United States Environmental Protection Agency

Modeling Nuclear Receptor Mediated Pathways in Liver Cancer

Society of Toxicology
March 9, 2010

Virtual Liver Project (v-Liver™)

I Shah, DC Wolf, K Houck, R Judson, J Jack, J Wambaugh, MT Martin, C Corton

This work was reviewed by EPA and approved for publication but does not necessarily reflect official agency policy.

Office of Research and Development
National Center for Computational Toxicology

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Predictive Models of Liver Cancer

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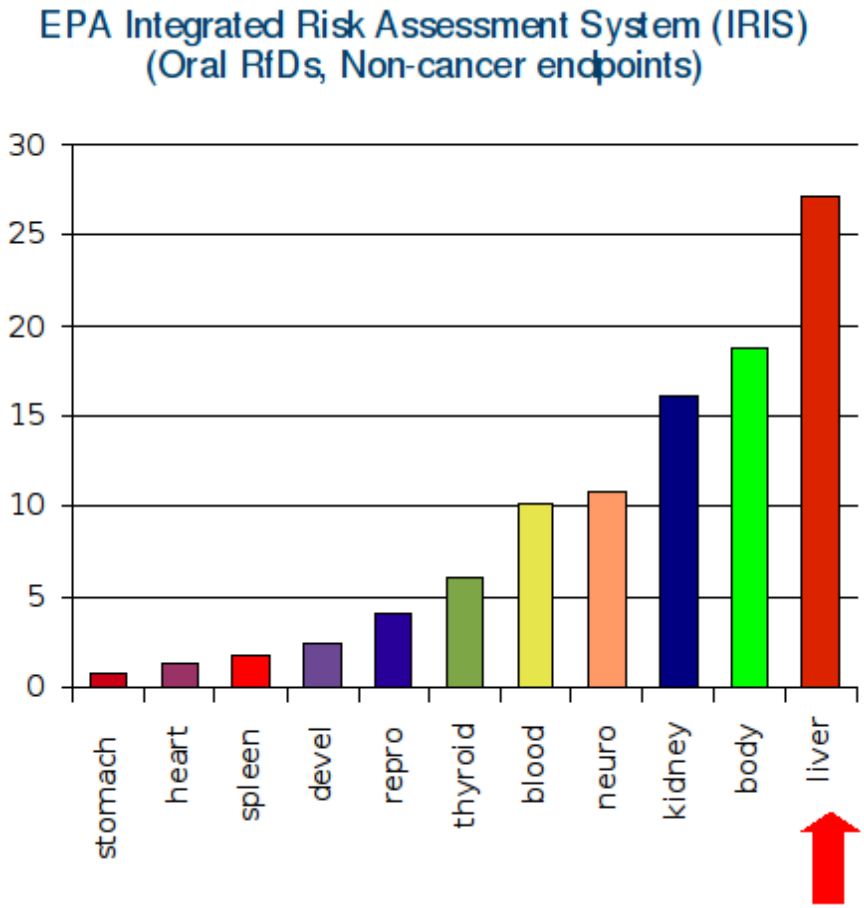
Motivation

- Thousands of environmental chemicals
- Insufficient information on human risk
- Rodent testing infeasible / uncertain results
- Need other decision support tools
- Develop proof-of-concept using
  - Existing *in vivo* data
  - New *in vitro* data
  - Predictive *in silico* systems

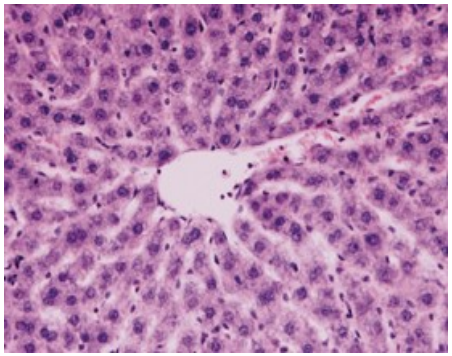
Hepatotoxicity

- Liver primary organ for detoxification
- Frequent site of adverse effects in rodents
- Human relevance of some effect uncertain
- Large amount of available data: *-omics*, histopathology, etc.

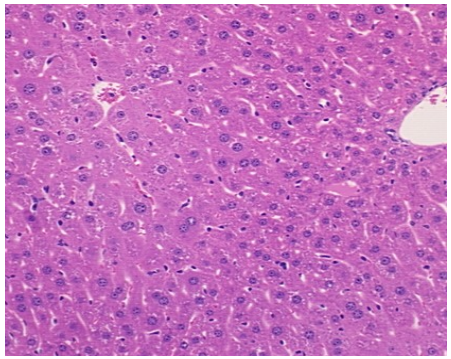


Goal: Predict Tissue Lesions

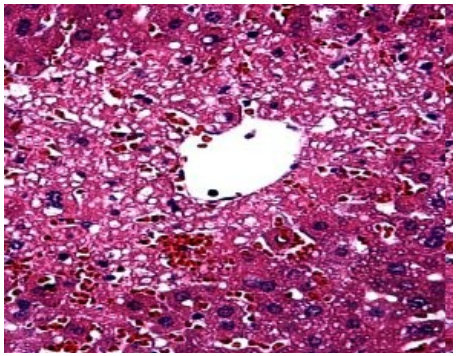
Hydropic Swelling



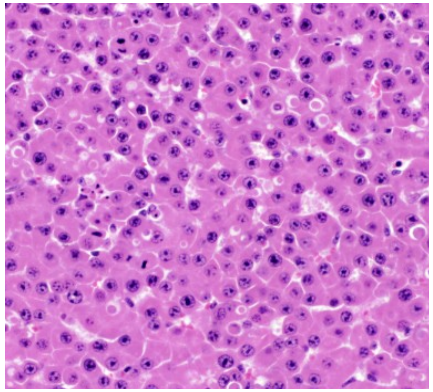
Hypertrophy



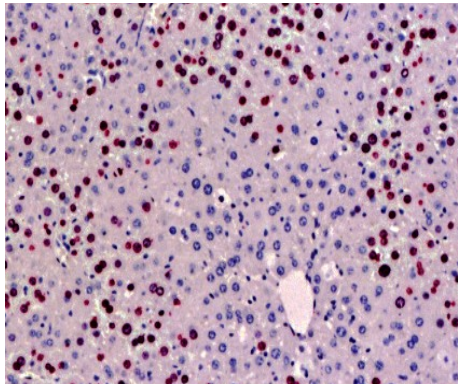
Necrosis



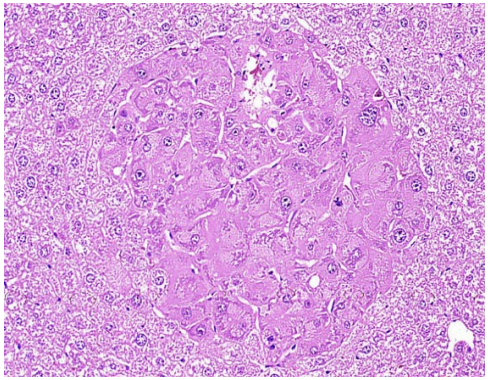
Apoptosis



Regenerative Prol.

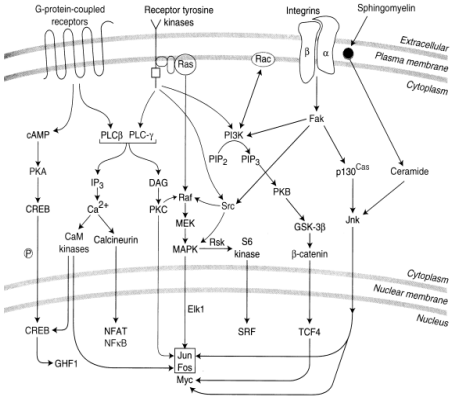


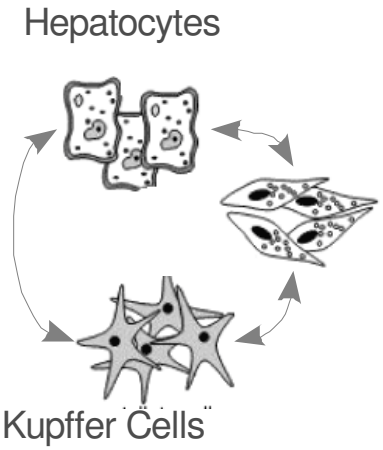
Altered Hep. Focus

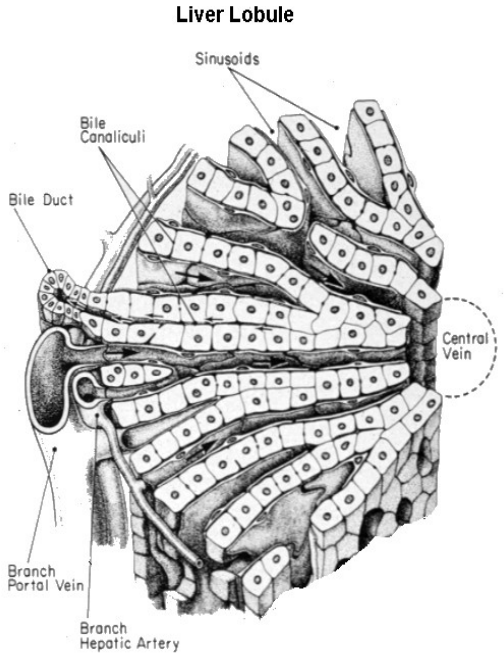


Images courtesy of Douglas C. Wolf, EPA
Fallsehr, *et. al.* World J Gastroenterol 2005 March 7;11(9):1303-1306
Jean-Paul Duong Van Huyen, *et. al. Modern Pathology (2006) 19, 1277–1288.0*
Uskoković-Marković, *et. al. J Pharm Pharmaceut Sci 10(3):340-349, 2007*

Systems View of Lesion Formation







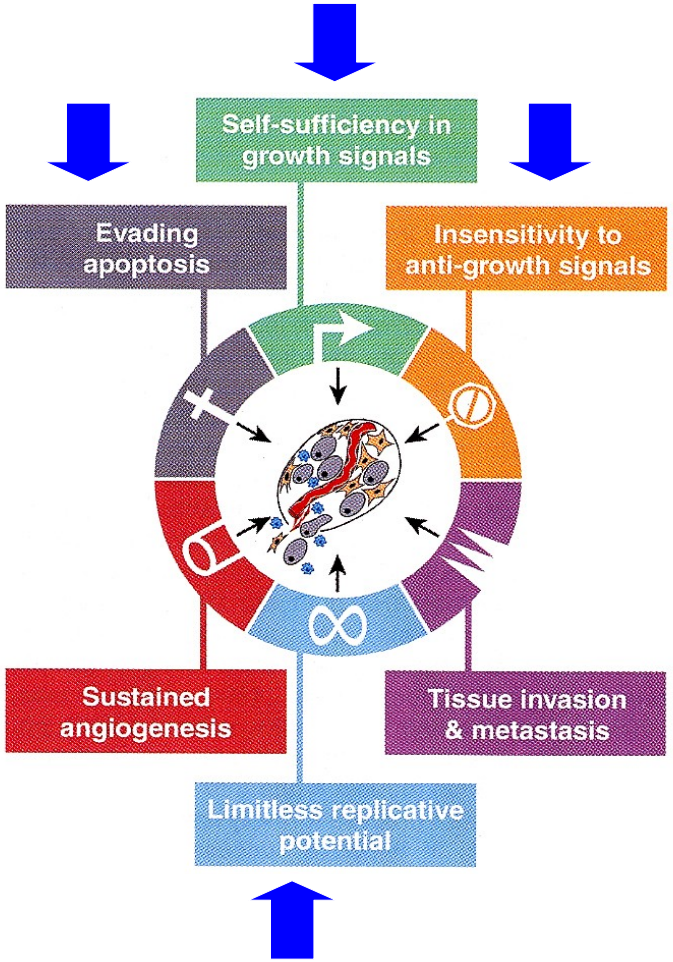
Internal Dose ↔ Pathway Changes ↔ Cellular Alterations ↔ Cell Interactions ↔ Tissue Injury/Repair → Chronic Lesions

Cancer: Modes of Action

Multiple MOAs: Mutagen, mitogen, cytotox
Nuclear Receptor (NR) activation relevant in rodent hepatocarcinogenesis

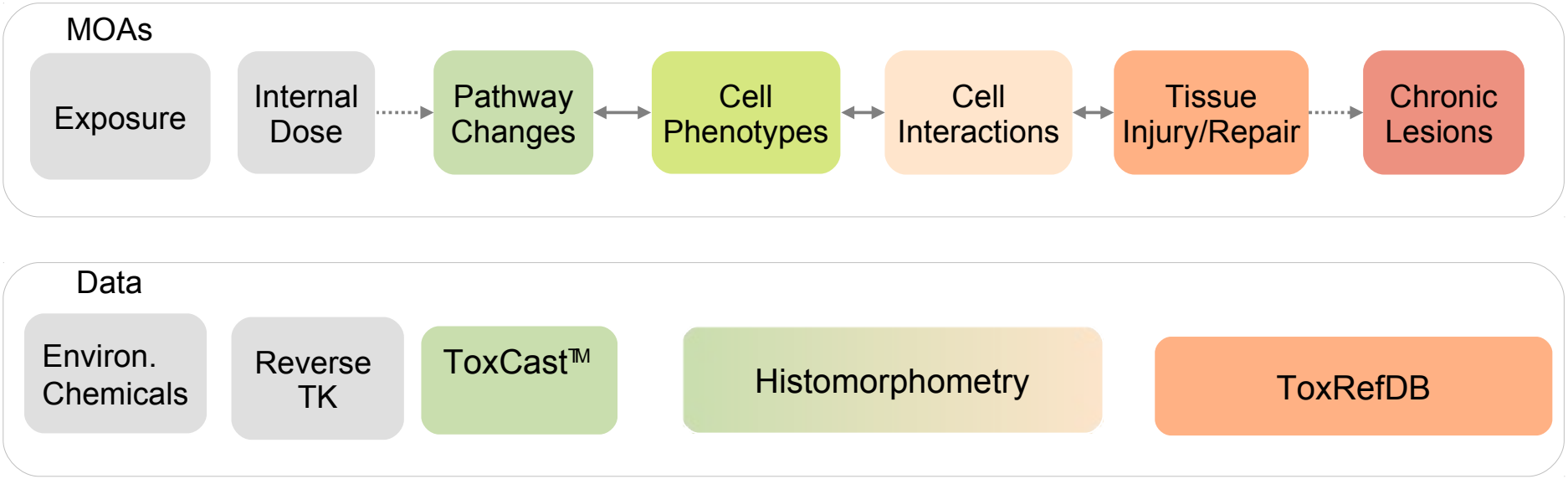
Can we extrapolate events to humans ?

- NR-activation
- Changes in apoptosis/proliferation
- Cancer lesion progression



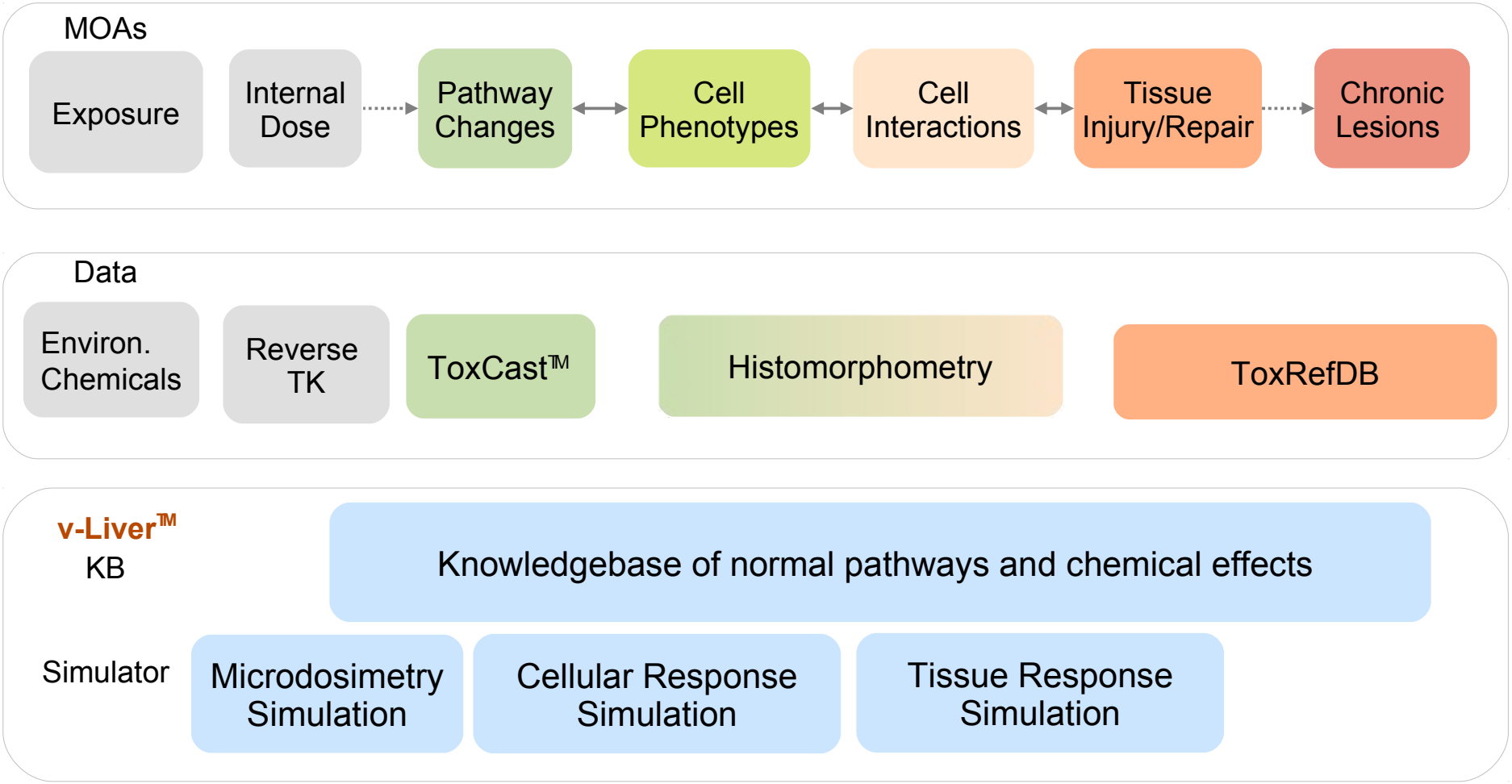
"Hallmarks of Cancer"
Hanahan & Weinberg, 2000

Virtual Liver Overview



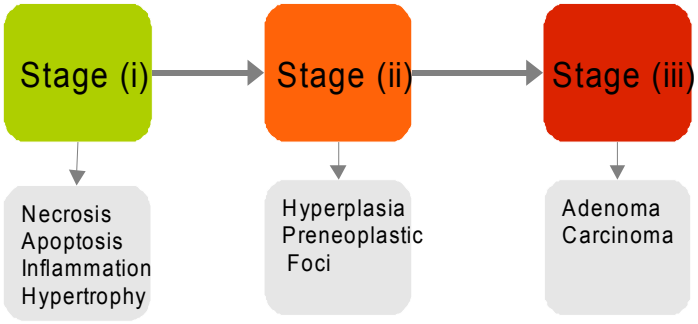
http://www.epa.gov/ncct/toxcast
http://www.epa.gov/toxrefdb

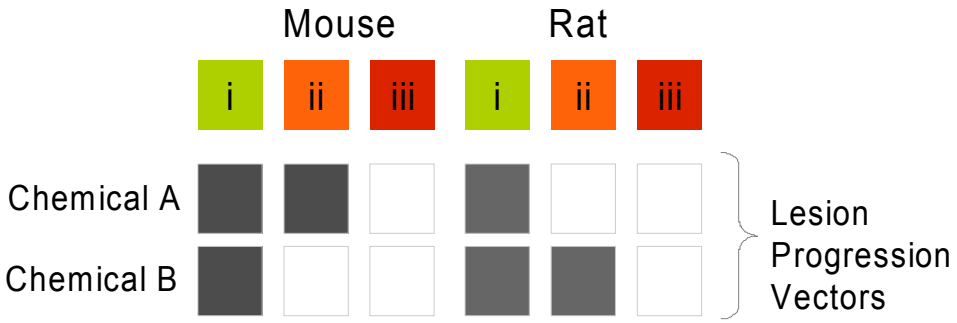
Virtual Liver Overview



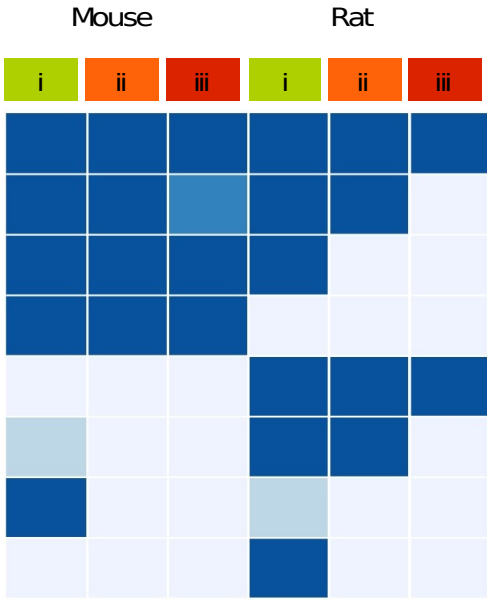
http://www.epa.gov/ncct/toxcast
http://www.epa.gov/ncct/toxrefdb
http://www.epa.gov/ncct/virtual_liver

Selecting Chemicals Using Rodent Histopathology





ToxRefDB

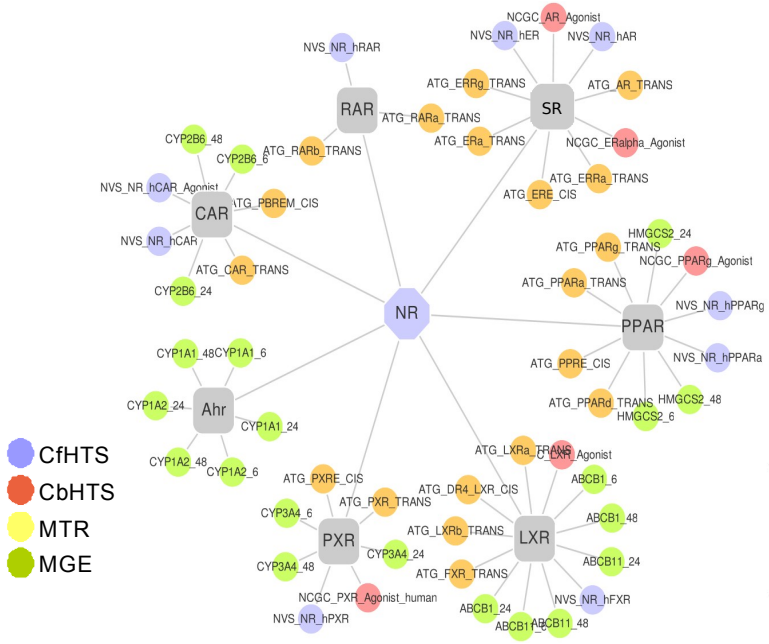


Shah, *et al.* (submitted)

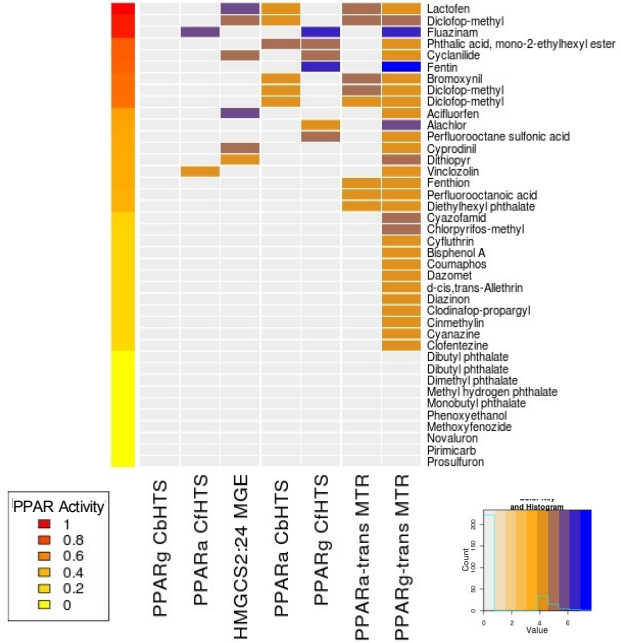
http://www.epa.gov/ncct/toxrefdb

Human NR Activity

(a)



(b)

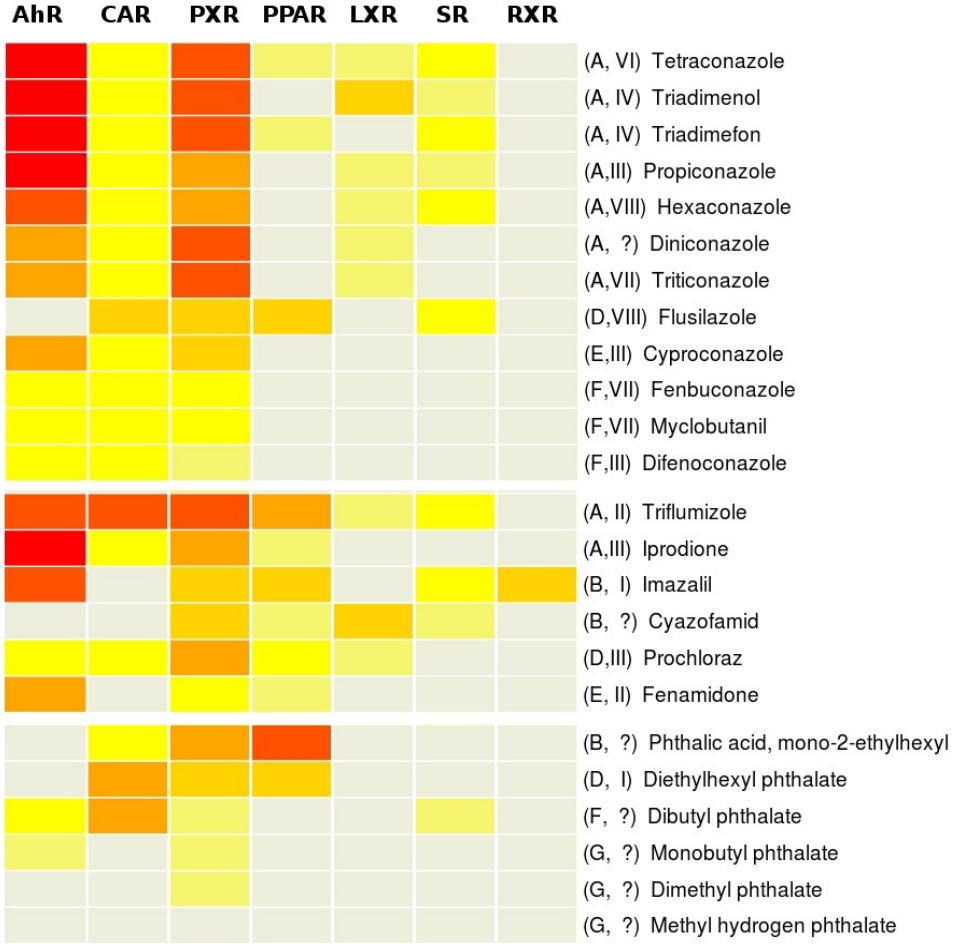


ToxCast: A combination of assays: cell free/cell-based NR bioactivity, multiplexed transcription reporters, multiplexed gene expression

Find the “aggregate” activity of each chemical across NRs e.g. PPARs

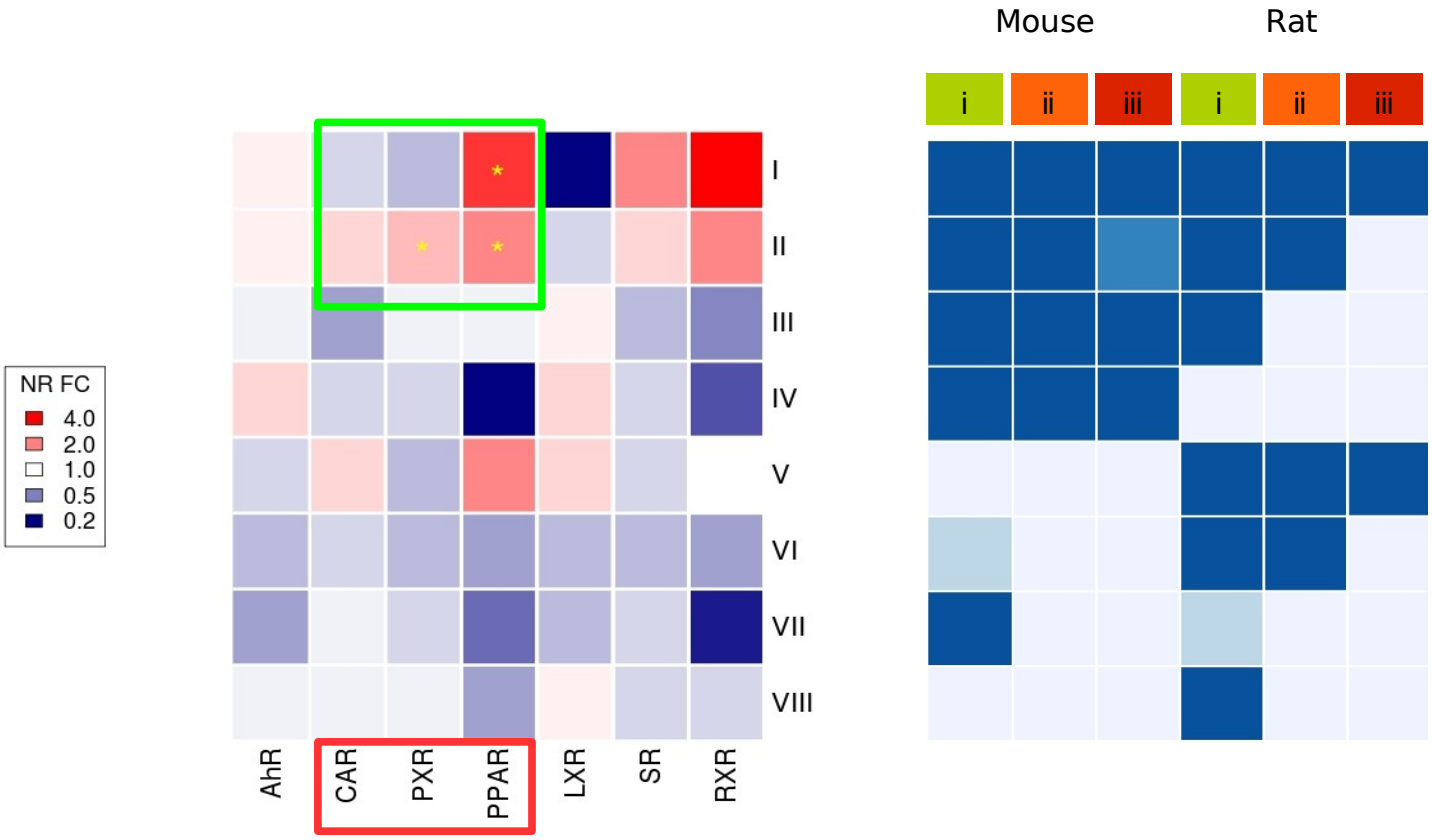
Shah, *et al.* (submitted)

Aggregate NR Activities: Examples



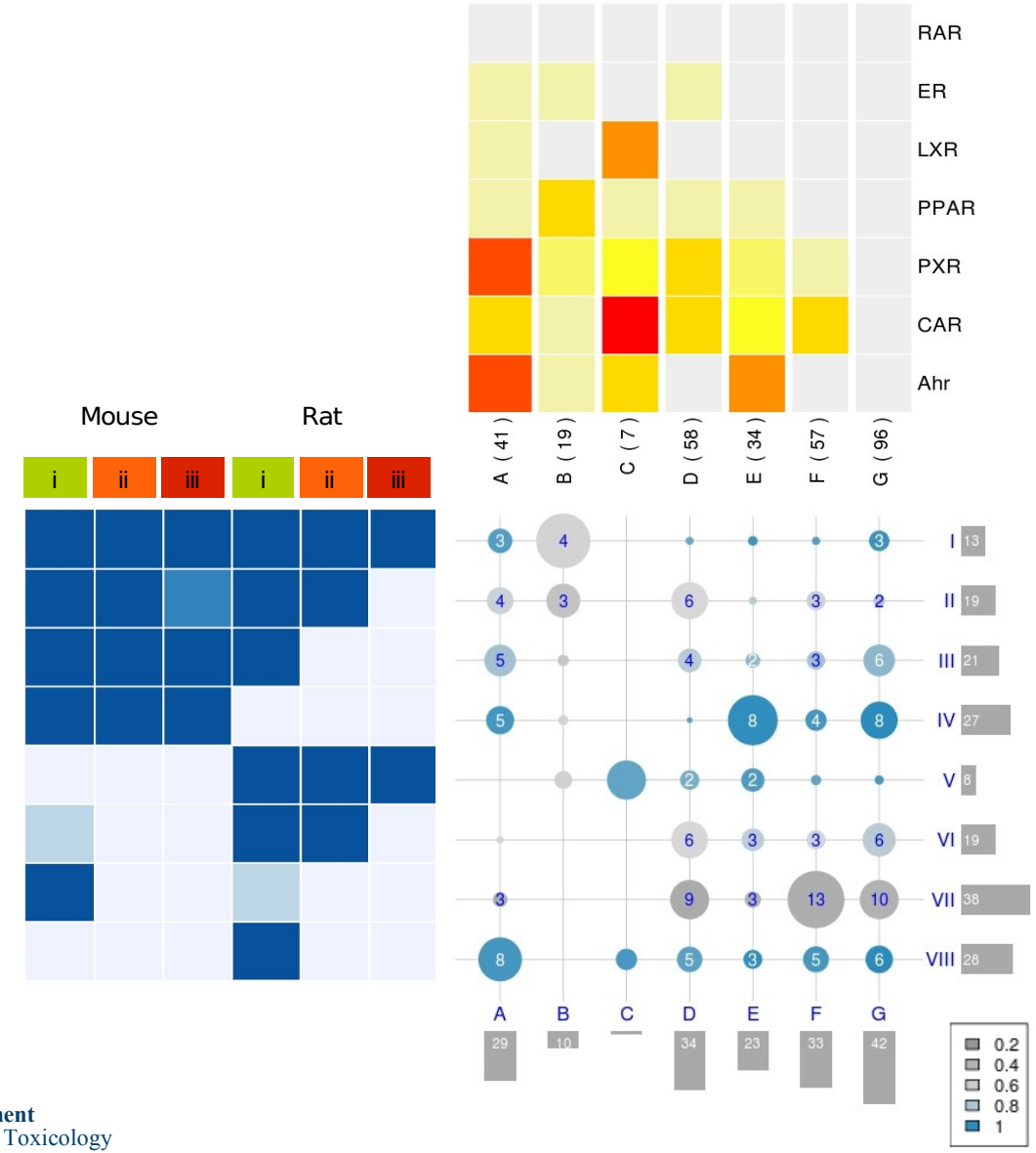
Shah, *et al.* (submitted)

Human NRs and Rodent Cancer



Shah, *et al.* (submitted)

NR activity vs. Rodent Cancer

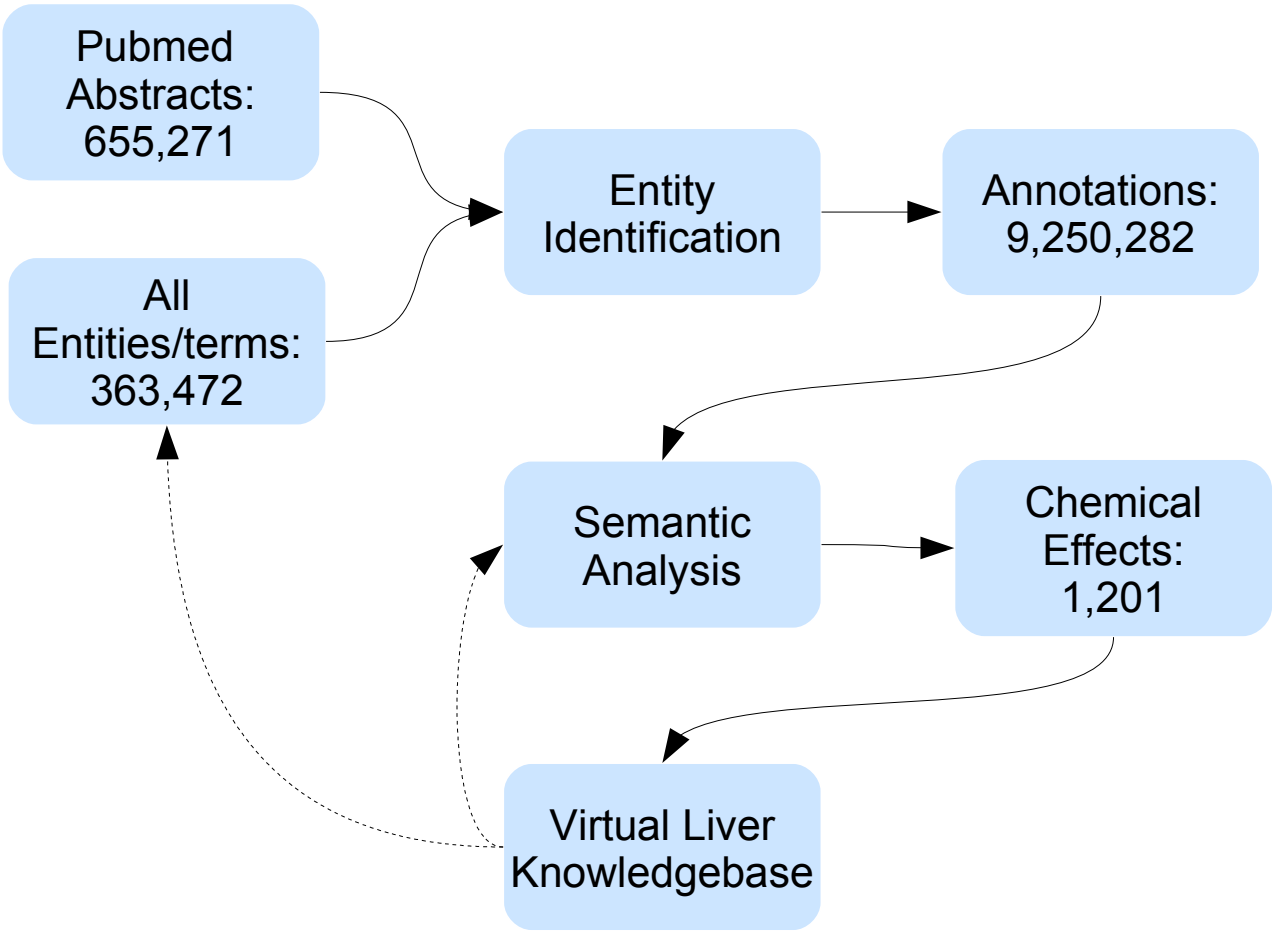


Shah, *et al.* (submitted)

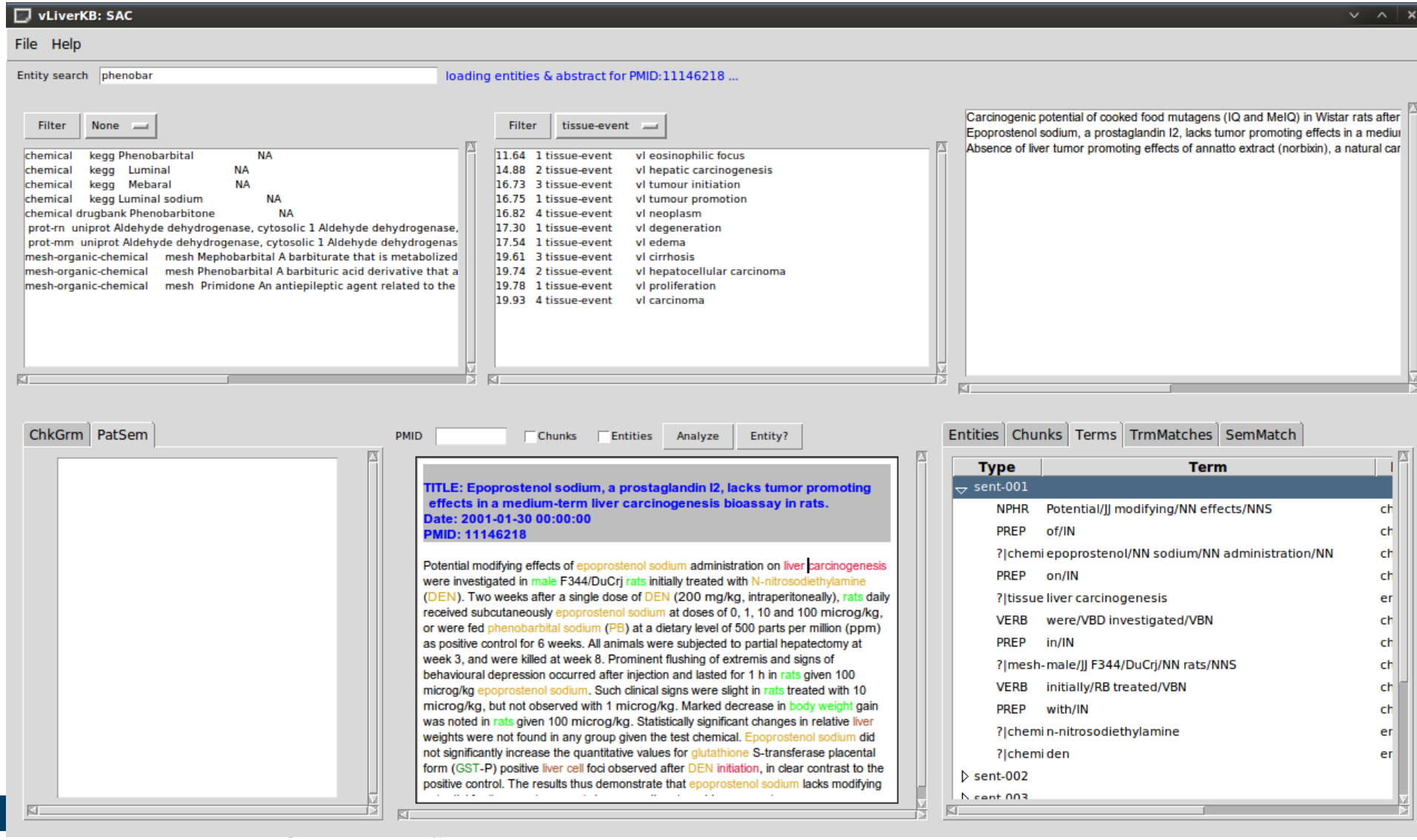
Relating NR Activity and Pathways

- Need to mine information from literature and public domain data
- Use natural language processing (NLP) tools
- Semi-automated curation of chemical-induced effects
- Store all information in v-Liver Knowledgebase

Literature Curation Overview

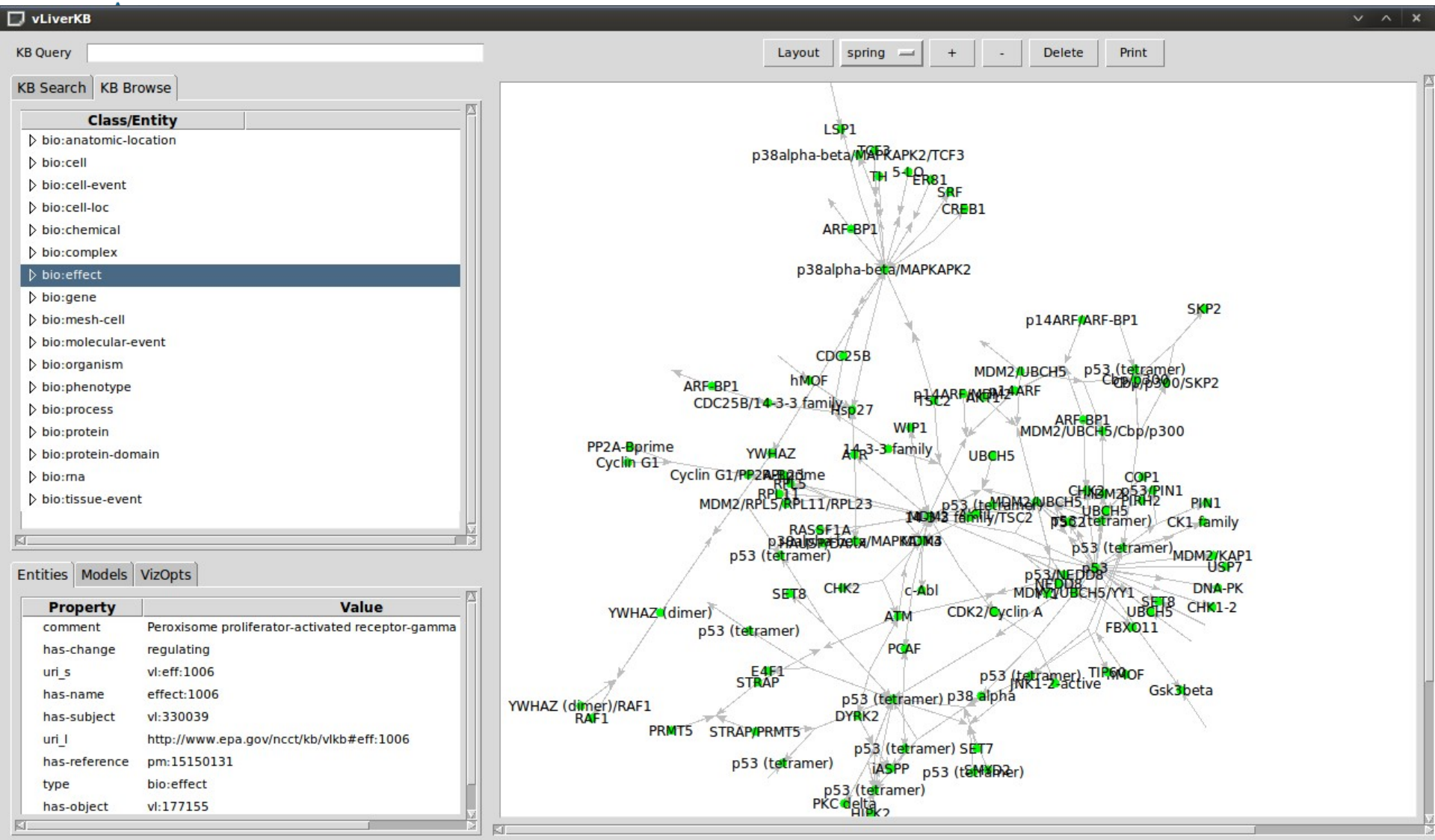


Semantic Analysis & Curation

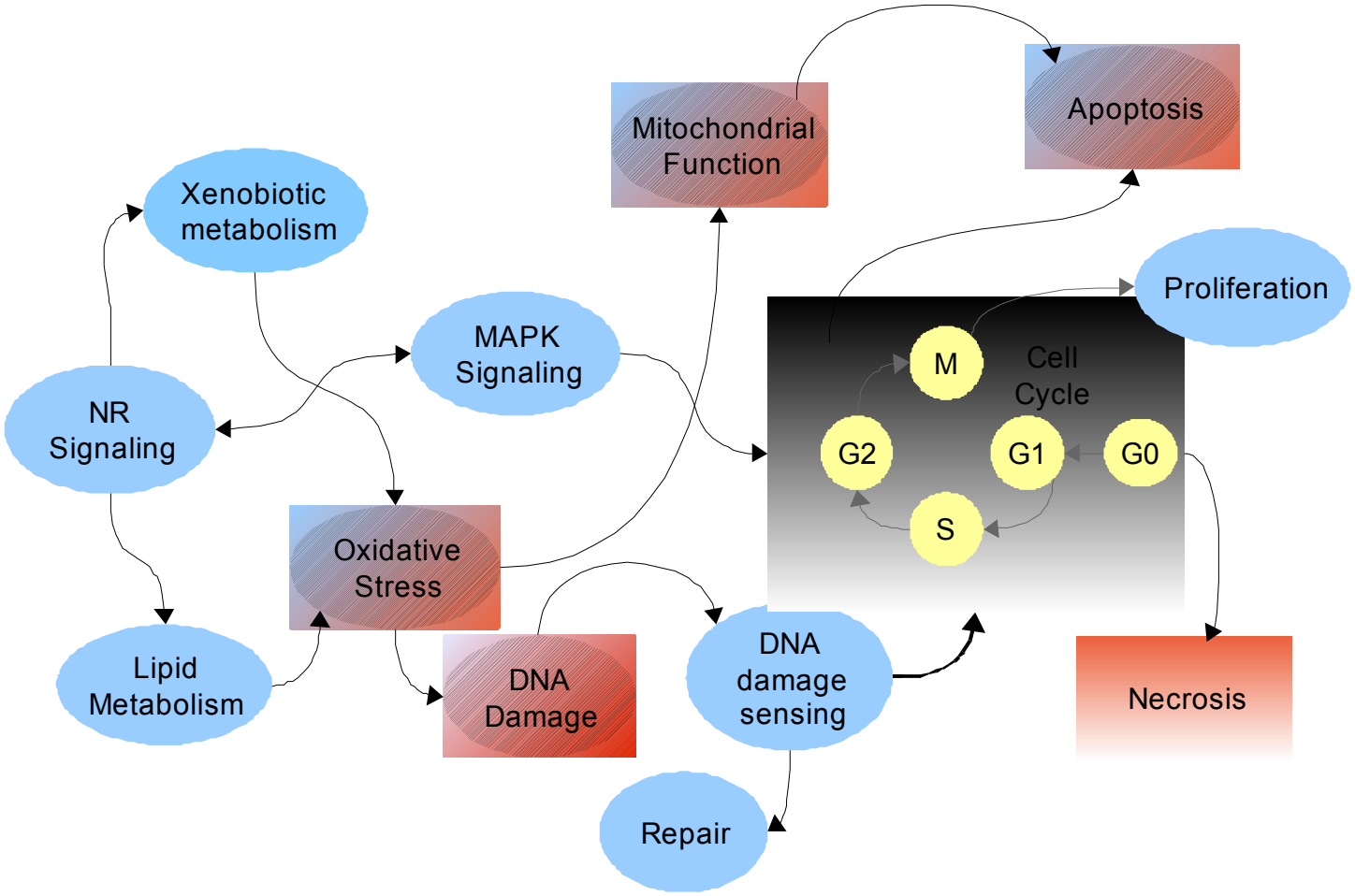


Shah & Haugh

Virtual Liver Knowledgebase



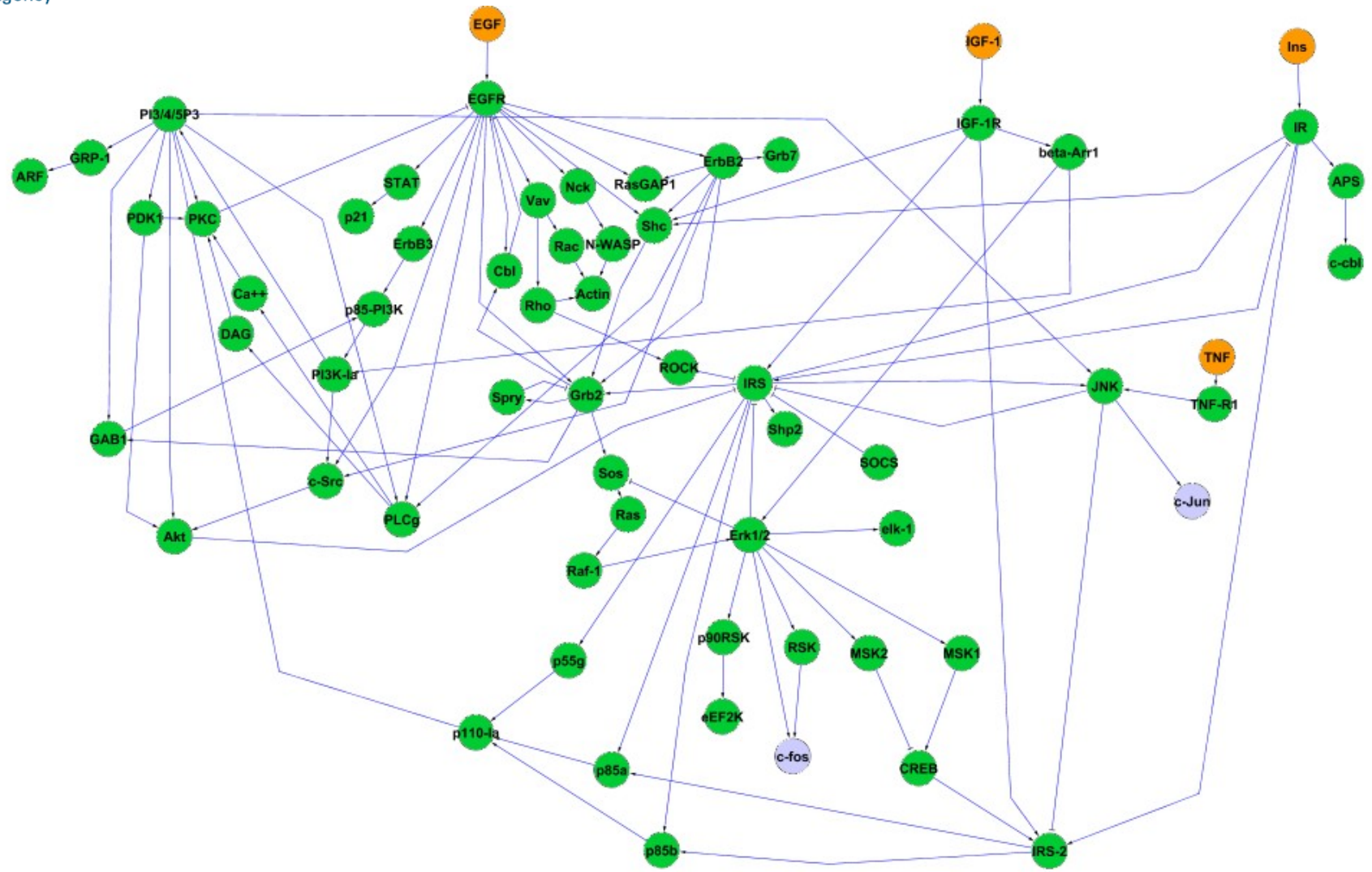
Knowledgebase: Cellular Processes



NR-Mediated Crosstalk Pathways

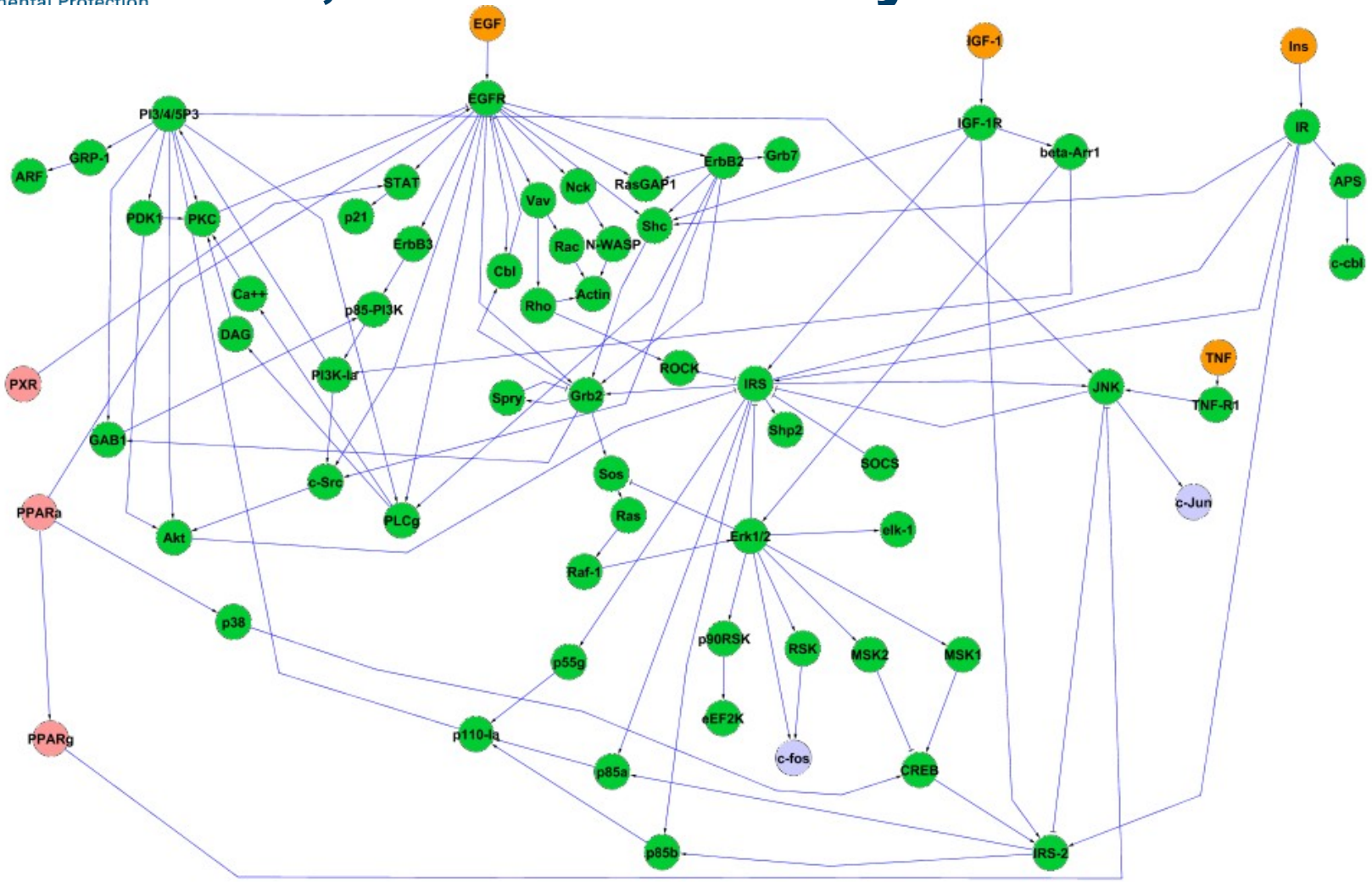
- Use KB to develop systems model of NR-mediated pathways
- E.g. synthesize evidence growth factor (GF) induced cell cycle changes (G1/S-transition)
- Evaluate potential crosstalk between NR and GF-signaling

Growth Factor Crosstalk: Cell Cycle



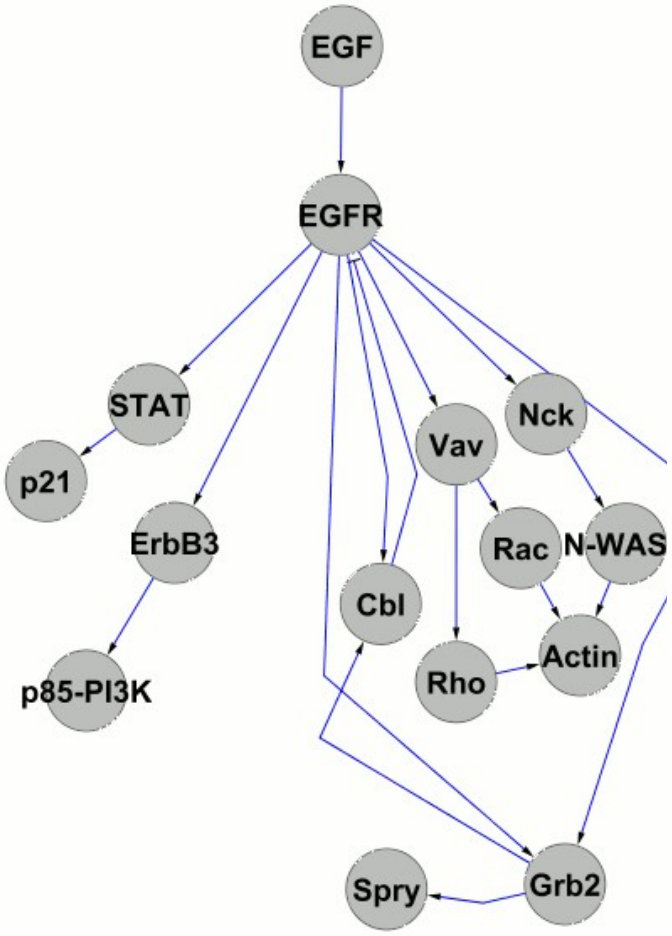
J. Jack

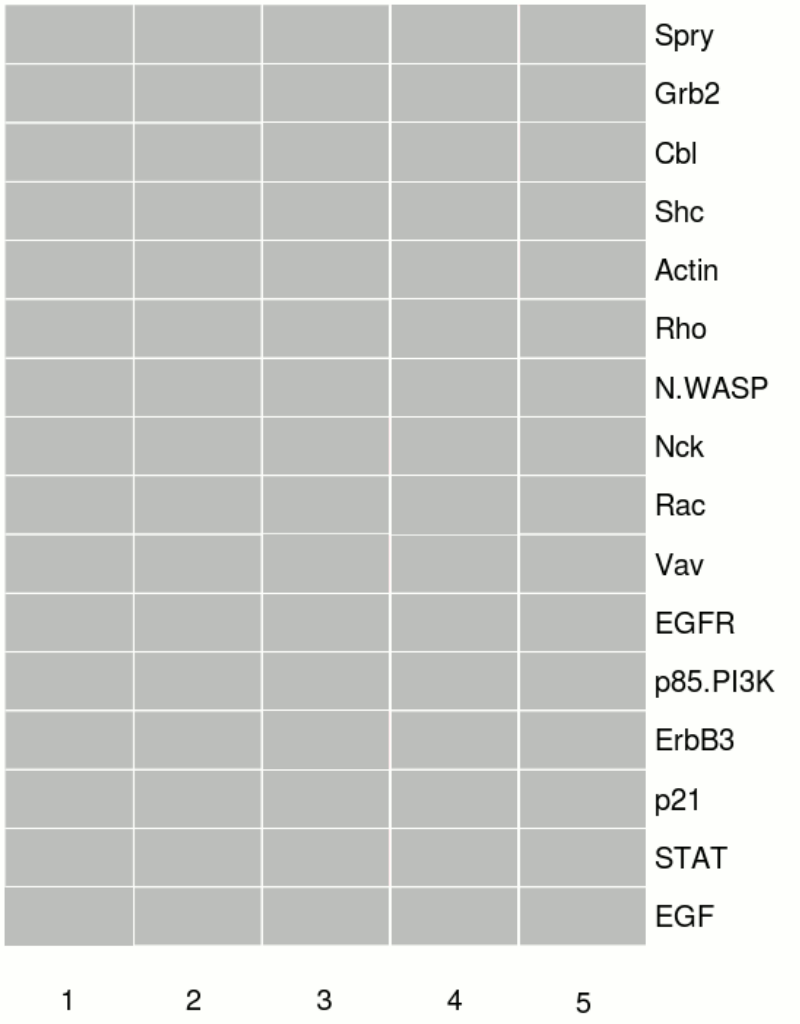
NRs, GFs and Cell Cycle



J. Jack

Simulating Signal Propagation



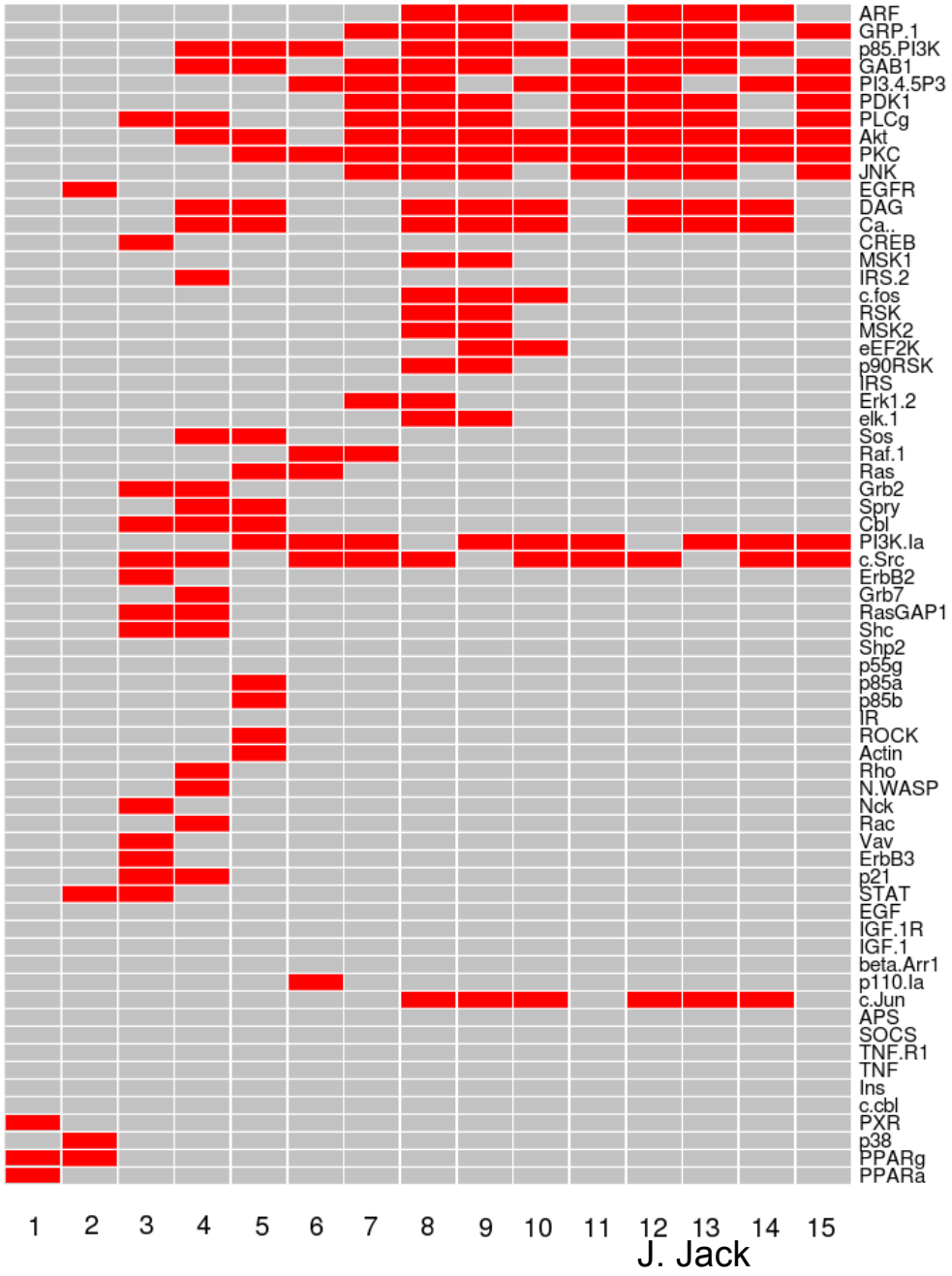


J. Jack

Simulate NR-Mediated Effects

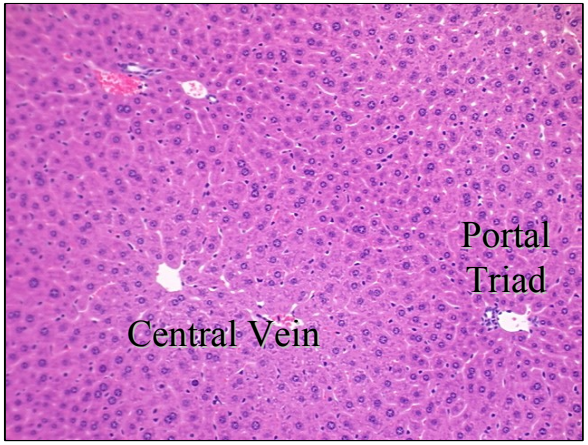
- Transient Activation of PPARa, PPARg, and PXR

- Effects:
  - cFos activation (t=8)
  - cJun activation (t=8)
  - Erk1/2 activity (t=7)
  - p21 activation (t=3)
  - Akt activation (t=7)

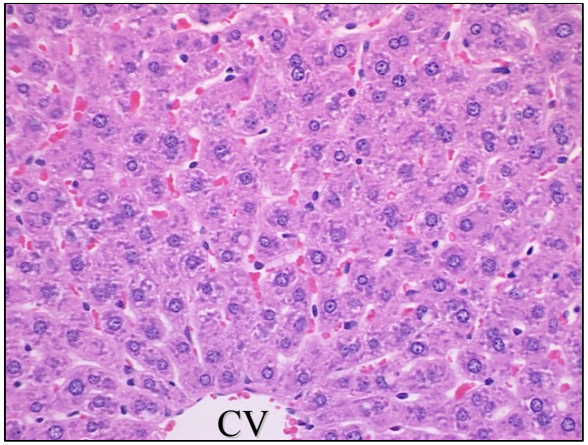


Quantitative Tissue Modeling

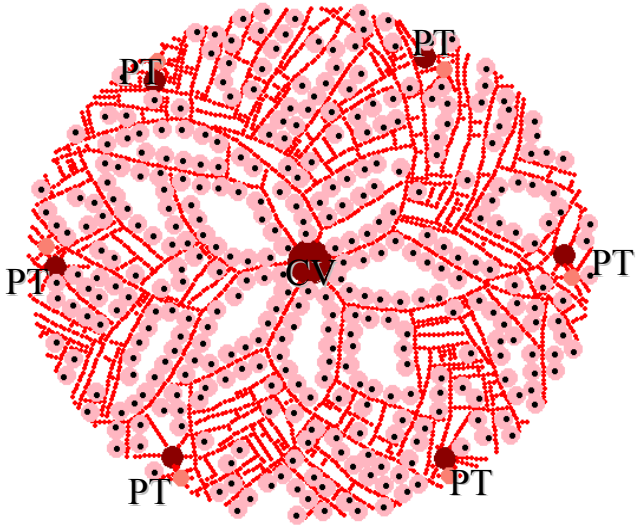
Dose-Response for a Virtual Lobule



The classic lobule consists of a single central vein fed venous and arterial blood via multiple portal triads



Blood flows through a network of sinusoids, supplying and exposing hepatocytes

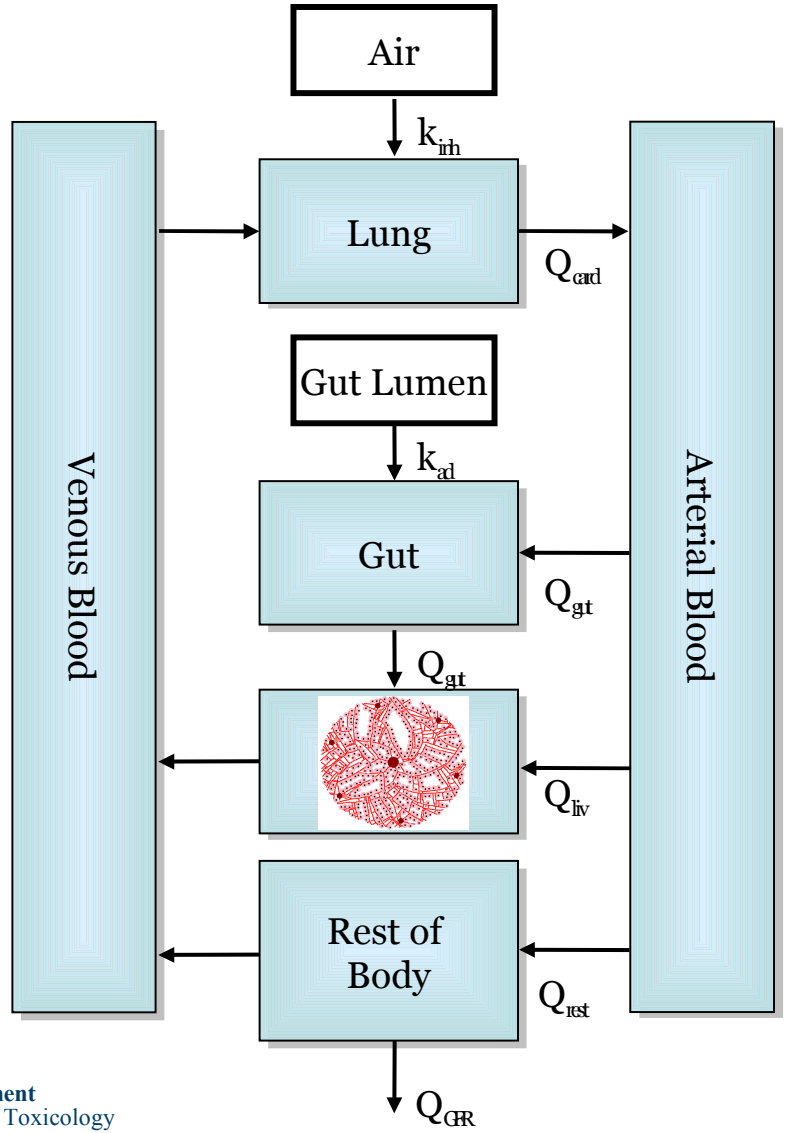


Synthetic lobule sufficiently complex to determine that approach would work with actual lobule morphology (e.g. Drasdo et al.)

Wambaugh & Shah (submitted)

photo credits: Rockett et al. (2006) Reproductive Toxicology 22 647-658

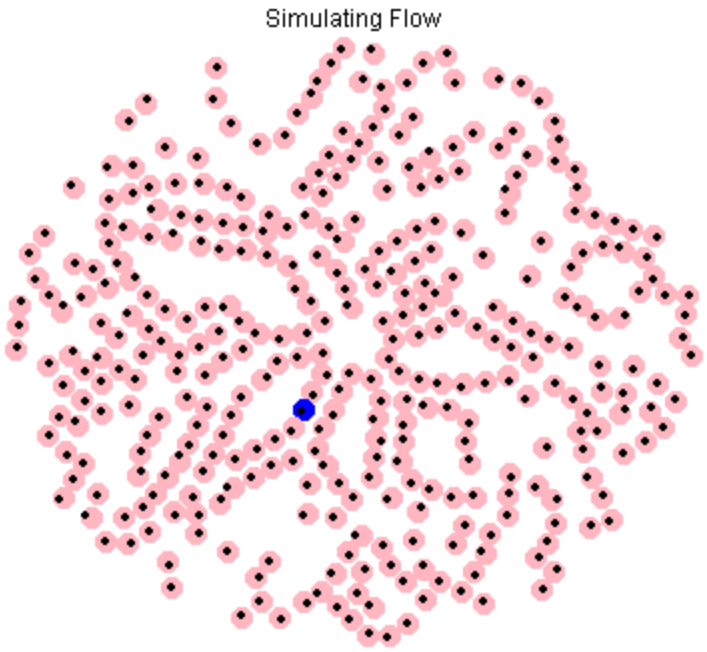
Integration with Pharmacokinetic Models



- Our lobule module is directly integrated with a pharmacokinetic model.
- Determines specific amount of chemical delivered to any given hepatocyte in µmol.
- Suitable for endogenous and xenobiotic compounds.

Simulating Microdosimetry and Effects

Slow Metabolism
(Cl_{int} = 1 µL/min/10^6 hep.)



Concentration
Above Threshold
Below Cytotoxicity Threshold

- H^{o} (Normal/Quiescent)
- H^{adpt} (Stressed/Adaptive)
- H^{inj} (Stressed/Injured)
- H^{nc} (Necrotic)
- H^{prl} (Proliferative)
- H^{app} (Apoptotic)

Wambaugh & Shah (submitted)

Summary

- Goal: NR-mediated cellular pathways, alterations and lesions
- Integrative computational and experimental paradigm is vital for success
- Next steps: *short-term in vivo* study design and assays evaluating *in vitro* data, MOA/dose-response

Multi-disciplinary Team: Cross-EPA/ORD & External Collaborations

v-Liver
John Jack
John Wambaugh
Chris Haugh
Woody Setzer
Lockheed Martin

v-Embryo
Thomas Knudsen
Amar Singh
Nisha Sipes
Nicole Kleunstreuer
Michael Roundtree

ToxCast
David Dix
Keith Houck
Richard Judson
Matt Martin
Holly Mortensen
David Reif
Ann Richard

ExpoCast
Elaine Hubal-Cohen

Robert Kavlock