U.S. FOOD & DRUG ADMINISTRATION

Integrated Response Markers

Dealing With the Elephant

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U.S. FOOD & DRUG ADMINISTRATION

Disclaimers

- Views expressed in this presentation are those of the speaker and do not necessarily represent an official FDA position

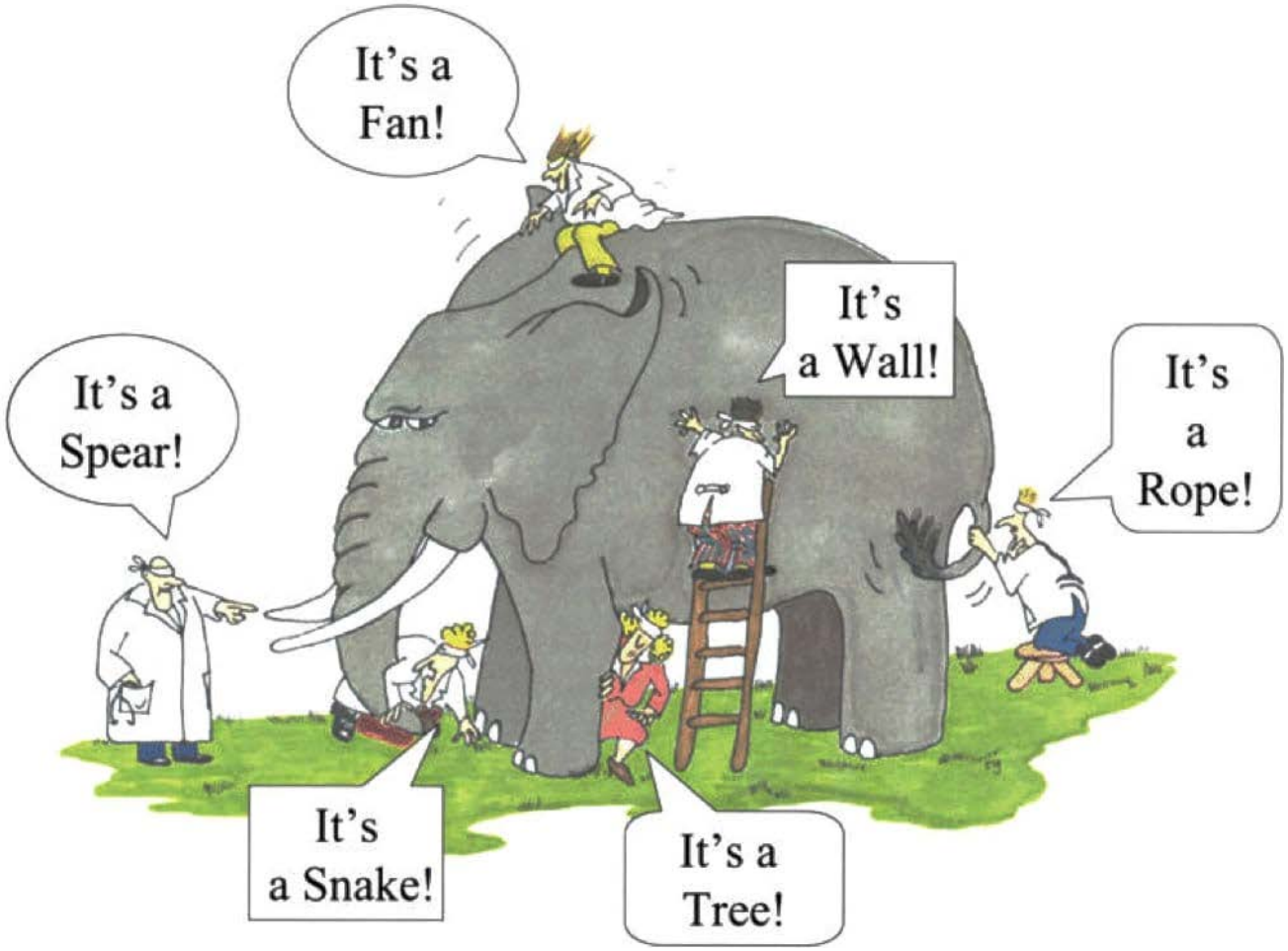
- I do not have any financial disclosures regarding FDA regulated products

Conclusions

- A holistic view of biology requires integrating multiple approaches and endpoints
- Such integration is a work in progress
  - Data acquisition
  - Technology integration
  - Simultaneous analysis of multiple endpoints
  - Incorporation of biological variation

The Elephant

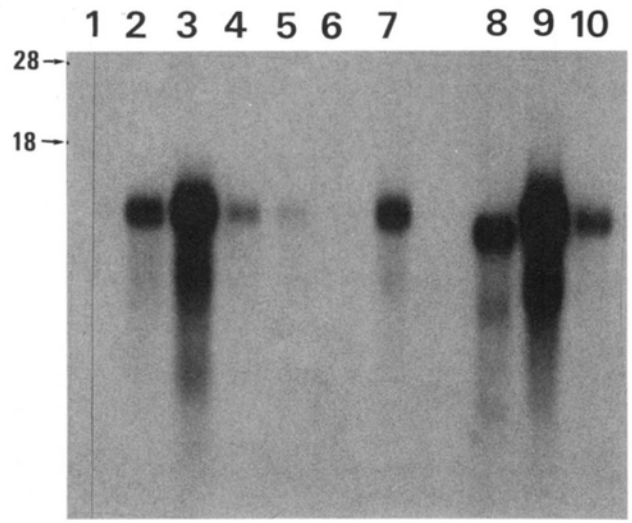
(as analyzed by blinded scientists)



Genomics in the Paleozoic Era

Gene Expression of Rat and Human Microsomal Glutathione *S*-Transferases*

DeJong, J. L., et al. (1988). J Biol Chem **263(17):8430-8436.**



One gene at a time

- ask your grandfather about Southern Blots

FIG. 3. A, rat tissue RNA blot hybridization with the ^{3Z}P-labeled XrMGSTl cDNA insert.

Evolution

Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray

Schena, M., et al. (1995). Science **270(5235): 467-470.**

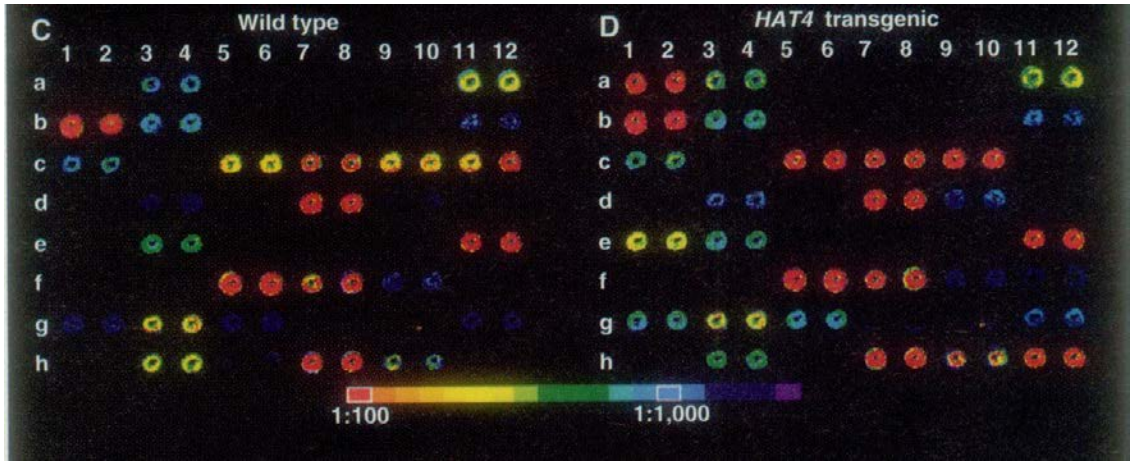


Fig. 1. Gene expression monitored with the use of cDNA microarrays.

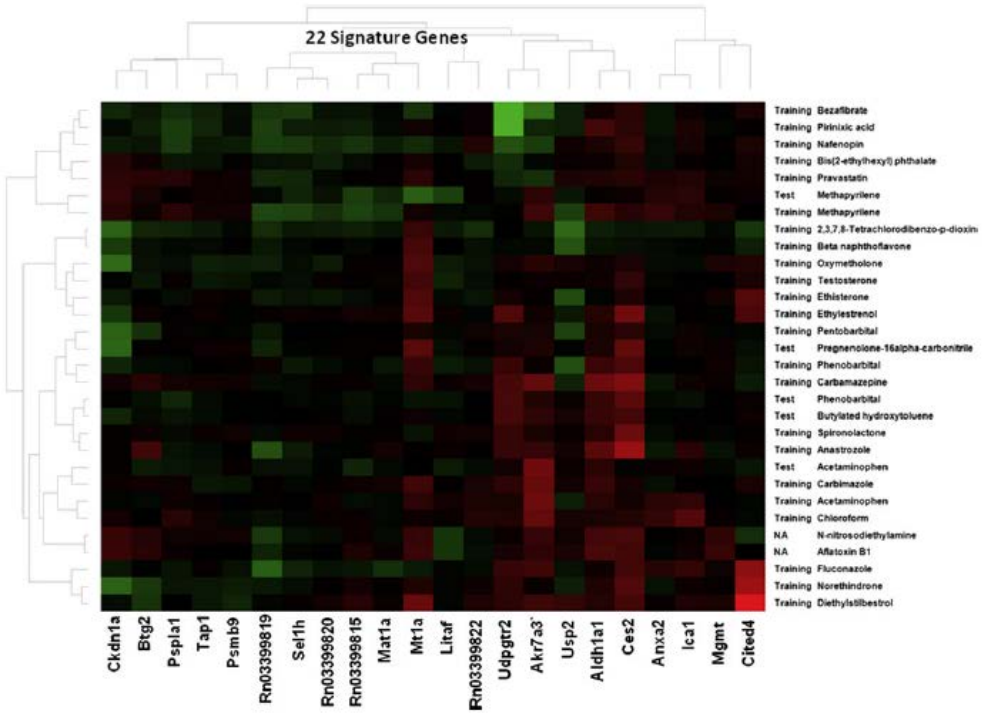
A bigger picture ….

But what to with all that data?

Toxicogenomic Signatures

Development and Evaluation of a Genomic Signature for the Prediction and Mechanistic Assessment of Nongenotoxic Hepatocarcinogens in the Rat

Fielden, M. R., et al. (2011). Toxicol Sci **124(1): 54-74.**



Model using expression of 22 transcripts (genes)

Training set:
72 compounds

Test set:
66 compounds

Developed and tested by a consortium of 12 organizations

Electrophysiological Models

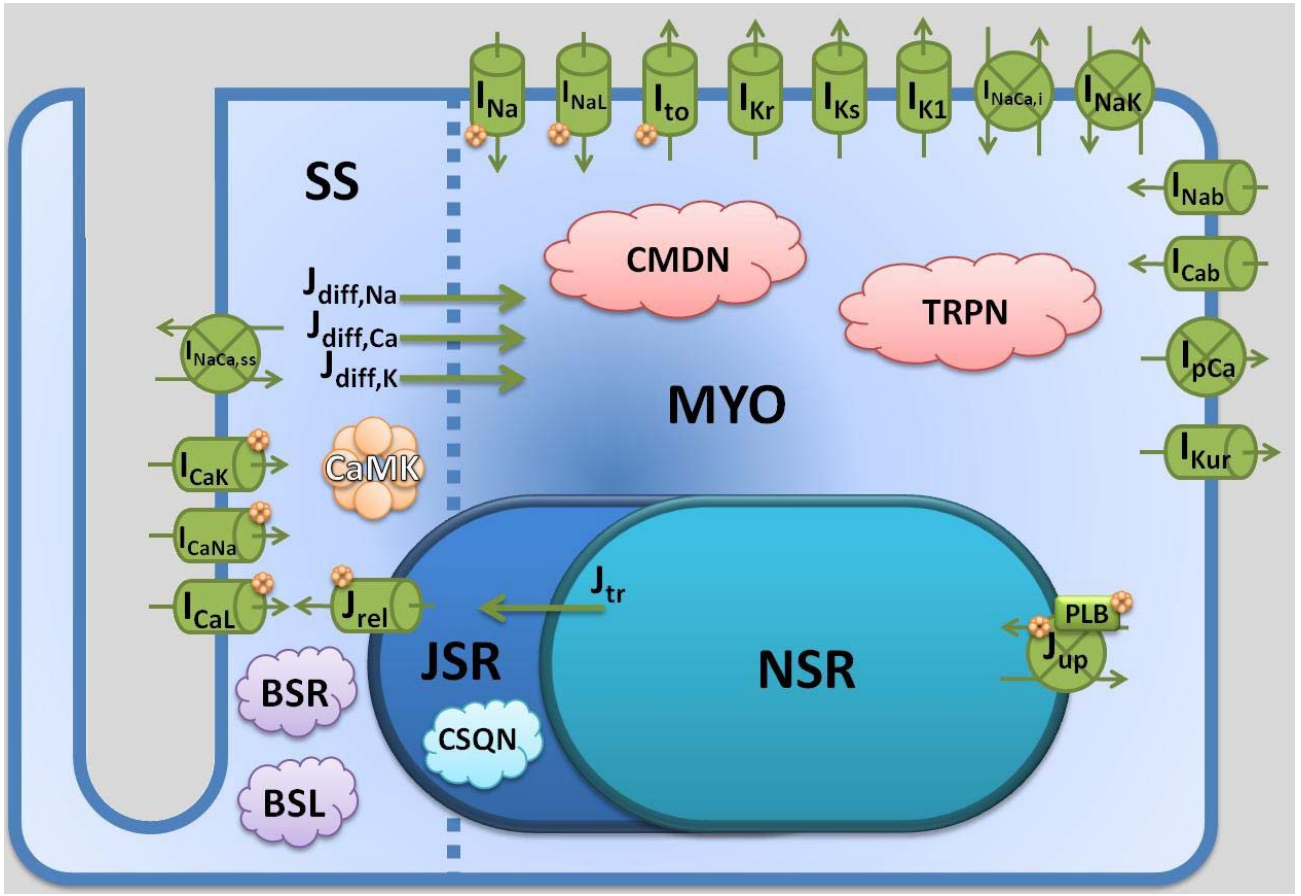
Optimization of an *In silico* Cardiac Cell Model for Proarrhythmia Risk Assessment

Dutta S., et. al. (2017)
Front Physiol. 8:616

Composite Biomarkers Derived from Micro-Electrode Array Measurements and Computer Simulations Improve the Classification of Drug-Induced Channel Block

Tixier E., et. al. (2018)
Front Physiol. 8:1096

Human Ventricular Myocyte Model



T.J. O'Hara, L. Virág, A. Varró, Y. Rudy, "Simulation of the undiseased human cardiac ventricular action potential: Model formulation and experimental validation" *PLoS Computational Biology* 2011; 7(5): e1002061. doi:10.1371/journal.pcbi.1002061

Division of Applied Regulatory Science

Holistic?

- The models and signatures described present a global view..
  - of one type of data
  - of one aspect of cellular physiology

Cardiotoxicity – Many Facets

Adverse Effects

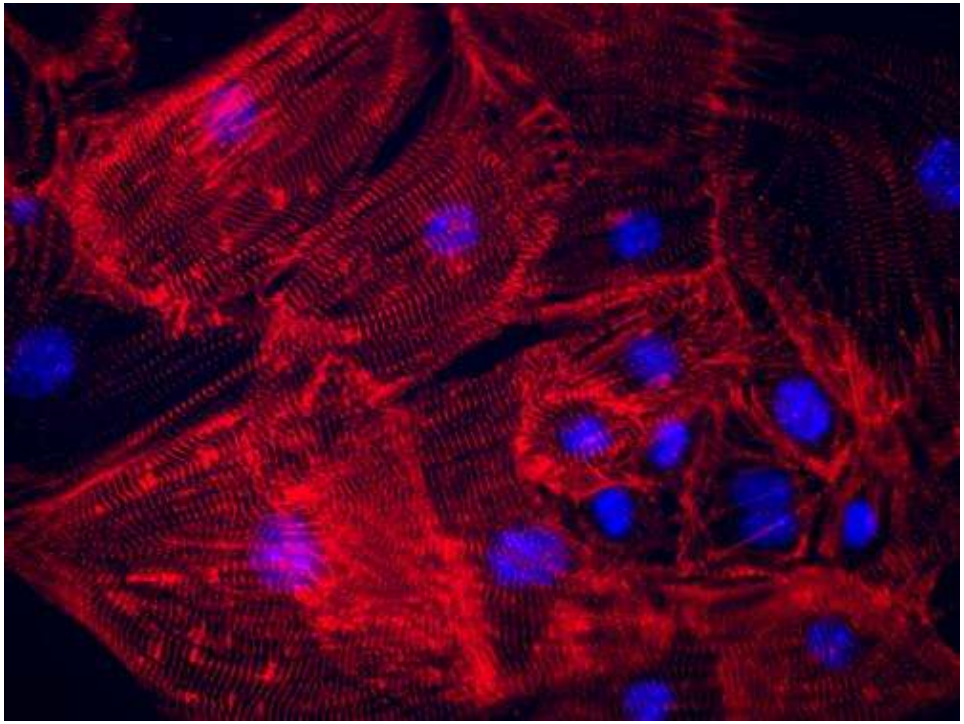
- conduction impairment
- QT interval prolongation
- impaired cardiac contractility and dilatation
- multifocal cardiomyocyte necrosis or apoptosis
- myocardial fibrosis
- hypertrophy

Clinical Manifestations

- arrhythmia
- valvular heart disease
- cardiomyopathy
- heart failure and/or sudden death.

From Yang and Papoian (2012)

The Model in Question



What can we measure?

In Vitro Endpoints

- Electrophysiological parameters
- Mitochondrial parameters
- Cytotoxicity
- Apoptosis
- cTroponin
- Ca handling

- Signaling pathways
- mRNA - cell
- miRNA - cell
- miRNA - media
- proteomics - cells
- kinomics - cells
- metabolomics - cells
- metabolomics - media

Studies to Date

- What has been measured?
- For which compounds?
- What endpoints have been integrated?

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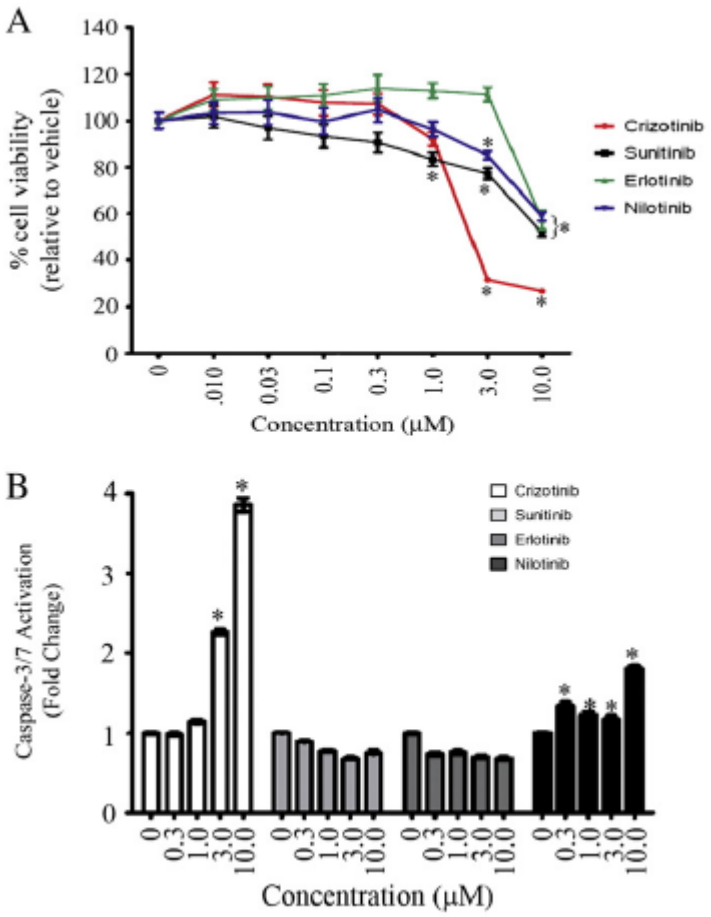
Crizotinib, Sunitinib, Erlotinib, Nilotinib



Gene Symbol	Gene Name	Crizotinib	Sunitinib	Erlotinib	Nilotinib
INSIG1	Insulin Induced Gene 1	10.40	1.98	1.07	1.18
SREBP2	Sterol Regulatory Element Binding Transcription Factor 2	2.52	1.19	1.14	-1.22
FADS2	Fatty Acid Desaturase 2	6.62	2.38	-1.06	1.29
FASN	Fatty Acid Synthase	3.16	1.49	1.02	1.31
HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1	16.19	2.17	1.03	1.01
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	6.27	1.51	1.04	1.23

- Crizotinib: increased ROS production, caspase activation, cholesterol accumulation, disruption in cardiac cell beat rate, and blockage of ion channels.
- Sunitinib: decreased cardiomyocyte viability, AMPK inhibition, increased lipid accumulation, disrupted beat pattern, and hERG block.
- Nilotinib: increased ROS generation, caspase activation, hERG block, and an arrhythmic beat pattern

Doherty, K. R., et al. (2013). Toxicol Appl Pharmacol **272(1): 245-255.**

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21 TKIs

Drug	Cessation of beating (μM)	Effective concentration (μM)	Amplitude of effect	LD_{50} (μM)	C_{max} (μM)	Cardiac safety index	Clinically reported cardiotoxicity
Vemurafenib	33	11.00	0.34	32.10	126.04	0.003	QT
Sorafenib	3.7	2.51	1.03	3.40	8.43	0.004	QT, LV, HF, MI, Hy
Doxorubicin	3.7	1.20	0.60	0.78	2.93	0.010	****HF, LV**
Regorafenib	11	3.70	0.84	7.10	8.08	0.010	#MI, Hy
Vandetanib	33	5.68	2.47	20.60	4.26	0.041	****QT, TdP, SCD, HF, Hy**
Crizotinib	11	1.91	0.59	8.60	1.24	0.063	QT, Brady
Nilotinib	100	8.31	2.65	29.00	4.27	0.104	****QT, LV, Vas**
Imatinib	100	33.00	1.59	78.20	5.11	0.126	LV (rare)
Lapatinib	33	11.00	0.40	100.76	2.30	0.209	#LV, QT
Sunitinib	3.7	0.81	1.33	12.70	0.18	0.218	#HF, LV, MI, QT, Hy
Bosutinib	33	4.73	1.92	12.39	0.51	0.315	PE
Gefitinib	33	3.11	1.24	26.30	0.45	0.409	None
Afatinib	3.7	1.65	1.11	12.30	0.10	0.444	None
Dabrafenib	100	36.75	0.71	100.68	4.16	0.459	LV
Ponatinib	3.7	3.70	0.54	4.30	0.14	0.483	****Vas, HF, LV, Hy**
Ibrutinib	33	10.01	1.54	11.90	0.37	0.507	Afib
Dasatinib	3.7	1.20	0.31	42.00	0.21	0.524	QT, PE, Hy
Erlotinib	N/A	63.38	0.51	87.60	3.11	0.653	MI (rare)
Pazopanib	N/A	73.86	1.19	N/A	103.08	0.671	#QT, LV (rare)
Cabozantinib	N/A	91.14	1.37	N/A	4.43	0.769	#None
Trametinib	100	33.00	2.37	66.80	0.02	1.000	LV
Axitinib	N/A	71.79	0.44	N/A	0.07	1.000	HF (rare) Hy
DMSO	N/A	100.00	0.58	N/A	N/A	1.000	None

a “cardiac safety index” to reflect the cardiotoxicities of existing TKIs

Sharma, A., et al. (2017). Sci Transl Med **9(377).**

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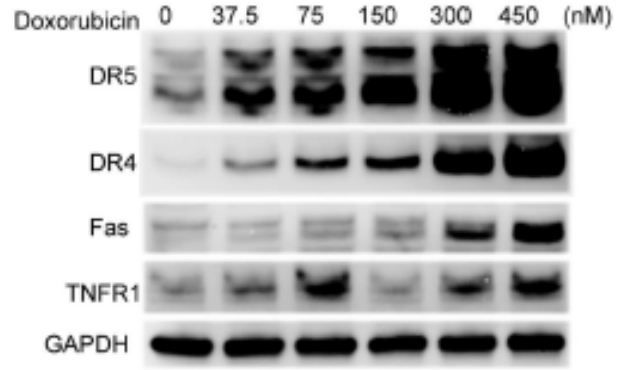
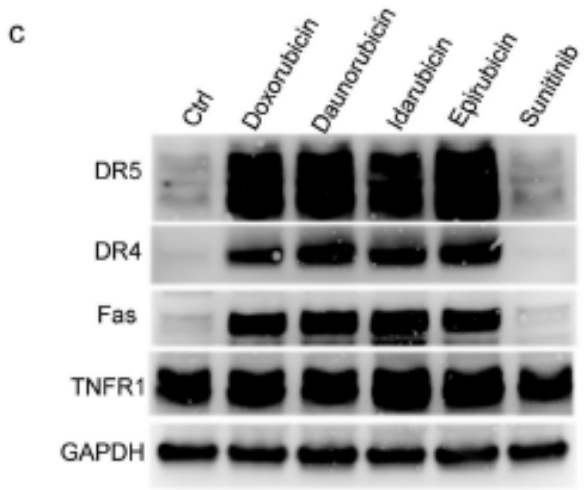
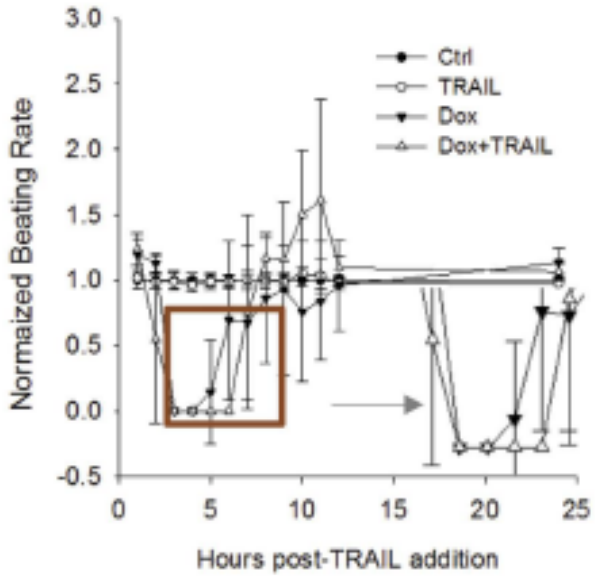
Anthracyclines







"Anthracycline agents upregulated the expression of death receptors (DRs) (TNFR1, Fas, DR4 and DR5) in iPS-derived cardiomyocytes at both protein and mRNA levels"

Zhao, L. and B. Zhang (2017).
Sci Rep **7: 44735.**

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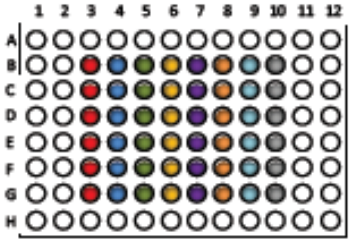
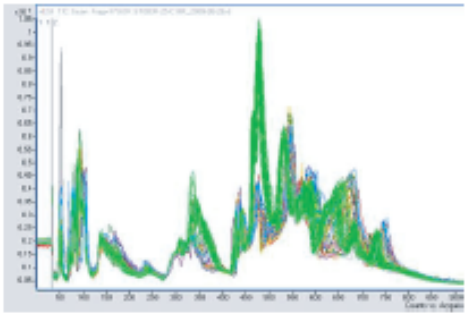
Multiple Agents



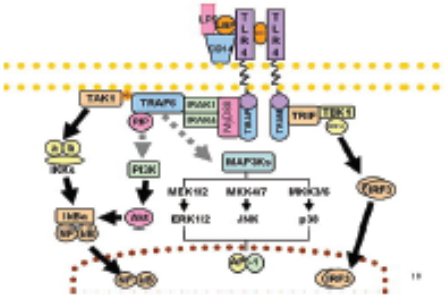
Dose cardiac precursors with drugs of known cardiotoxicity

Metabolites are secreted in response to treatment



Metabolomic profiling

Identify Biomarkers and use for:
- toxicity assay
- diagnostic tools



Identify biochemical pathways to understand disease mechanisms and use for:
- disease models
- disease treatments

Stemina BIOMARKER DISCOVERY

A metabolic signature of toxicity for predicting the cardiotoxic potential of pharmaceutical compounds

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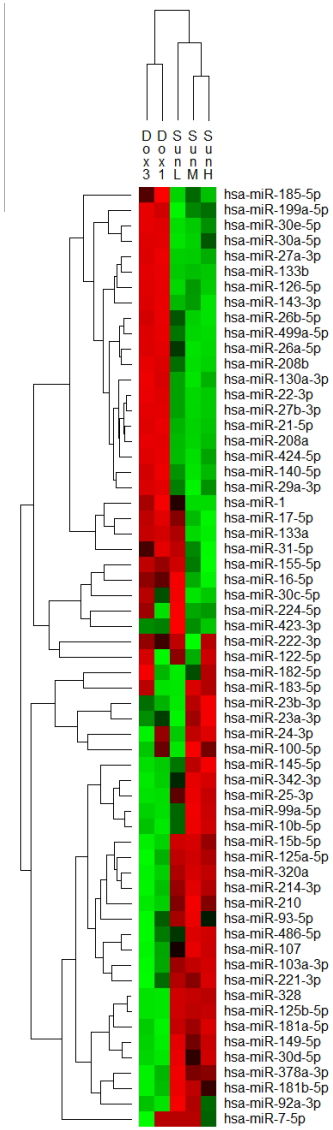
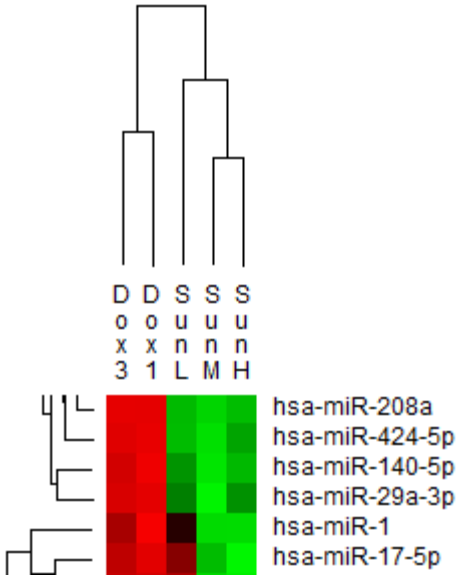
Doxorubicin & Sunitinib





"Cardiac Associated" miRNA panel

Doxorubicin & Sunitinib induce very different changes in cellular miRNA

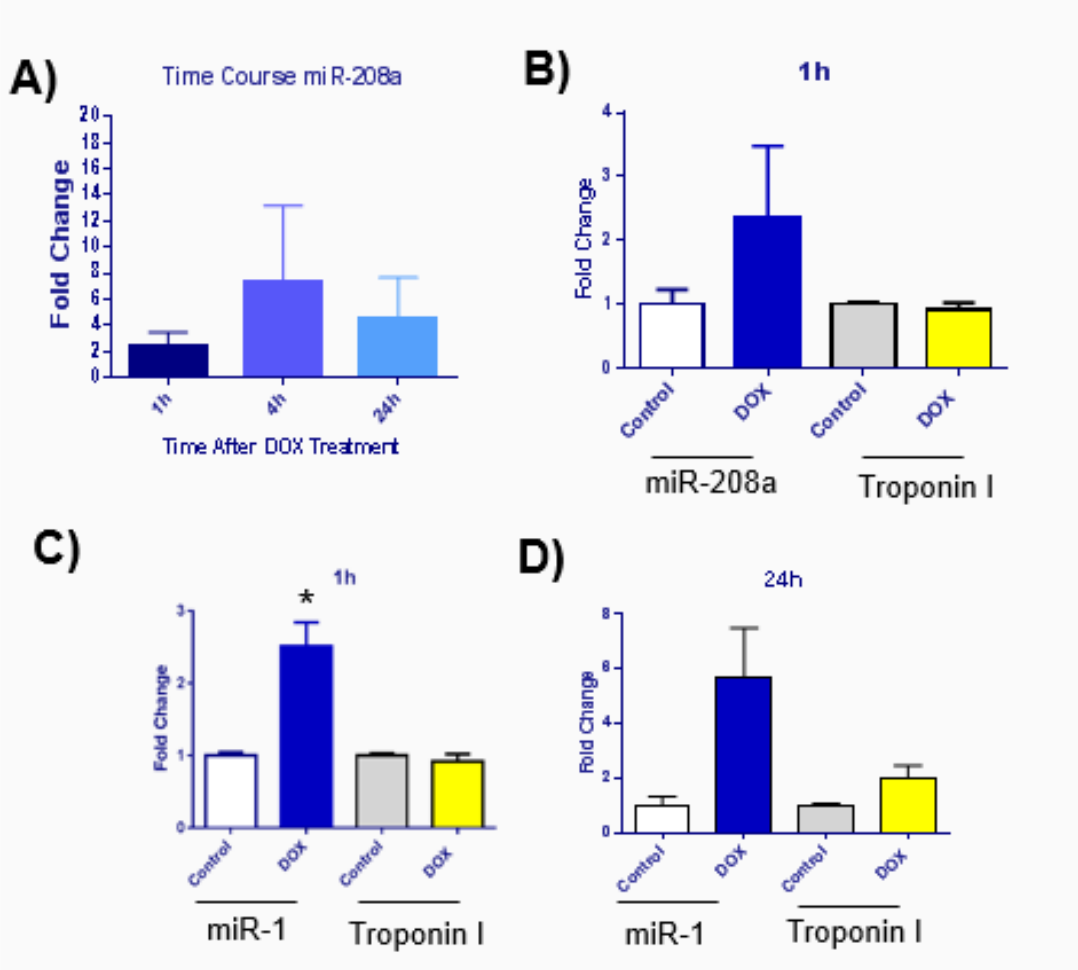
miR-1 and miR-208a appear to be Dox specific

Comparative cardiotoxic effects of doxorubicin and sunitinib in human iPSC-derived cardiomyocytes

Matthew C. White, Lijun Ren, Xi Yang

Gordon Research Conference 2016

Doxorubicin



Extracellular miR-208a, miR-1 and Troponin I

miR-1 and miR-208a appear to be early response markers

PSTC Studies

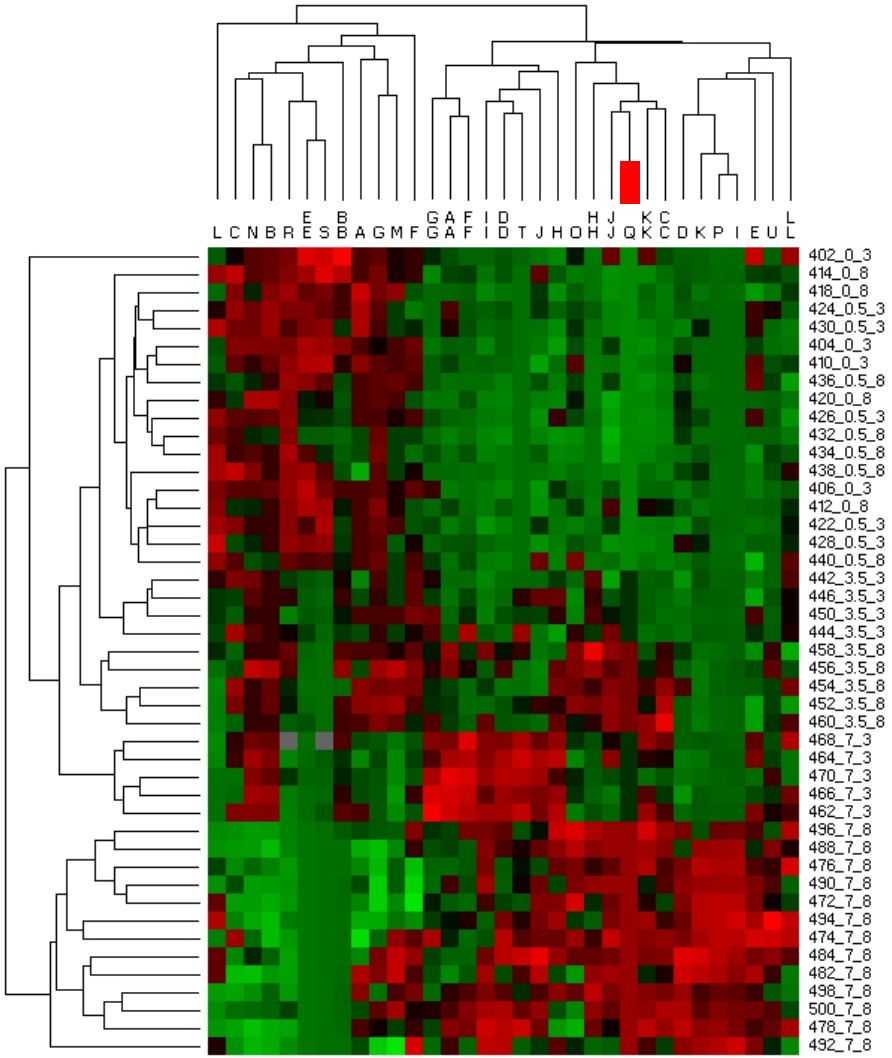
nature biotechnology

Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium

Dieterle, F., et al. (2010). Nat Biotechnol **28(5): 455-462.**

- Multiple rodent studies
- Multiple compounds
- Multiple doses / time points
- Clinical Chemistry
- Histopathology
- Multiple blood and urinary biomarkers

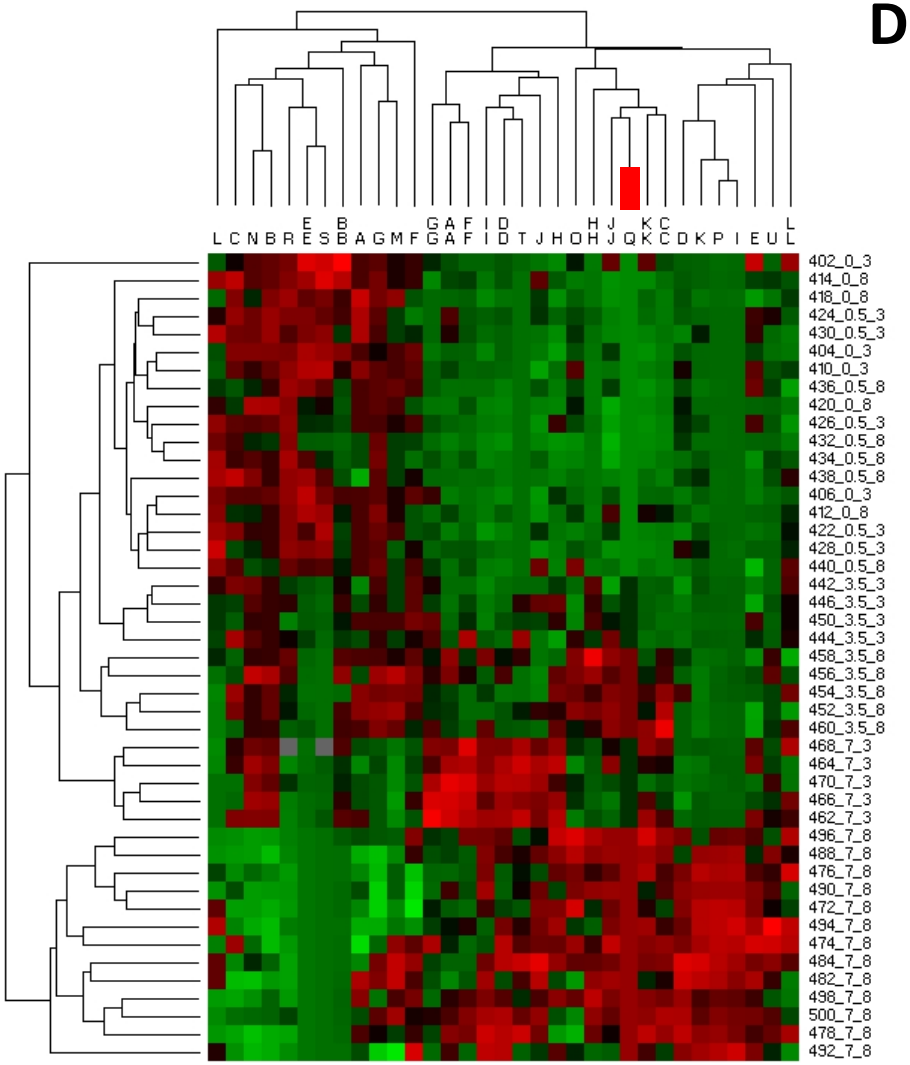
Clustering of Data from One Study



- One compound
  - Control (0)
  - 3 doses (0.5,3.5, 7)
  - 2 days (3, 8)
- Serum clinical chemistry endpoints (A-P)
- Histopathology (Q - Red column)
- Urinary biomarkers (S,T, U, AA – LL)

- All endpoints and samples clustered

Clustering of Data from One Study



Discoveries from this approach

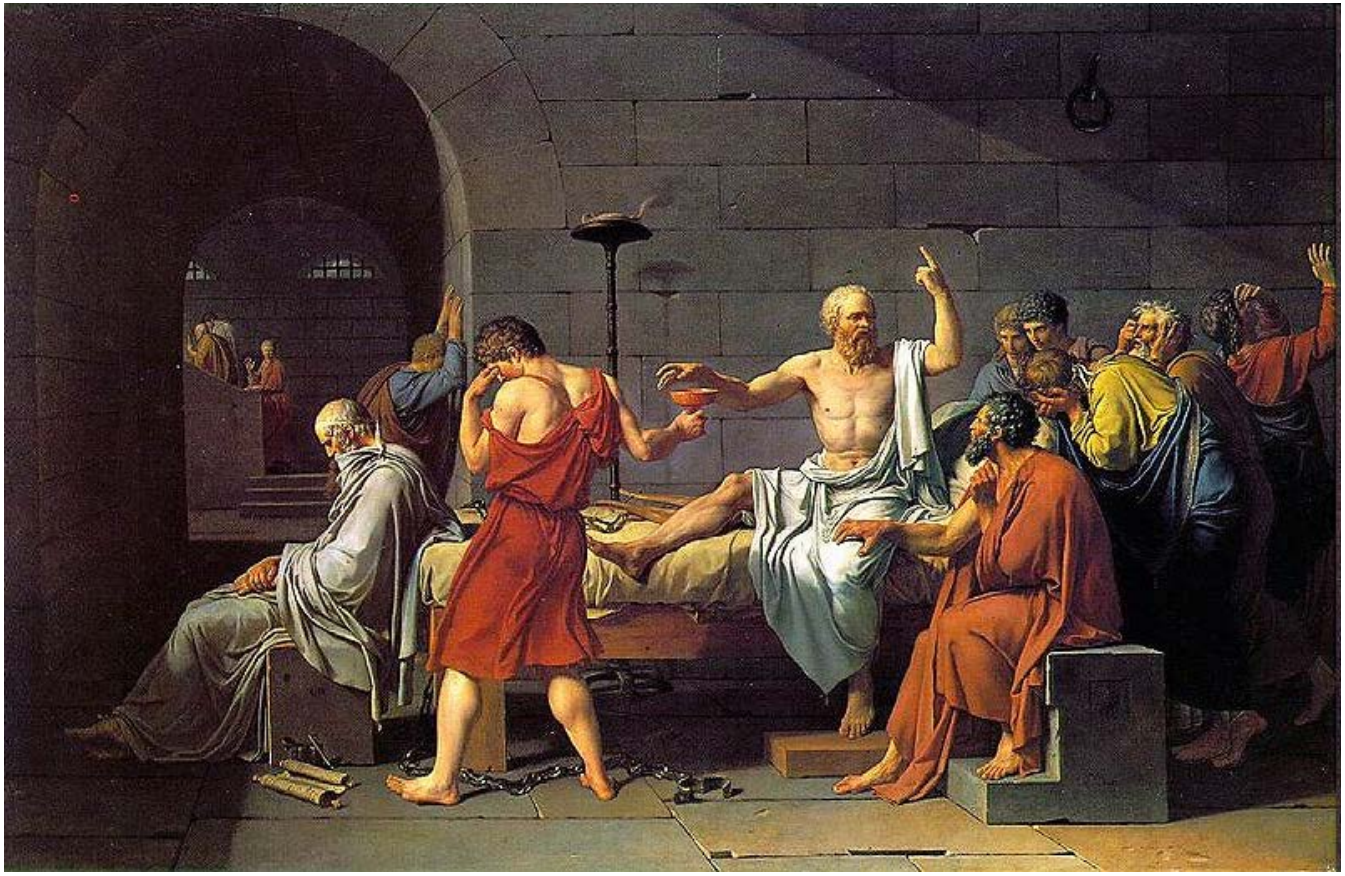
- Not all animals responded the same
- Classes of markers could generally distinguish time and dose groups
- Some markers clustered with histo, some did not
- Many classical clinical chemistry markers clustered with the new markers!

Conclusions

- A holistic view of biology requires integrating multiple approaches and endpoints
- Such integration is a work in progress
  - Data acquisition
  - Technology integration
  - Simultaneous analysis of multiple endpoints
  - Incorporation of biological variation

- **But it offers the potential of elucidating, mechanisms, biology, and predictive biomarkers**

Toxicity Assessment – 399 B.C.

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