

# **Bridging Exploratory Biomarkers into Drug Development**

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# Introduction

**All biomarkers are not created equal !**

- ◆ **diagnostic vs. predictive**
- ◆ **different bar for GLP vs. exploratory use **or** clinical vs. preclinical?**
- ◆ **what experimental evidence suffices for a given purpose? e.g., correlative vs. qualified**

# Questions

**Sound business decisions require some level of understanding. This varies with purpose, so....**

**What level of understanding do we need before integrating 'omics data into various stages of drug development ?**

**How might the level of understanding (extent of qualification) impact submission to regulatory agencies (whether to submit, what to submit, process to use – VXDS or regulatory filing) ? Future liabilities ?**

# **As president of JJB, Inc., I want to:**

- Discover disease targets with reliability**
- Identify at an early stage programs / compounds with highest probability of success (efficacy, safety profile)**
- Establish toxicity profile, evaluate risk to people, and move compounds to registration quickly & efficiently**
- Identify endpoints of potential adverse affects to monitor in early clinical work**

**Currently, JJB, Inc. is working on a sex-linked disease. A number of human males spend an excessive portion of evenings and weekends 'vegged out' in front of the TV.**

**In the interest of increasing productivity (as well as marital harmony) we wanted to intervene in this disease process**

# Approach

## ● **In Discovery:**

- ◆ **identify basis of disease to establish target for intervention**
- ◆ **screen libraries of compounds to identify leads**

## ● **In Safety:**

- ◆ **establish toxicity profile in routine species**
  - are there toxicity issues ?**
  - what is the margin of safety ?**
  - do species differences exist ?**

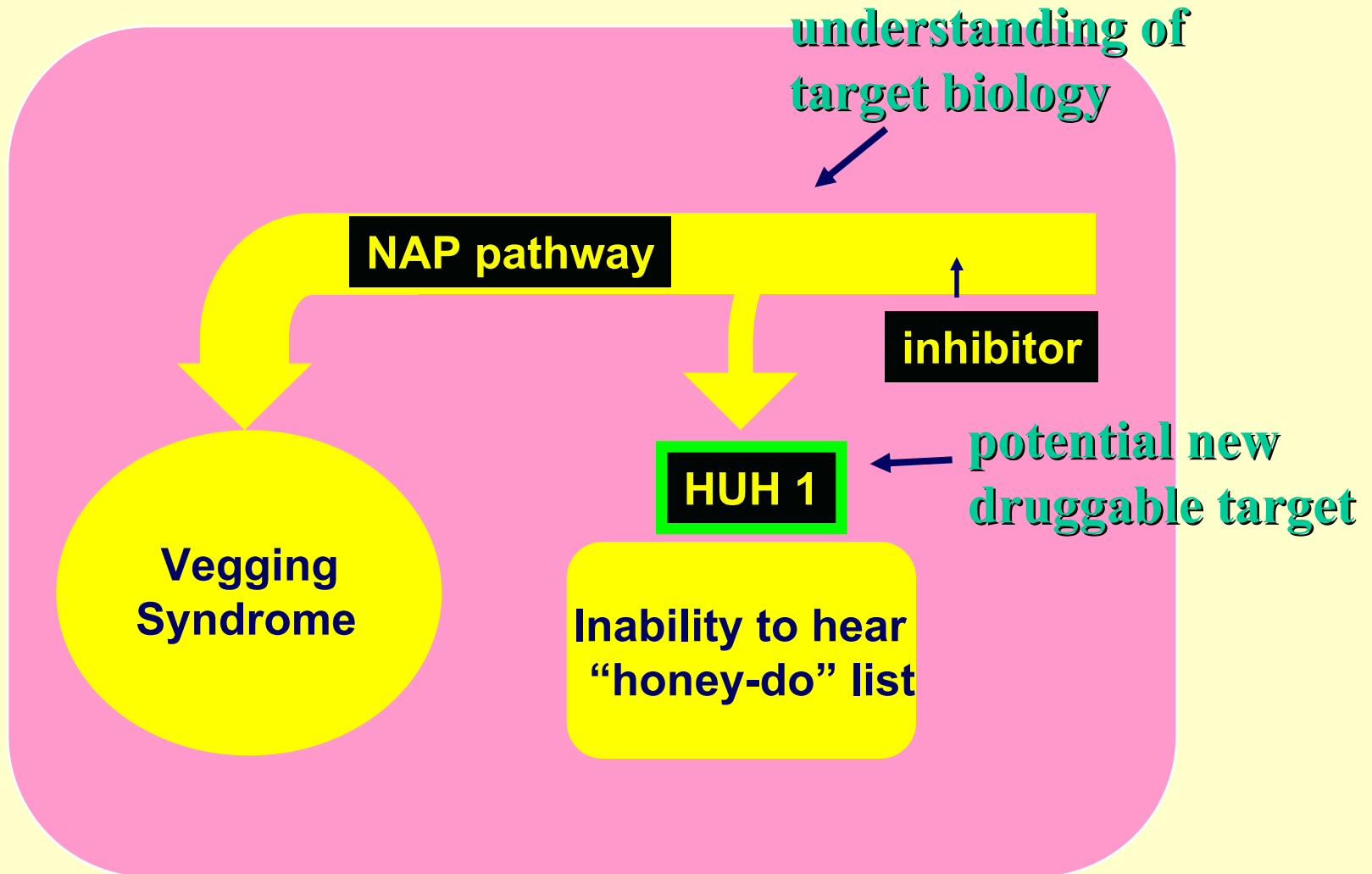
# Early Discovery

**Toxicogenomic analysis of brain tissue, (control v. 'vegged out') suggested similarities with disease genotypes affecting a signaling pathway, termed NAP**

**Blocking phosphorylation of one of the NAP kinases alleviated the syndrome in male rats; screening compound library yielded promising candidates to bring forward.**

**[presumably submission of early, exploratory 'omics & biochemical data would be through VXDS, if at all]**

# Pathways Discovered Through By Incorporating Genomic Profiling



# **Early Compound Selection**

**Basic Research provided 5 Leads around 2 different patentable core structures with approximately equal efficacy in in vitro screens**

**How did we choose a compound ? We'd like both efficacy in vivo and indication of toxicity (if possible.) Biomarker set #1 informs toxicity to some target tissues.**

# What is biomarker set #1?

- **Set #1 derived from correlation of profiling data with histopathology (which genes best diagnose positive / negative) for major target organs (liver, kidney, muscle, for eg.) in 10 studies; set has been reduced to 48 genes analyzed on qPCR platform**
- **Question of which series is least likely to show toxicity if taken forward can now be addressed**
  - genes add to weight of evidence of other, standard endpoints
  - doesn't need to predict that I will have toxicity but diagnose that I do [to save histopathology resources]

# Initial In Vivo Efficacy Study

**Core Structure #1**



**Core Structure #2**



**Rat study:**

**0, 30, 100, 300 mg/kg**  
**3 day dosing**  
**3 animals/group**

**Endpoints:**

**efficacy measures**  
**physical signs**  
**clinical chemistry**  
**biomarker set #1**  
**(no histopathology assessment)**

**Results suggest that structures #1 & #2 are equally**

**efficacious – achieves my primary goal**

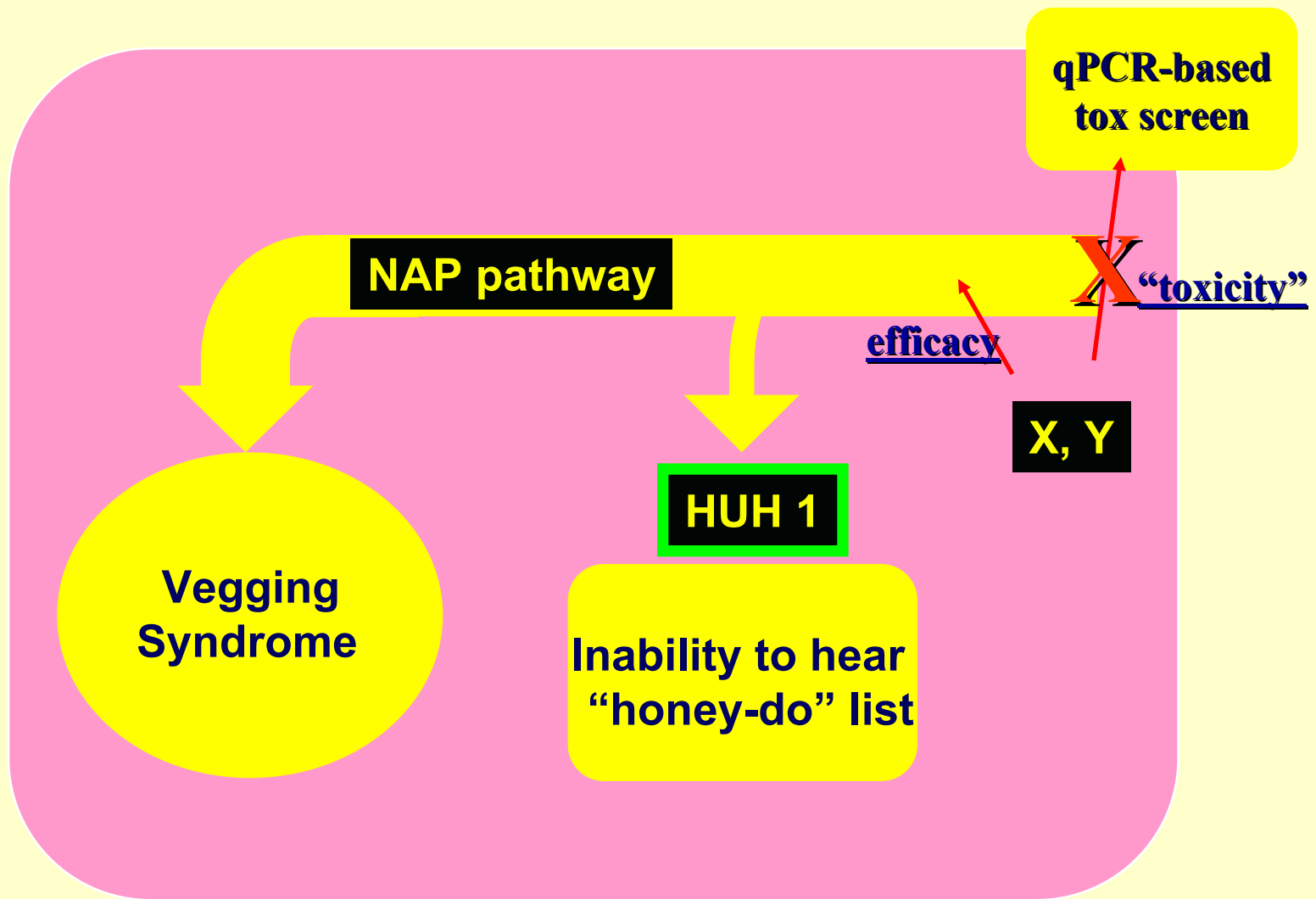
**qPCR data suggest that structure #1 may have muscle**

**liability, but not #2 – adds more information per study**

# **Questions Around Set #1**

- **Set is “filtered” subset from earlier ‘omics work**
- **Not sure of sensitivity, specificity around these genes**
  - **Risk is that I waste in-house resources**
  - **Data being used for internal decision-making, not to support safe conduct of a clinical trial**
- **Is this work for compound selection reportable?**
  - **Presumably submission would be through VXDS, if at all, and qPCR markers, not genomics work leading to this biomarker set**

**This approach provides JJB, Inc. with flexibility in making early development decisions & enables exploration of novel approaches / technologies**



**Ready to move into Safety Evaluation !**

# **Preclinical Safety Studies** **(X & Y in parallel)**

- **2 week rat study – no toxicity w/ either compound**
- **14 week rat study – kidney tubule toxicity w/ both**
- **14 week dog – confirms kidney toxicity**

**How do I approach selection of backup?**

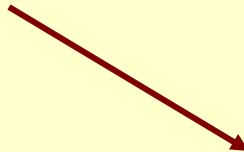
# Two Approaches

**animal studies to screen out kidney toxicity**



**resource intensive  
tox shows between  
2 & 14 wks**

**3<sup>rd</sup> compound tested:  
no issues in rat, dog  
no issue in monkey**



**evaluate kidney from 2, 14  
wk GLP rat studies to try to  
understand mechanism**

**time intensive to get to  
understanding**



**screen backups based on  
understanding developed**

**enhanced understanding of  
what to monitor in clinic?**

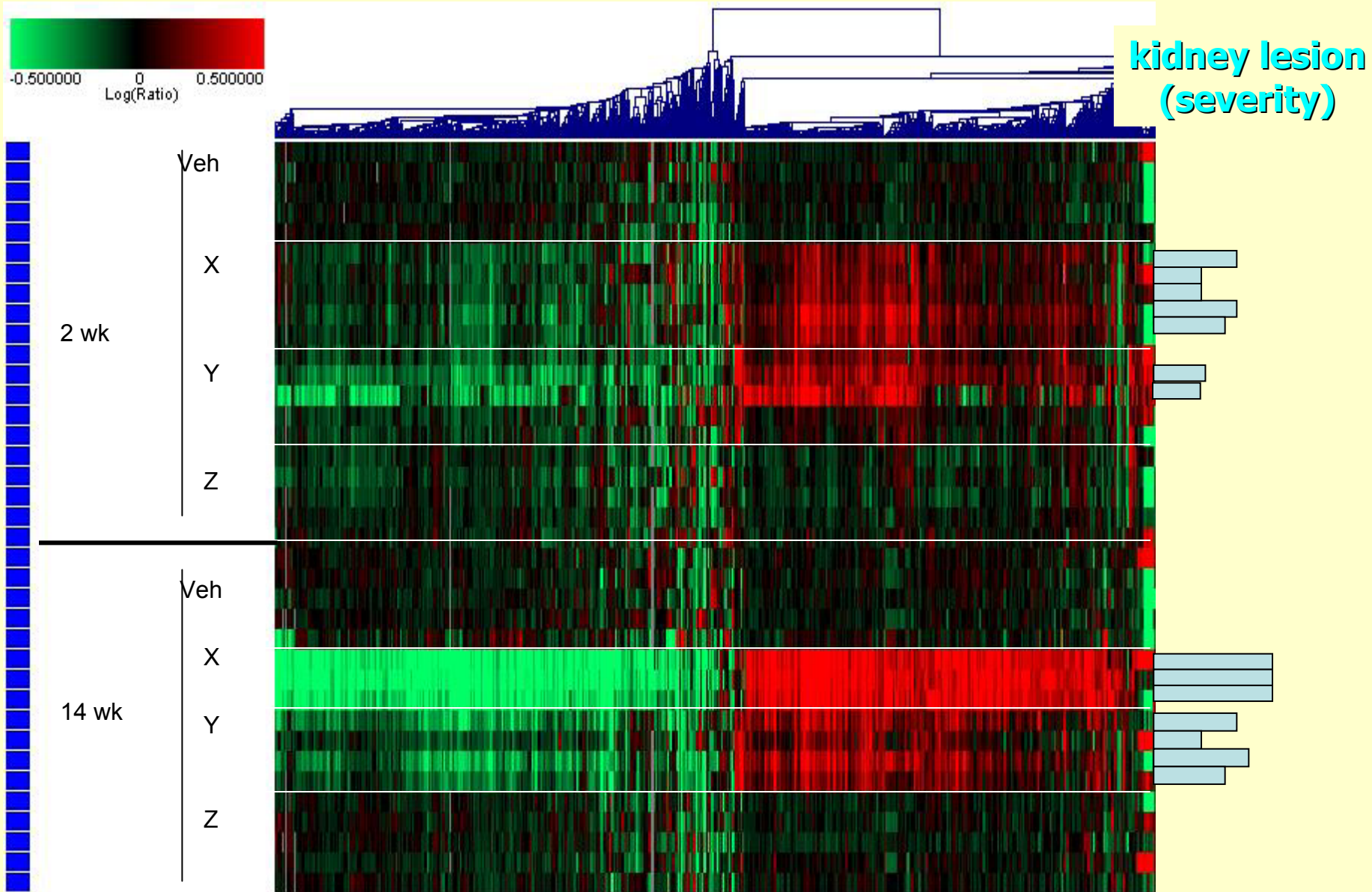


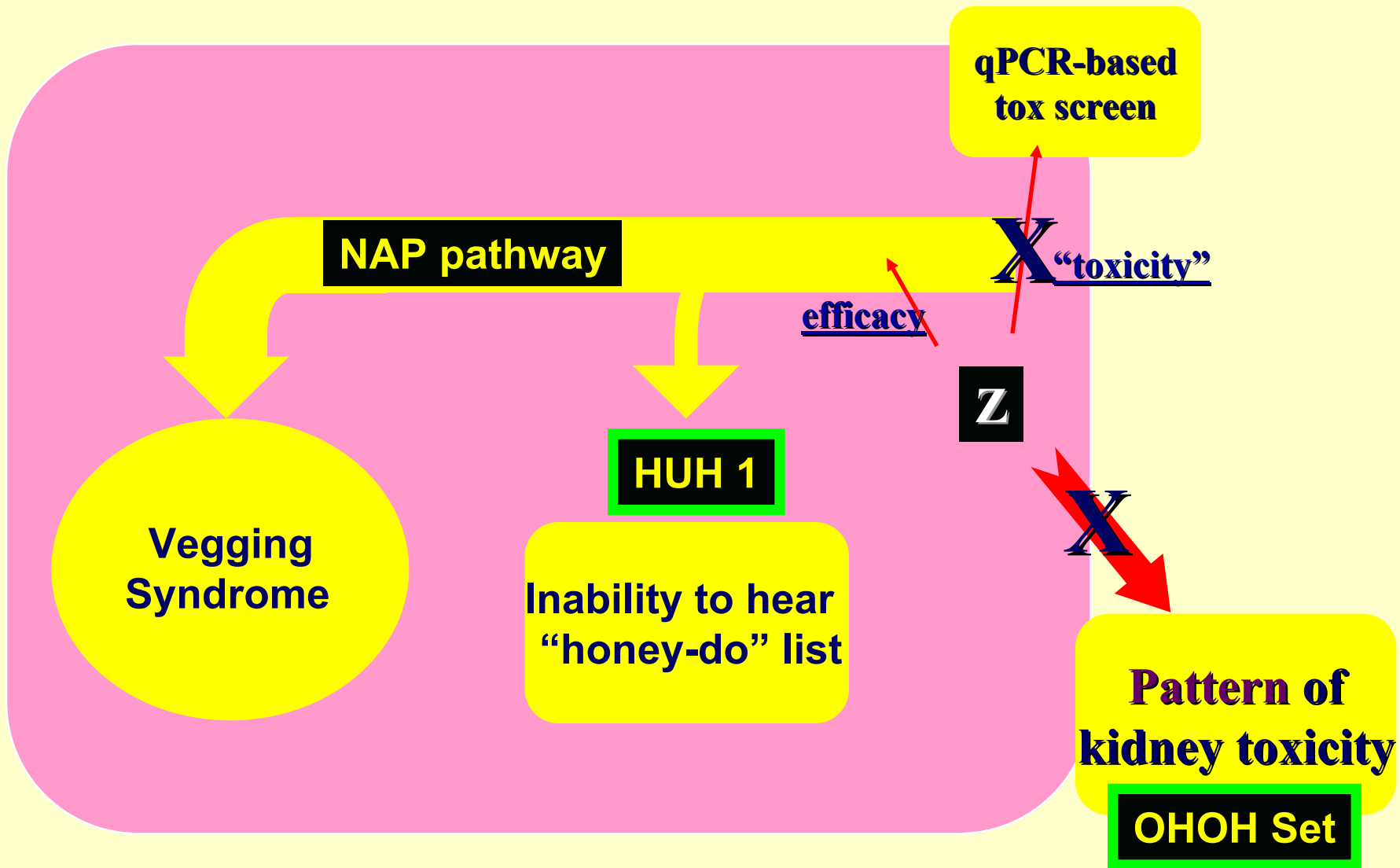
**animal studies to  
confirm lack of toxicity**



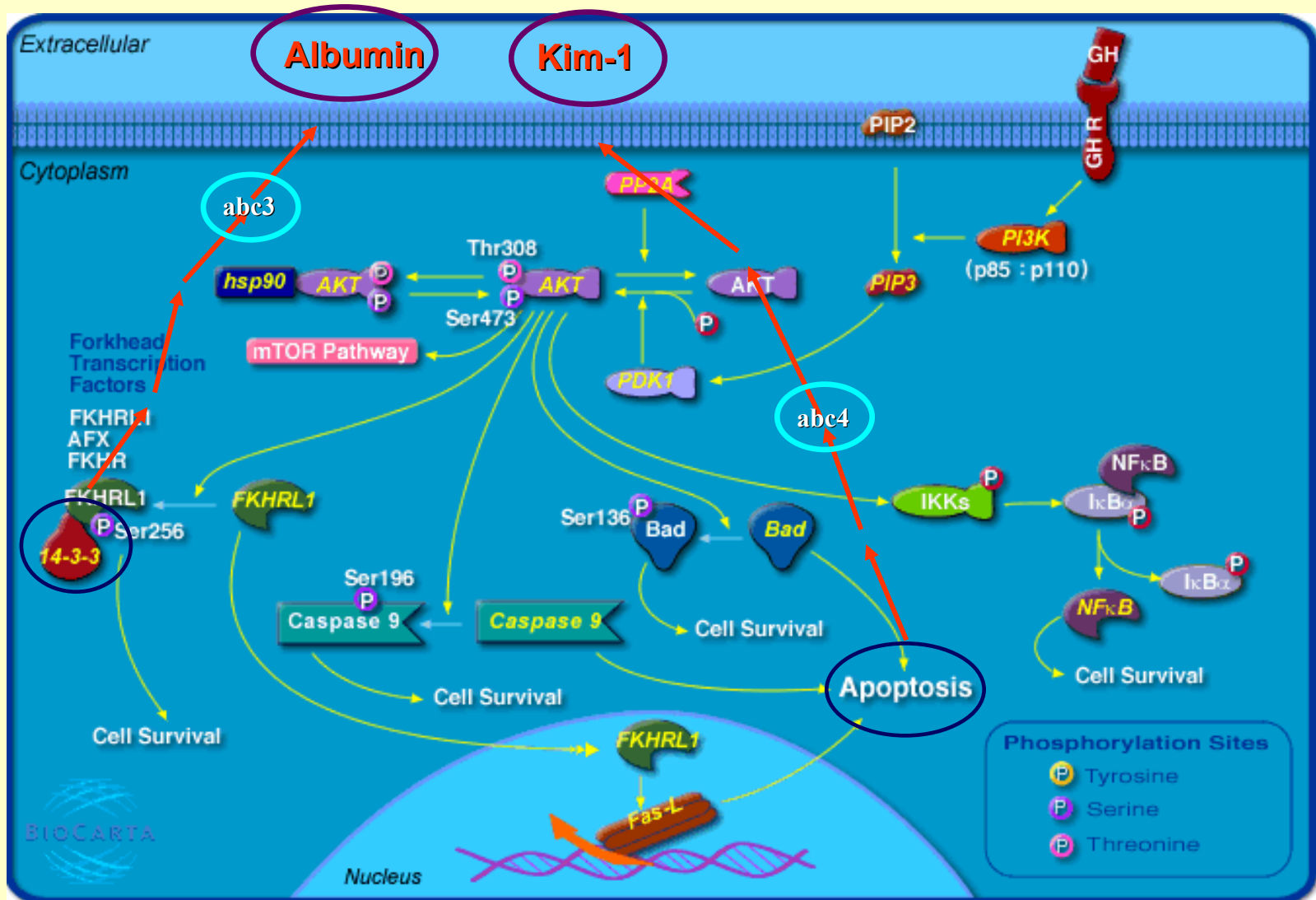
**clinical candidate**

# Profiling: pattern recognition-based compound selection





# Pathway Analysis



Mock Kidney cell

from: Biocarta.com

**Table 1: 81 annotated genes among the 219 genes that differentiate compound X and Y from Z across weeks 2 and 14**

Sequence or Gene		Day 4			Day 12		
Name	Code	Xd4 AFC	Yd4 AFC	Zd4 AFC	Xd12 AFC	Yd12 AFC	Zd12 AFC
Kim-1	NM_007500	3.4801	2.124561	0.1800112	3.00011	3.35001	0.18432
albumin	M92059	3.1123465	2.7024351	0.0611205	3.6657631	3.2511	0.1612
abc3	NM_024360	-1.5835624	-2.2116768	0.2477168	-1.455613667	-2.11506675	0.1819202
abc4	NM_019218	5.0764574	3.5569432	1.2268218	3.602745333	3.6453945	1.191378
abc5	600510684R1	0.8717196	0.9340476	-1.1572642	2.949614	1.8043085	-0.2837008
abc6	AB001321	-1.4684788	-1.5770714	-0.686734	-9.262257667	-2.28789875	-1.1801484
abc7	AB032551	1.4171508	1.9772702	-1.120923	1.837448333	2.00085625	-0.2474462
abc8	AB062135	1.273224	0.7473766	-1.1278078	2.233502667	1.42685625	-0.5992188
abc9	AF014503	1.919542	1.367877	-0.9570788	6.612407333	3.171801	-0.2437412
abc10	AF228917	1.9409794	1.5964512	-0.6490912	3.666386	1.87568525	0.673965
abc11	AF359355	-1.2942324	-1.4890574	-0.2304996	-7.647647	-2.29679775	-0.2676778
abc12	AI502080	-1.4218198	-1.433535	0.653768	-2.658683333	-0.70656	1.1475466
abc13	AW141985	1.3258792	1.2899682	0.2116792	2.211431	1.37927325	0.6642708
abc14	D10041	3.5884042	4.0979948	0.695588	4.260271	2.697035	0.745463

**First 4 genes followed up by PCR. No qualified biomarkers among 219 gene OHOH signature set**

# Some genes (many CYP) suggest similarity to a hepatotoxin in internal database

		Hepatotoxin		Compound X		
		rat1	rat2	rat1	rat2	rat3
Cluster	Gene Name	323	324	429	430	431
1	MURINE_B2_at					
1	rAlbum_at					
1	mApoC3_at					
1	rApoC1_at					
1	rApoE_at					
1	rCYP2D1_s_at					
1	rCYP2D2_at					
1	rFABP_at					
1	rGSTmic1_at					
1	rPST_at					
2	rCYP2B1_s_at					
2	rCYP2B2_c_at					
2	rCYP2B2_d_at					
2	rCYP2B2_s_at					
2	rEHm_at					
2	rGSTM1_at					
2	rUGT2B1_at					
3	mApoA4_at					
3	rMCACD_at					
4	mCYP3a11_g_at					
4	rGSTA1_s_at					
5	mGSTA2_at					
5	rGSTA2_at					
6	rApoA4_at					
6	rPEPCK_at					

**But...no liver toxicity  
seen in any  
exploratory or  
preclinical study !**

# Questions Around OHOH Set

- **Am I sure of sensitivity, specificity around these markers?**
  - **No. Looking at relative differences (pattern recognition) to enable decision-making. Animal studies followed; these considered definitive**
- **How many of these genes are really biomarkers?**
  - **Only have annotation on <50%**
  - **annotated genes fit broad pathways; interactions within pathways only partially understood**
  - **integration of pathways into whole organism response (systems toxicology) is in infancy**

# Questions Around OHOH Set

- **Where is the bar ? For instance, don't see liver toxicity, but see genes "associated" with a known toxic compound (but the signature is driven by CYP genes, so is it toxicity or metabolism?)**
- **What is reportable now (and later) ?**
  - **I used GLP samples. Submission through VXDS, or full report? Guidance suggests review of gene list as new markers are qualified ??**
  - **Entire gene list? Those confirmed experimentally? Only those with annotation and/or known biology?**
  - **Too much ambiguity for comfort – should I just have avoided the 'omics work ?**

# Monitoring Clinical Studies

- **Based on traditional drug development path, I have Compound Z which is efficacious and does not show toxicity in preclinical species with 50-fold margin**
- **I want to monitor for unanticipated kidney toxicity**
  - **traditional endpoints – BUN, creatinine, etc.**
  - **OHOH genes? But I don't have experience with these except in tissues**
  - **subset of OHOH genes (e.g., Kim-1, albumin) in accessible fluids? If so, what level of work is required for qualification ?**

# Discussion Points

- **Point:** How to I qualify markers for clinical use ?
  - Probably cannot perform as extensive a battery, so more weight on individual markers ...
  - Level of confidence clearly needs to be greater than for exploratory or preclinical applications if we are going to make definitive safety judgments
  - Defer to the following speakers

# When is a biomarker really a biomarker ?

**target discovery,  
efficacy studies**

**preclinical  
studies**

**clinical  
studies**



**Degree of Ambiguity**

## Requirements:

**hypothetical or  
correlative acceptable**

**correlation and/or  
mechanistic  
understanding needed**

**well understood,  
accessible marker**

# Discussion Points

- **Point: What is Biologically Relevant ?**

- **The fact that we see changes in genes, proteins does not bring us closer to ‘prediction’ or ‘understanding’ at level of fully integrated animal (system) accounting for synergism & antagonism**
- **Because we can measure a change, does it mean that it must be relevant? Is it qualitative or quantitative?**
- **Solid paradigms and prediction models based on phenotypic anchor(s) required for appropriate (fit for purpose) level of confidence**

# Discussion Points

- **Point:** Toxicogenomics data is only one piece of evidence supporting risk assessment
  - What is expectation of level of understanding in ‘omics submission, and how would it impact what is included ? (all gene sequences, relevant ones, experimentally confirmed genes?)
  - Only “known and probable valid” gene sequences submitted to IND – those interpreted to have biological meaning and whose context was confirmed by follow-up experiments
  - If not all gene changes are required for IND submission, should NDA ‘full report’ include these more speculative genes, or is bar for consideration only qualified markers?

# Backups

# Factors Driving IND Data Submission

## Level of Qualification

<u>Study Purpose</u>	<b>Low</b> (Validity not established)	<b>Med.</b> (Probable valid)	<b>High</b> (Known valid)
<p>GLP Study w Potential for Data w Regulatory Impact</p> <p>Used by sponsor in decision to support the safety of a clinical trial</p> <p>Not used to assess prognosis of animal findings (eg, explore mech.)</p>	<b>Vol*</b>	<i>FuR</i>	<i>FuR</i>
	<b>Vol*</b>	<b>Vol*</b>	<i>AbR</i>
Non-GLP Exploratory Study for Internal Decisions	<b>Vol</b>	<b>Vol</b>	<b>Vol</b>

\*If additional information becomes available, sponsor must submit

# NDA Data Submission

## Level of Qualification

<u>Study Purpose</u>	<b>Low</b> (Validity not established)	<b>Med.</b> (Probable valid)	<b>High</b> (Known valid)
GLP Study w Potential for Data w Regulatory Impact			
Used by sponsor to support a safety claim	<i>Synop</i>	<i>AbR</i>	<i>FuR</i>
Not used to assess prognosis of animal findings	<i>Synop</i>	<i>AbR</i>	<i>AbR</i>
Non-GLP Exploratory Study for Internal Decisions	<i>Synop</i>	<i>AbR</i>	<i>AbR</i>

**\*314.50 CFR: ..the [NDA] application is required to contain reports of all investigations of the drug product sponsored by the applicant.”**