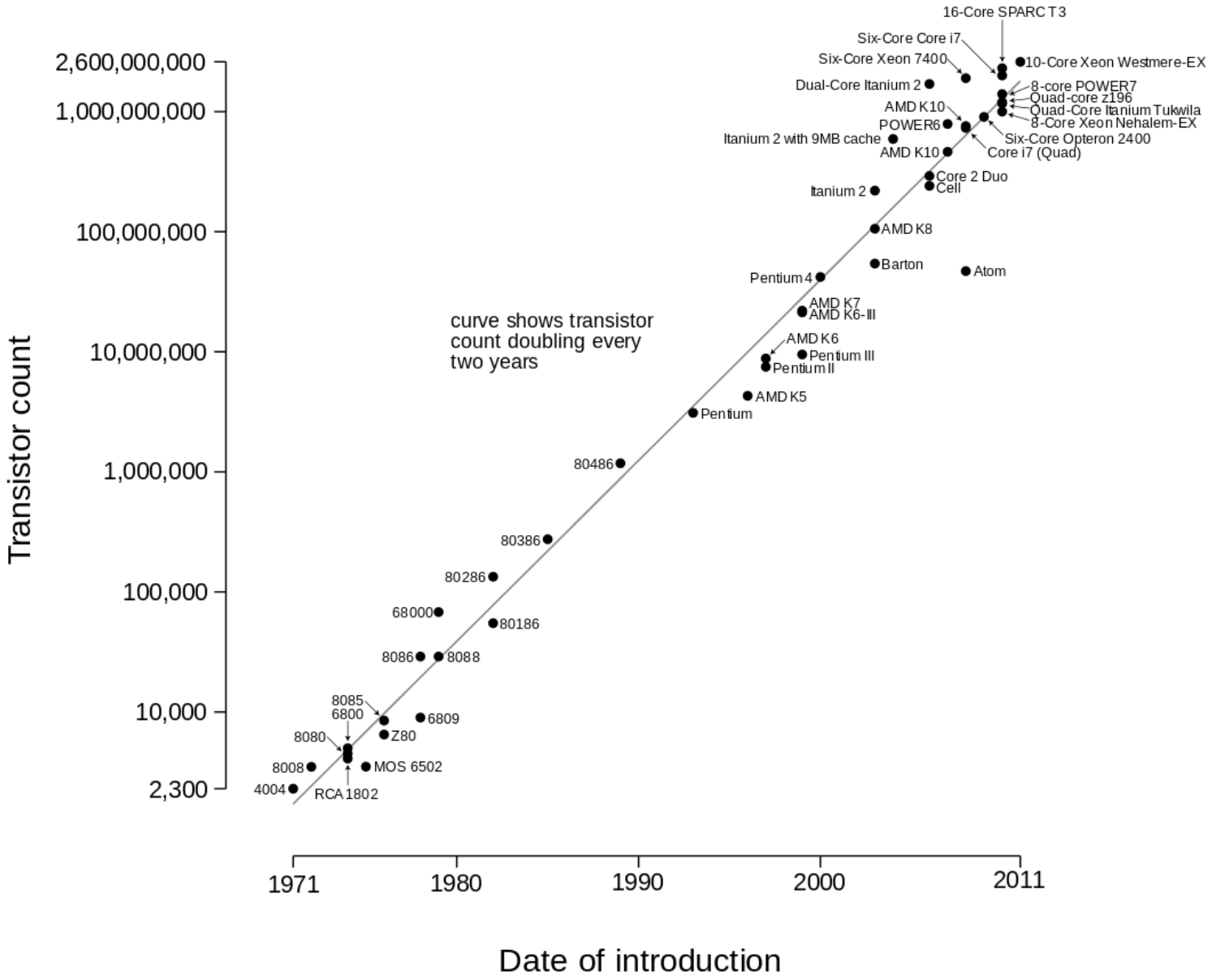
Byte Club Meeting

Thursday, June 16



Laura Ferguson
Graduate Student
Laboratory of Dr. R. Adron Harris
University of Texas at Austin

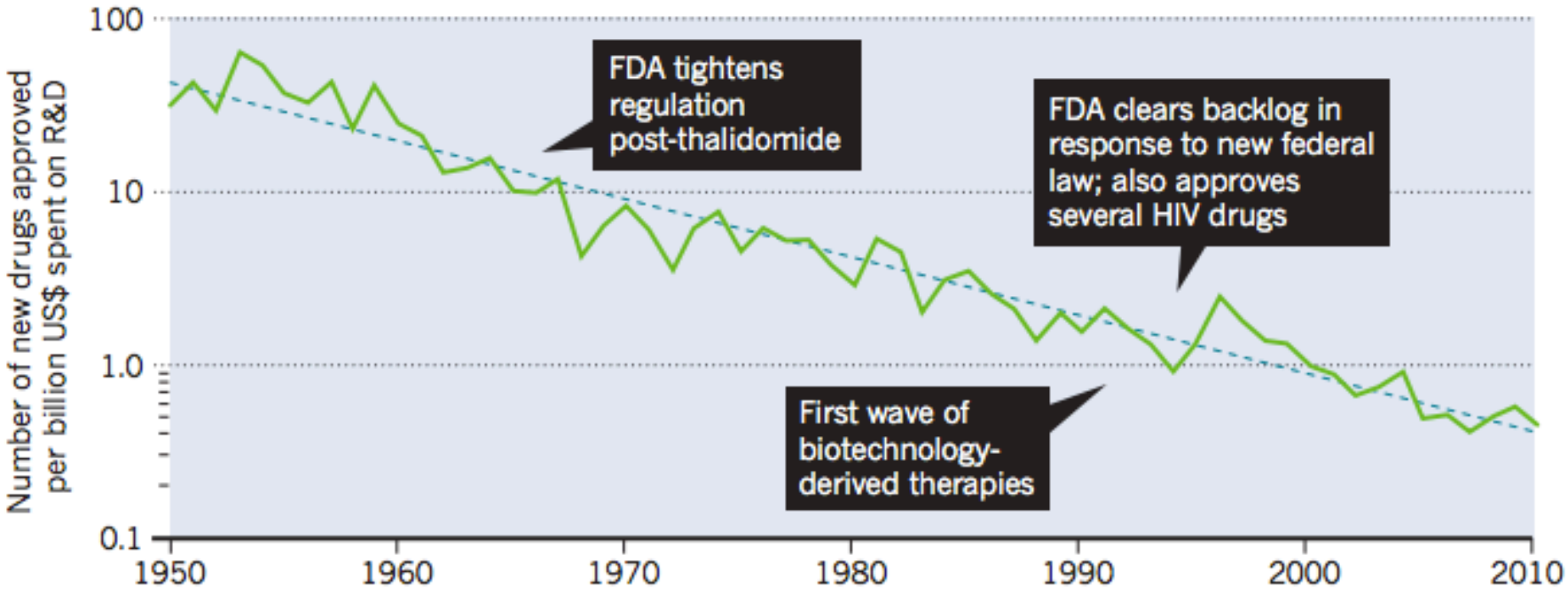
Microprocessor Transistor Counts 1971-2011 & Moore's Law

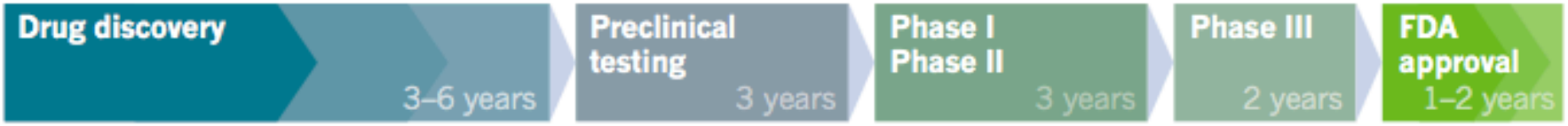


June 14, 2016
Can You Teach Old Drugs New Tricks?
Nicola Nosengo, Nature News

EROOM'S LAW

The efficiency of research and development of new drugs in the United States halves every nine years or so. Drug developers sometimes call this Eroom's law — Moore's law for microprocessors in reverse. Repositioning drugs could help to counter this decline.





A SHORTER TIMESCALE

Because most repositioned drugs have already passed the early phases of development and clinical testing, they can potentially win approval in less than half the time and at one-quarter of the cost.

Can we use genomics to predict drugs that will correct complex disorders (like alcoholism)?

Dudley et al., 2011. **Computational repositioning of the anticonvulsant topiramate for inflammatory bowel disease.** *Sci Transl Med.*

Used gene expression data from IBD patient intestine biopsies to predict drugs to treat IBD– found topiramate which was validated in vivo.

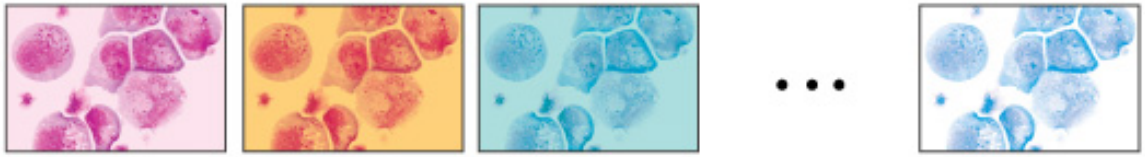
Liu et al., 2015. **Treatment of obesity with celastrol**. *Cell*

Used gene expression data to predict celastrol, which led to a 45% weight loss in diet-induced obese (DIO) mice

LINCS Dataset

9-78 cell types

5 neuronal cell types



Chemical & Genetic Perturbations

Includes many FDA-approved Drugs (e.g., naltrexone)

Gene over expression

> 3,000 ORFs

Chemical compounds

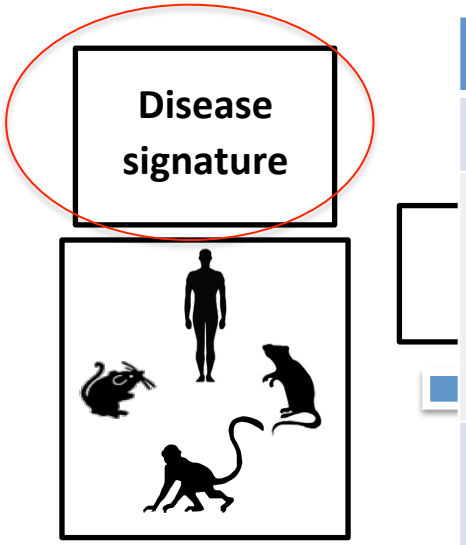
> 19,000 small molecules

Gene knock down

> 12,000 x3 constructs

Expression profile

Identifying a compound to treat alcoholism



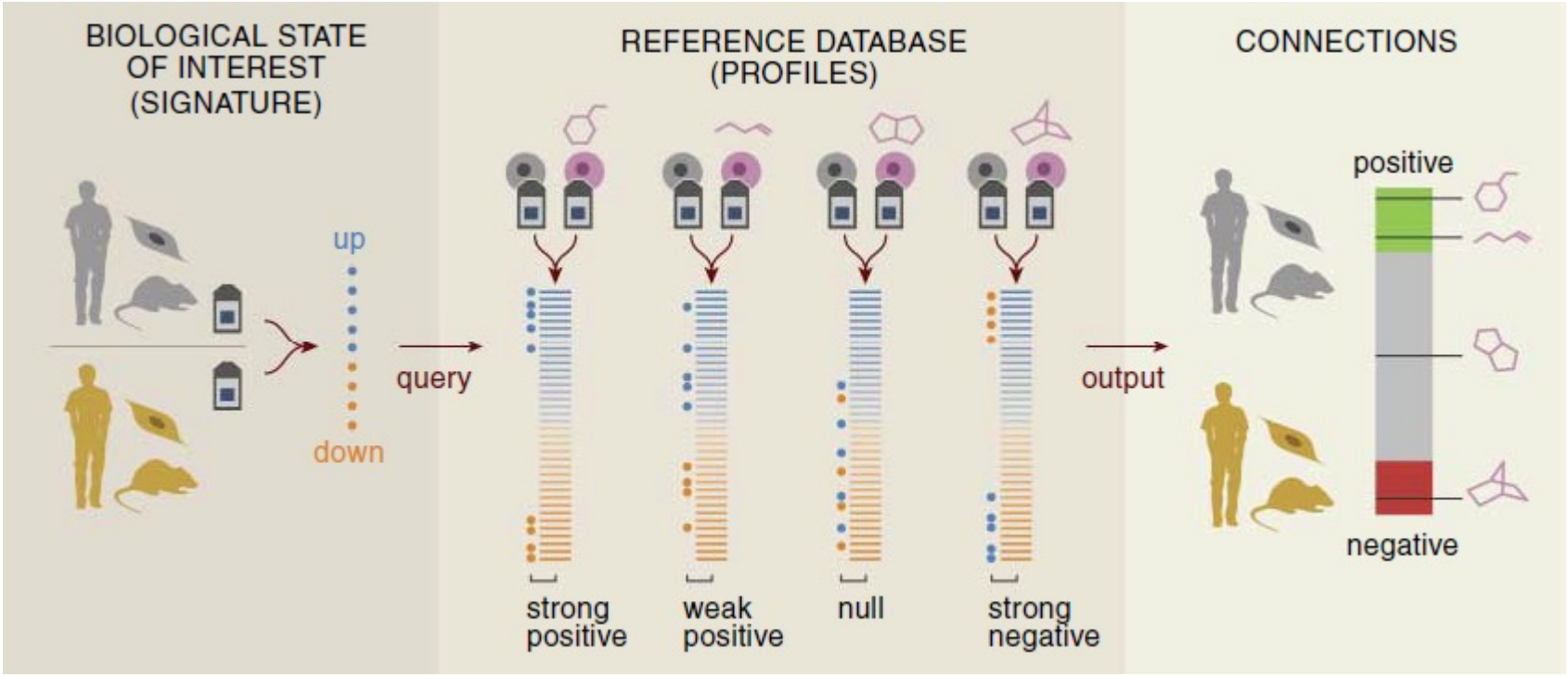
Datasets		
Species	Brain area(s)	Treatment
HDID mice	PFC, NacC, NacSh, BNST, BLA, CeA, VTA, VS, OC	etoh naïve
C57Bl/6J mice	PFC	Continuous 2BC DID 2BC EOD 2BC
Macaque	PFC, CeA	chronic/ withdraw
Human	FC, CeA, BLA	Alcoholics
Rat	various	various

1. Generate a "disease signature," i.e., a list of genes that have increased or decreased expression in human alcoholics or animal model vs controls

SCIENCE VOL 313 29 SEPT 2006

The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease

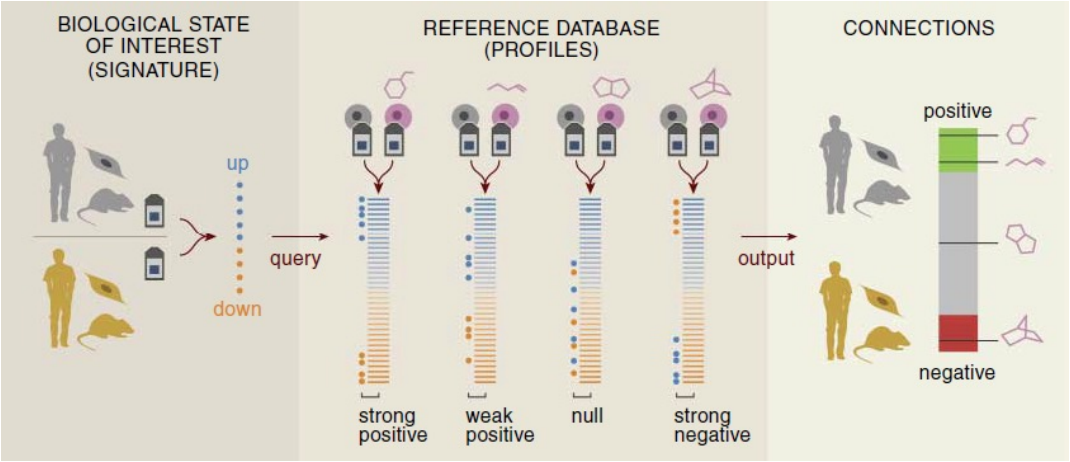
Justin Lamb, (Eric Lander, Todd Golub), et al. Harvard/MIT/Broad



Kolmogorov Smirnov non-parametric rank statistic

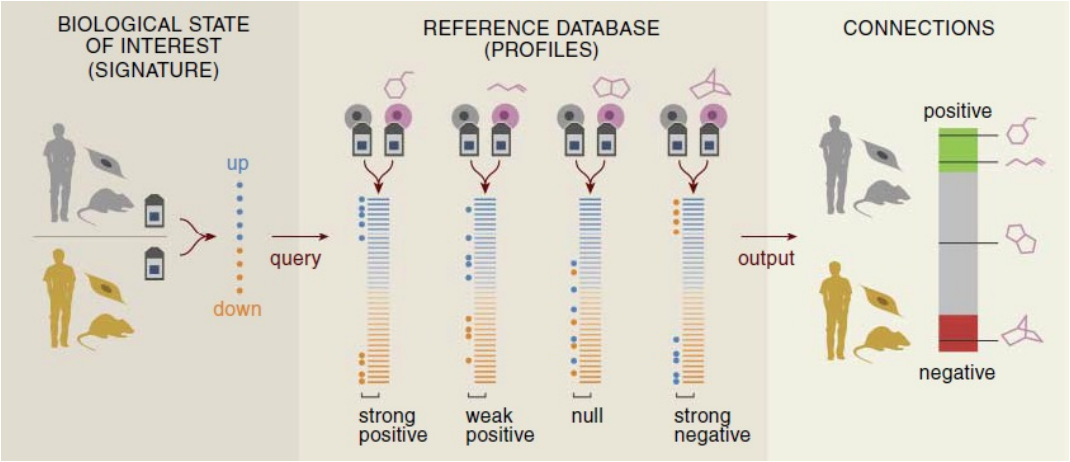
http://www.broadinstitute.org/cmap/help_topics_linkified.jsp#how connectivity score is calculated

Current Algorithm



Even though the up score is very high
Score = 0
Because the up and down genes are being changed in the same direction overall

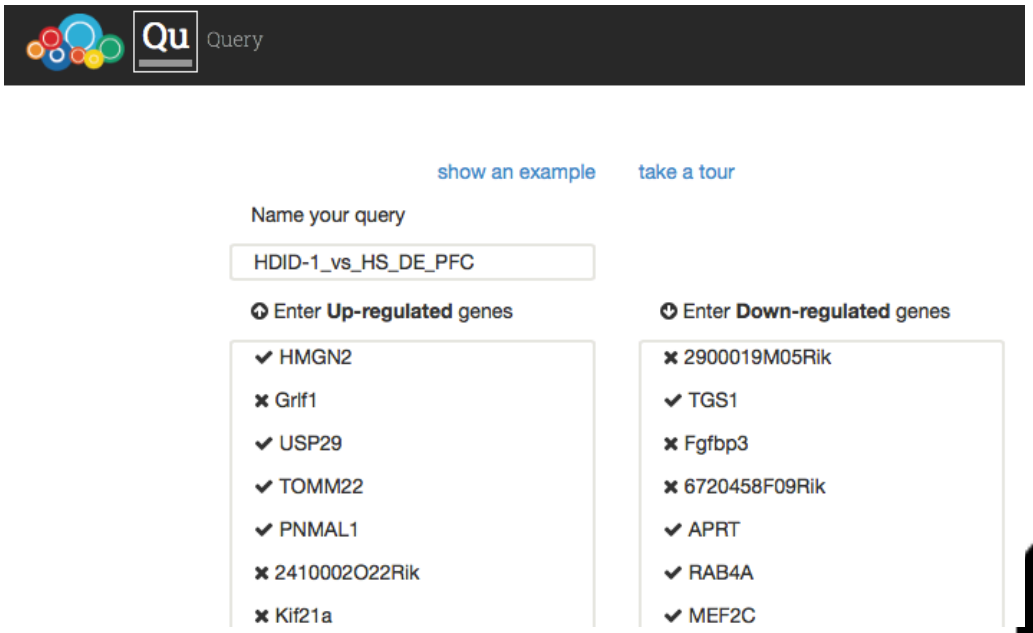
Better Algorithm?



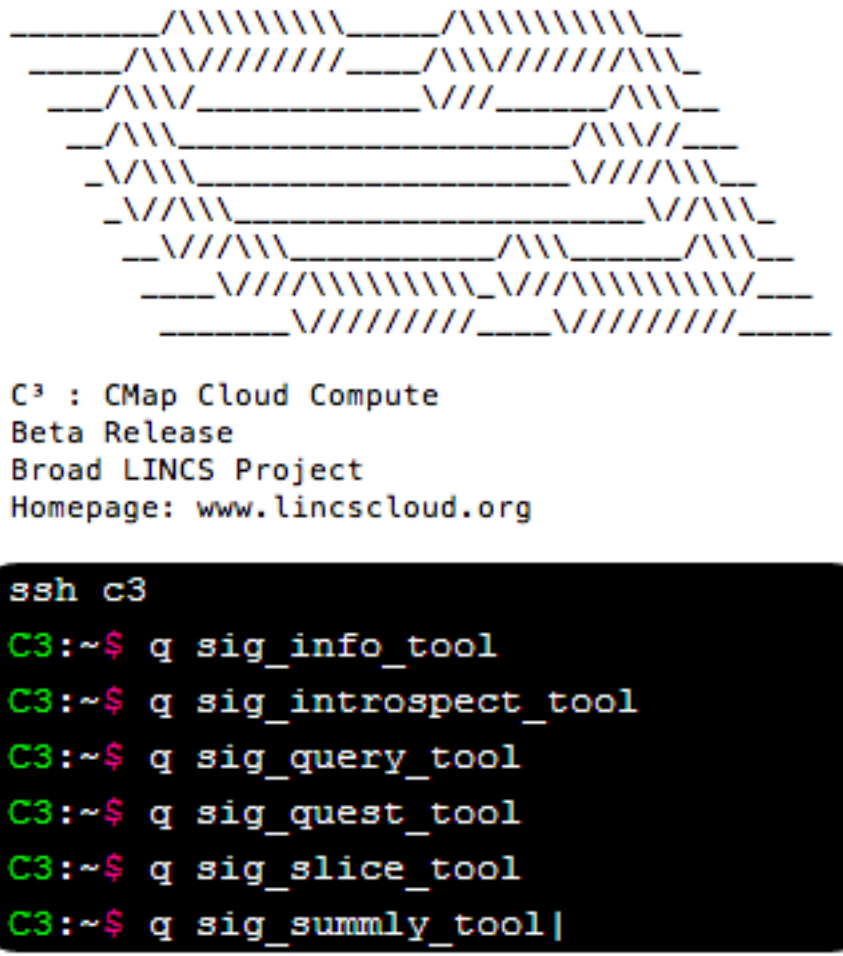
Take max between up and down score

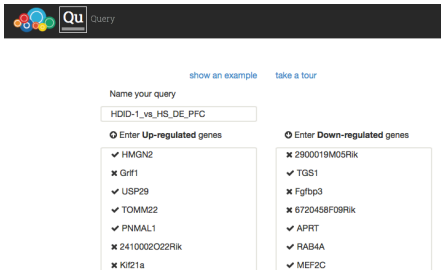
2 ways to query LINCS

Query using the web app
http://apps.lincscloud.org/query



Query using C3 – Compute Connectivity on the Cloud






```
ssh c3
C3:~$ q sig_info_tool
C3:~$ q sig_introspect_tool
C3:~$ q sig_query_tool
C3:~$ q sig_quest_tool
C3:~$ q sig_slice_tool
C3:~$ q sig_summly_tool
```

	Query with web app Summly (not corrected)	Query with C3 Gutc (corrected)
Which experiments are used for analyses	perturbagens that give reproducible signatures	well-characterized perturbagens that give reproducible signatures ('touchstone' set)
Whether scores include correction	None	provides a measure of how unusual the connectivity score for a given drug is, relative to that drug's score across all cell lines, times and doses
How scores are summarized and reported	summarizes results in the 2, 4, and 6 cell lines in which the connections have strongest magnitude	uses the max quantile summarization method, so it produces just a single score

Parameter	Options
Input gene signature	• All DE genes • Top 100 DE genes • L1000 landmark genes • DE genes within modules or pathways of interest
Genes in LINCS database	• L1000 landmark genes • “bing” (best inferred) genes • All
Cell types	• Brain cell types • All cell types
Experiments	• Touchstone • Gold • All • (“touchstone” and “gold” are subsets of experiments that the BROAD has determined are the most reproducible)

LINCS API

http://api.lincscloud.org/

Lots of meta-data – you could answer a lot of interesting questions just by querying the LINCS meta-data!

Query examples:

- **Lookup expression of a gene across core cell lines**

/a2/geneinfo?q={"pr_gene_symbol":”OPRM1"}&l=1

- **Return meta-information for terreic-acid**

/a2/pertinfo?q={"pert_iname":”terreic-acid"}

- **Return only the specified fields – for a drug get signature cell ids, signature strength, and top regulated genes**

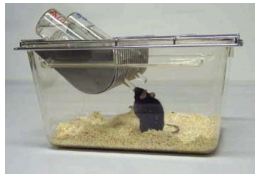
/a2/siginfo?q={"pert_desc":”terreic-acid"}&f={"cell_id":1,”distil_ss":1, “up50_lm”: 1, “dn50_lm”:1}

Identifying a compound to treat alcoholism

- Existing FDA approval / clinical trial
- Cross BBB – brain access
- Acceptable side effects
- Predicted to be effective in multiple brain areas or across species

Prioritize compounds

Test candidates in vivo



3. Test the compounds in preclinical model

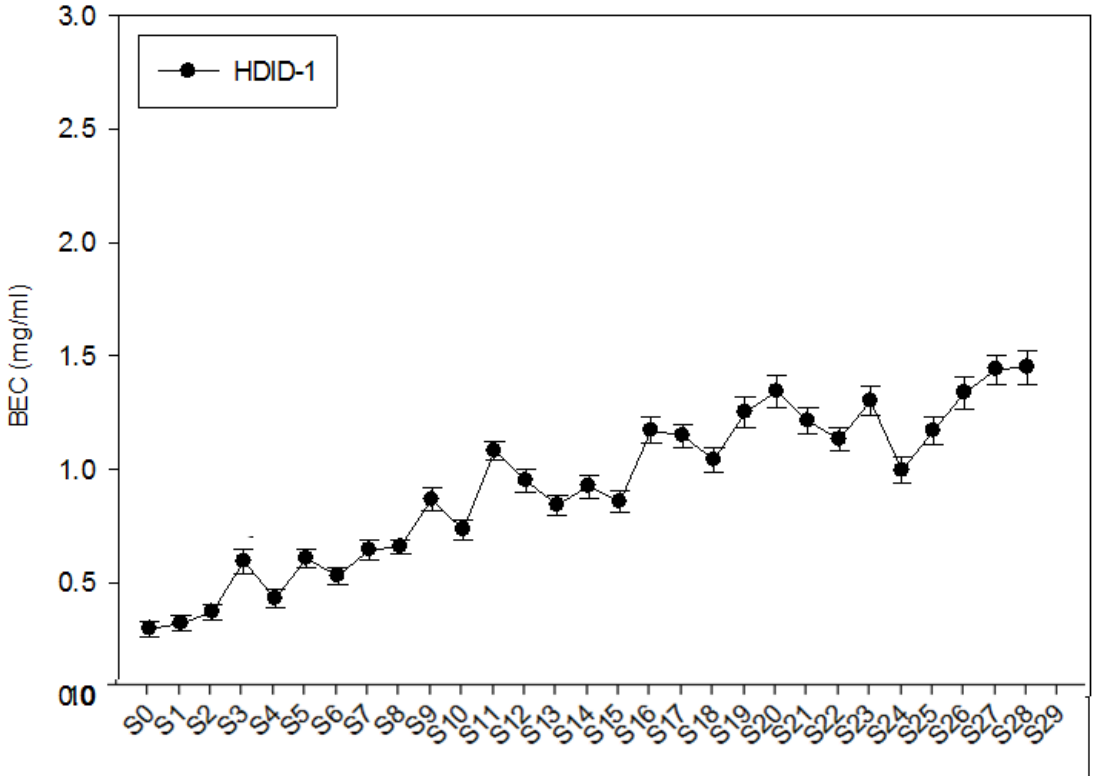
Proof-of-principle

HDID MICE

HDID mice
(High Drinking in the Dark mice)

Genetic model for “binge-drinking”

- Drinking in the Dark
- Selected for high BEC



Identifying a compound to decrease excessive ethanol consumption in HDID mice

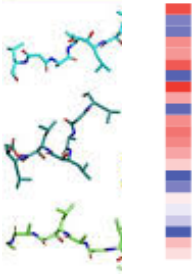
Disease signature

etoh naïve HDID mice

PFC, NacC, NacSh, BNST, BLA, CeA, VTA, VS, OC, whole brain

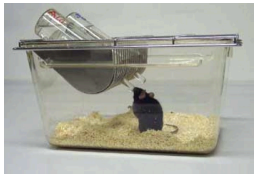
LINCS

Candidate Drugs

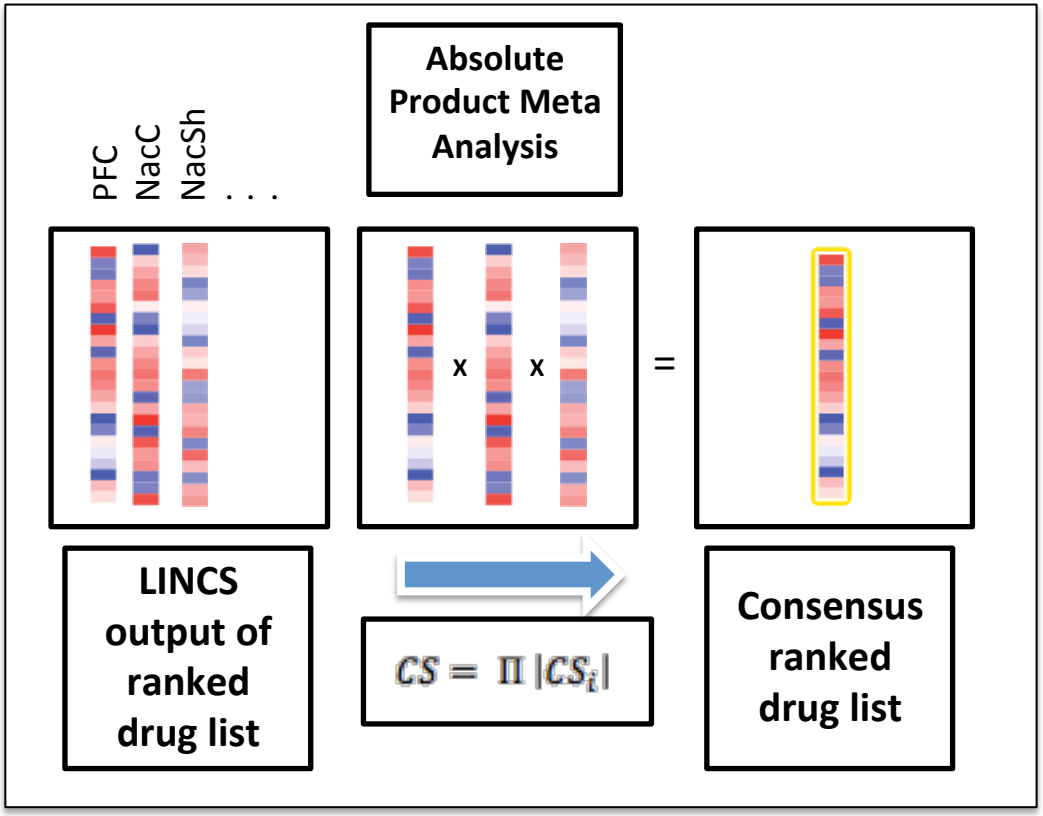


Prioritize compounds

Test candidates in vivo

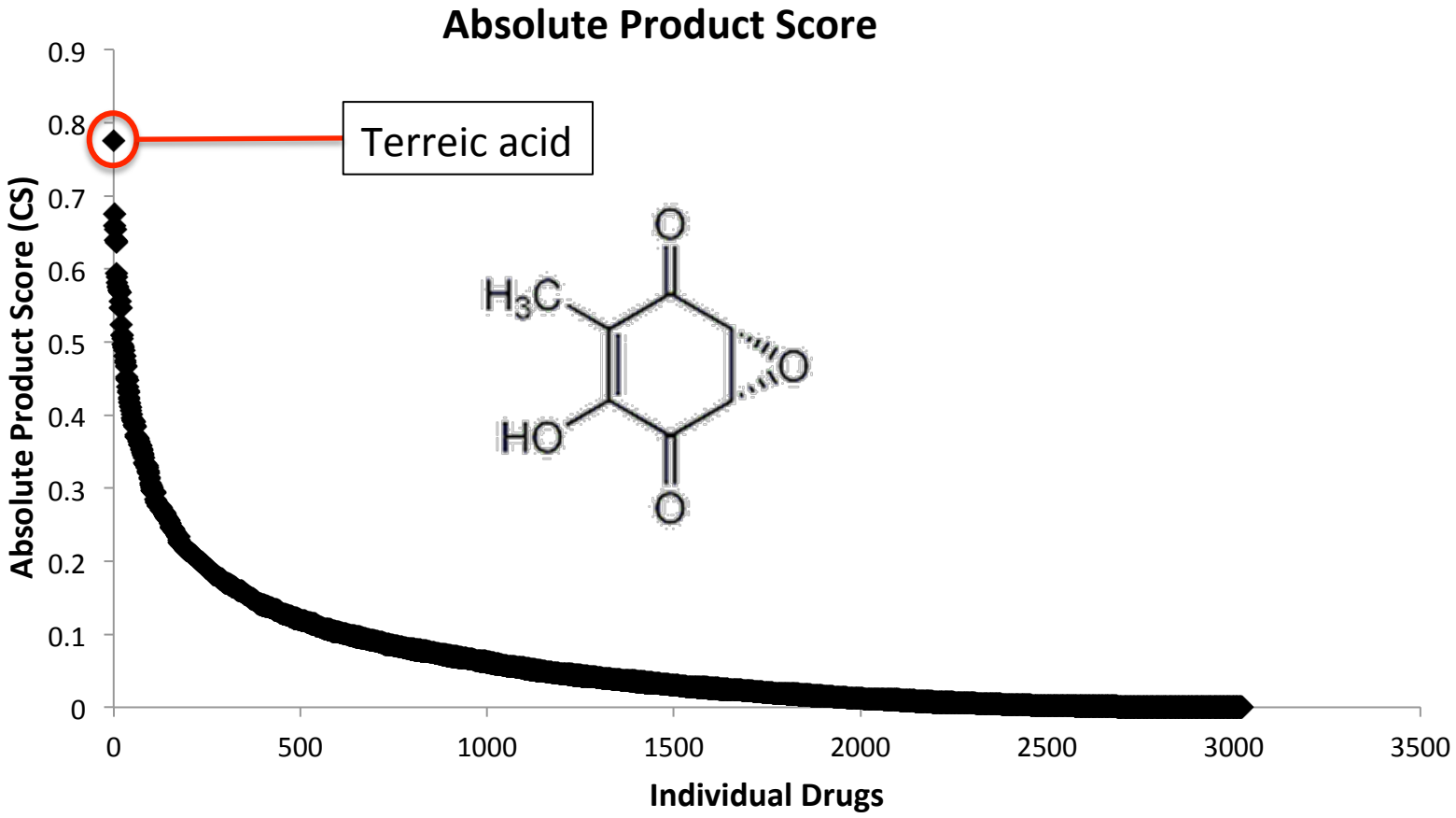


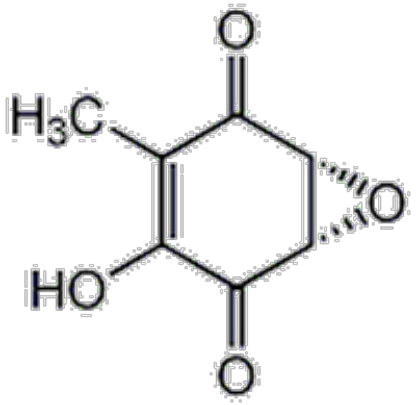
1. Generate a "genomic signature," i.e., a list of genes that have increased or decreased expression in etoh naïve HDID mice vs controls
2. Identify compounds with signatures that have a strong connectivity to the disease signature
3. Test the compounds in HDID mice



Fortney et al., 2015. **Prioritizing Therapeutics for Lung Cancer: An Integrative Meta-analysis of Cancer Gene Signatures and Chemogenomic Data**. *PLOS Comp Bio*

Liu et al., 2015. **Treatment of obesity with celastrol**. *Cell*

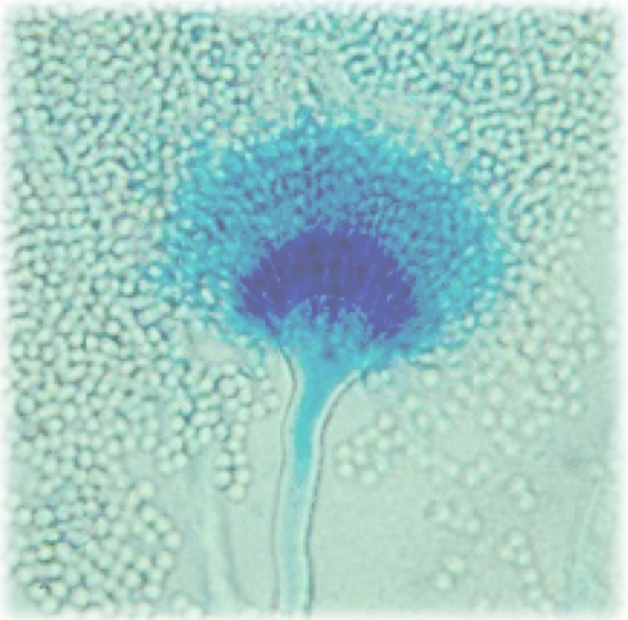

Absolute Product Score
Absolute Product Score (CS)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Terreic acid
H3C
HO
O
O
O
0
500
1000
1500
2000
2500
3000
3500
Individual Drugs



- [illegible] of compound… no known function for btk in alcholism….etc
  - Ito M, et al. Nat Commun 2015

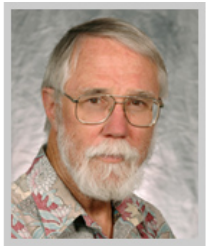
Proof-of-principle

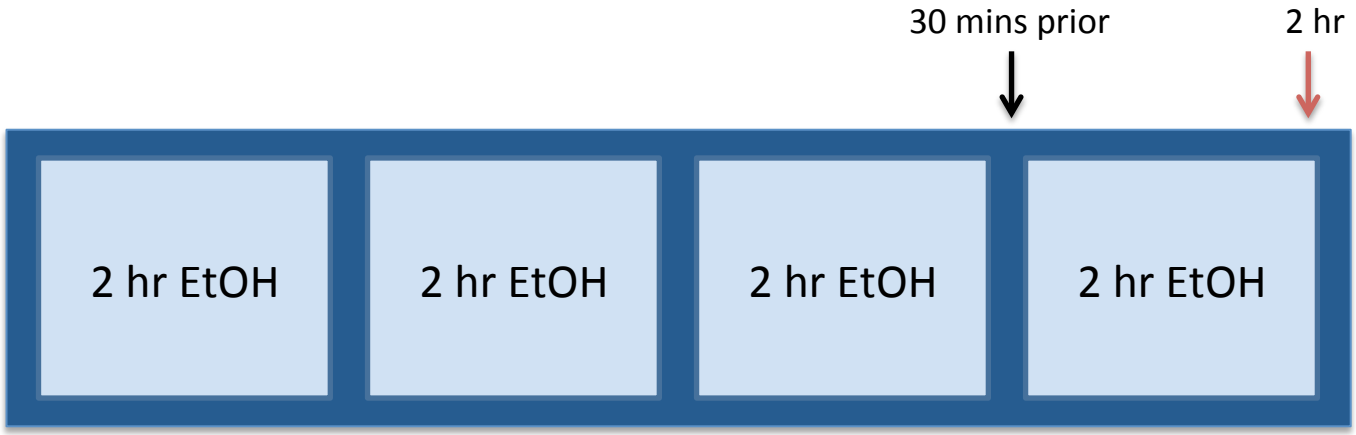
TERREIC ACID

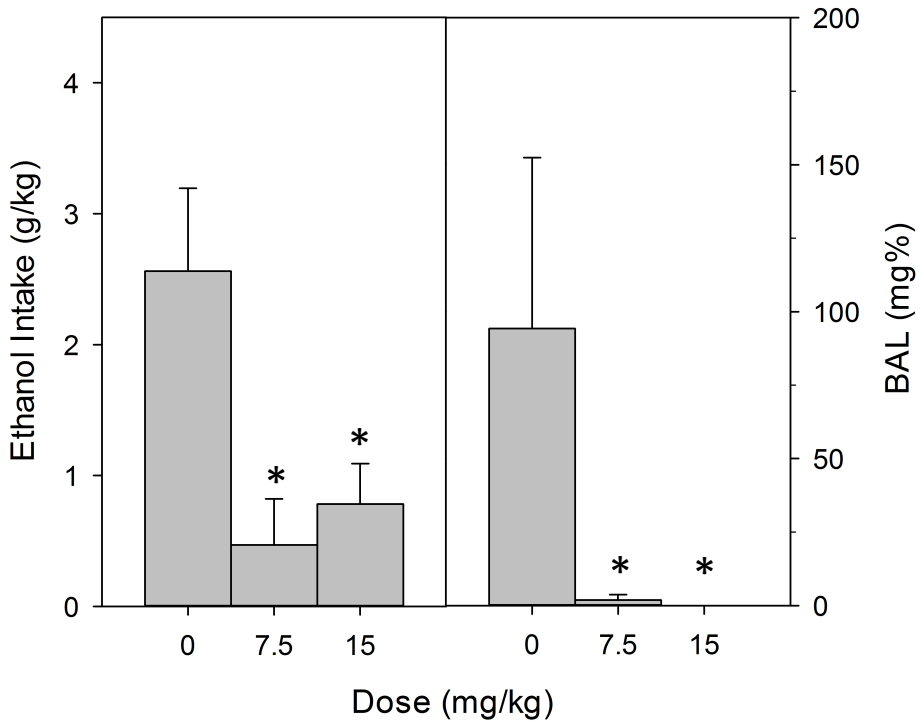


Aspergillus terreus

Testing terreic acid in vivo



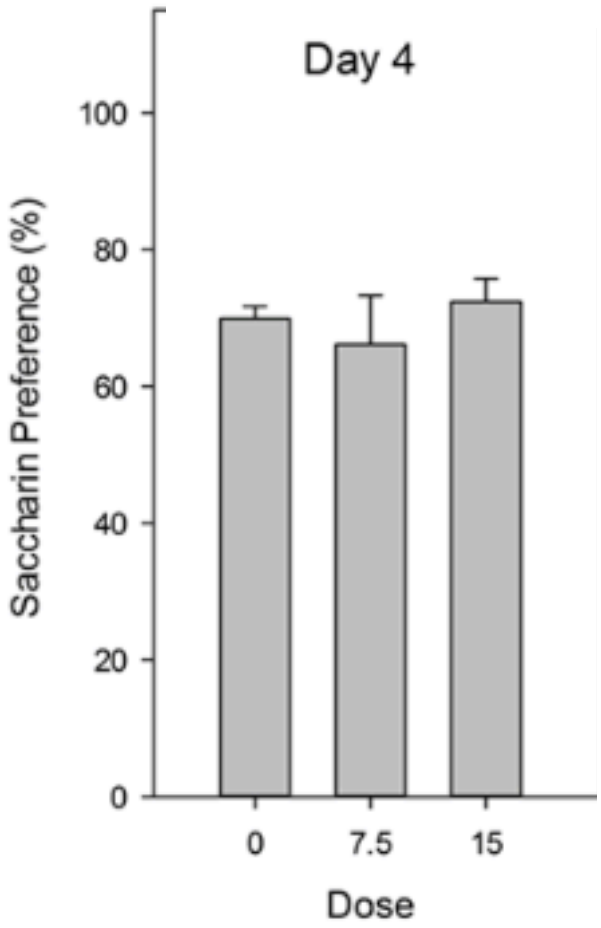




Graphs by John Crabbe and colleagues

* P < 0.05
N=5-6 per dose

Day 4 Saccharin Preference



Note that there was a decrease in overal saccharin consumption that is being investigated…

Graph by John Crabbe and colleagues

Summary

- We used gene expression signatures of HDID mouse brain and gene expression signatures of compounds from the LINCS database to predict drugs that might decrease BALs in HDID mice

- Terreic acid emerged as a top hit from our drug prediction analyses

- When tested in vivo, terreic acid decreased BALs by ~100%

Future Directions

- Use qPCR to determine if genes we predicted to be "corrected" by terreic acid are being "corrected"
- Determine if terriec-acid's effects on a 2-bottle-choice test (20% EtOH vs H2O)
- Select other drugs for HDID mice using different computational strategies

Conclusion

Our successful POC suggests these algorithms could predict other drugs for alcohlism and other psychiatric illnesses

The University of Texas at Austin
Waggoner Center for
Alcohol & Addiction Research

Thank you!

People

- Dr. Adron Harris
- Dr. Dayne Mayfield
- Dr. Igor Ponomarev
- Dr. John Crabbe
- Dr. Angela Ozburn
- Ted Natoli
- Lab mates
- Waggoner Center
- INS

Funding

- NRSA F31 - AA024332-01
- Alcohol training grant - NIH/ NIAAA U01 AA13520
- Bruce/Jones endowed Graduate Studies in Addiction Research
- Waggoner Center
- INS