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Evaluating Cardiotoxicity Potential:

Translational Approaches and Models

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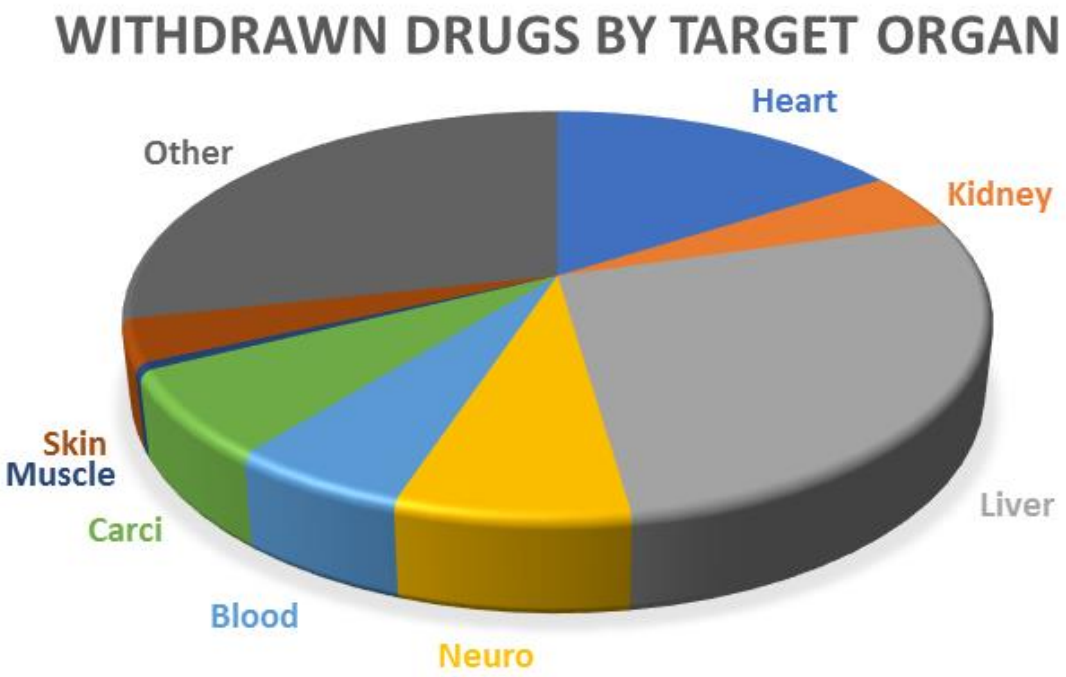
U.S. FOOD & DRUG ADMINISTRATION

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

Cardiotoxicity



Data from Wikipedia

"List of Withdrawn Drugs" - 1979-2011

Cardiotoxicity is a concern for drug development and environmental chemicals

Cardiotoxic Agents

- Anticancer drugs
- Antiretroviral agents
- Antidiabetic drugs
- Cocaine
- Ethanol
- Metamphetamines
- Carbon monoxide
- Metals
  - Lead
  - Cobalt
- Venoms / Toxins

Mechanisms of toxic cardiomyopathy

Hantson, P. (2018). Clinical Toxicology **57:** 1-9

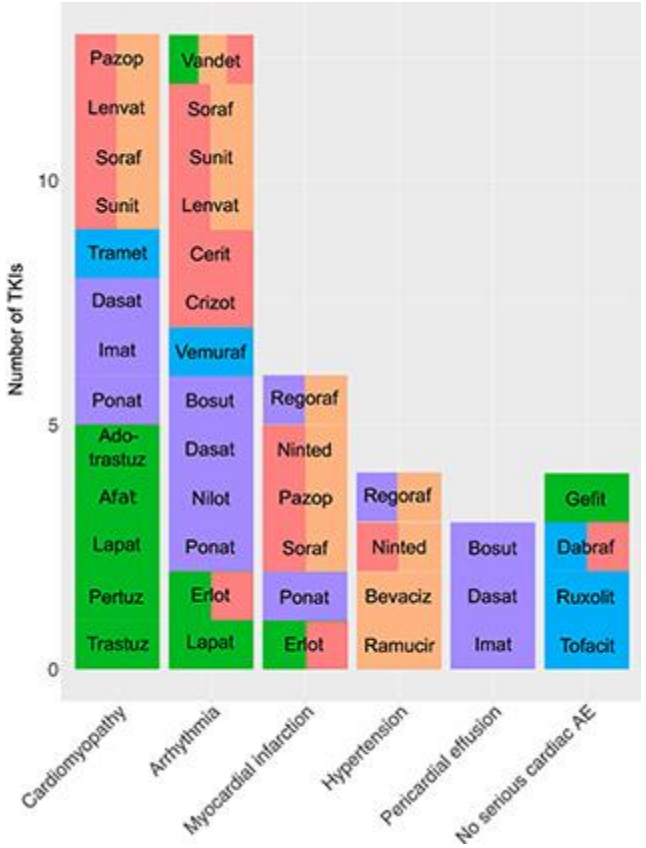
Cobalt Cardiomyopathy
A Critical Reappraisal in Light of a Recent Resurgence

Packer, M. (2016). Circulation: Heart Failure **9**:e003604

Low-level lead exposure and mortality in US adults: a population-based cohort study

Lanphear, BP. (2018). Lancet Public Health **3**: e177–84

Cardiotoxicity - Manifestations



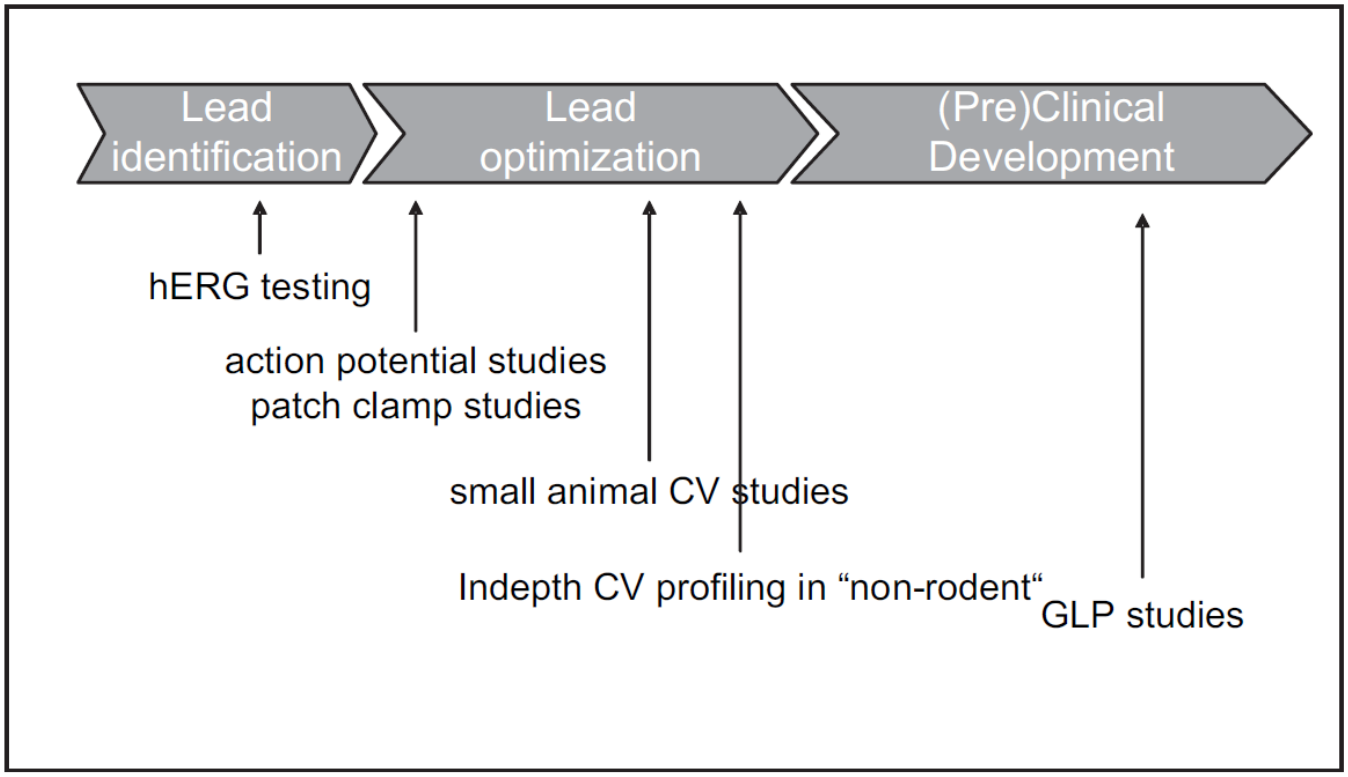
Shim, J. V., et al. (2017). Front Physiol **8:** 651.

Adverse Events Elicited by Tyrosine Kinase Inhibitors

- Cardiomyopathy
  - cardiac dysfunction
  - congestive heart failure
  - left ventricular dysfunction
  - cardiomyopathy
- Arrhythmia
  - prolonged QT interval
  - cardiac bradyarrhythmia
  - cardiac arrhythmia
- Myocardial infarction
- Hypertension
- Pericardial effusion
  - pericardial/pleural effusion
  - cardiac tamponade
- Hypertrophy

Cardiotoxicity Assessment

Drug Development Safety Pharmacology Studies
For Cardiovascular Liabilities

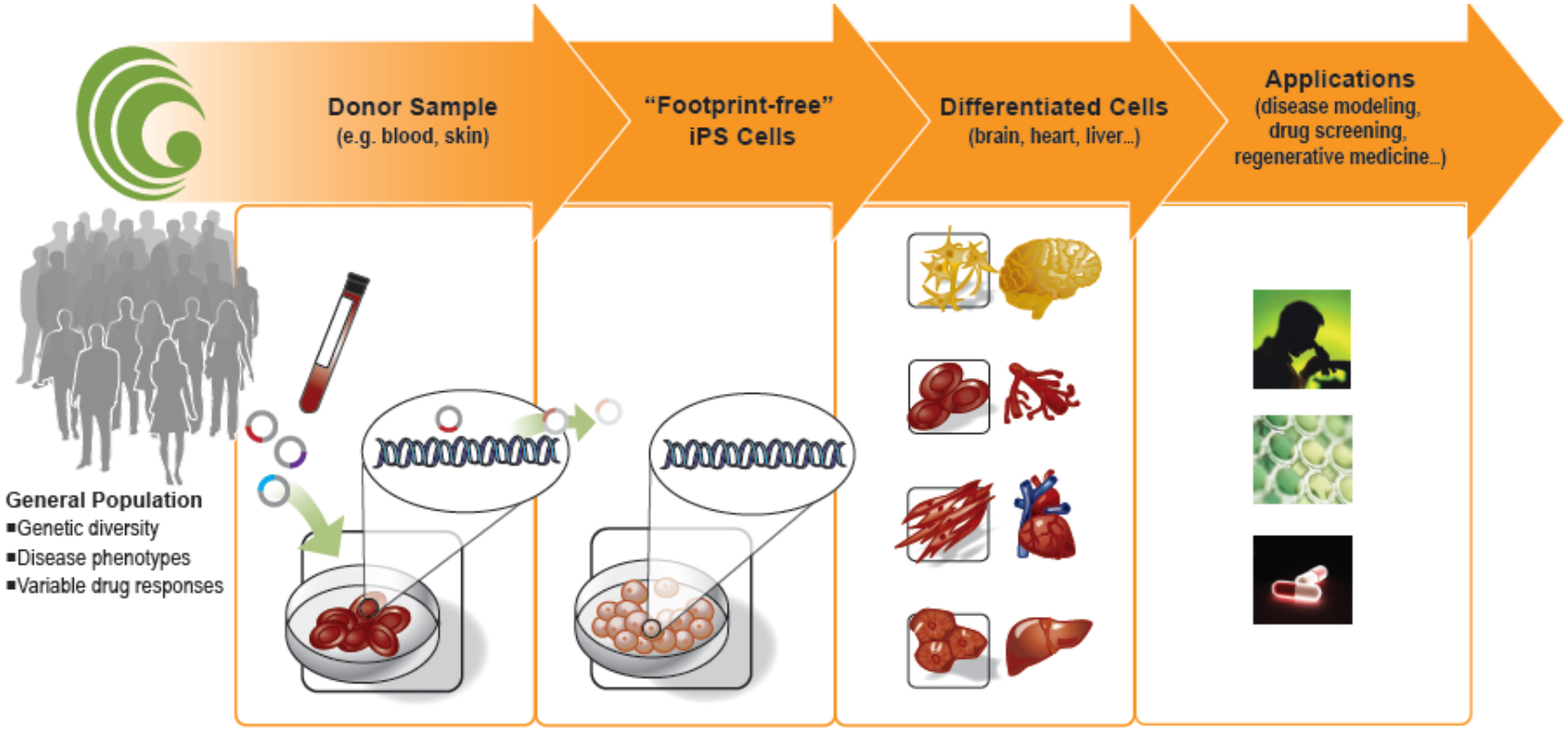


Guth, B. D. (2007). Toxicol Sci **97: 4-20.**

New *in vitro* Tools and Approaches

- “Cardiomyocytes” from induced pluripotent stem cells from human donors: iPSC-CMs
- Noninvasive electrical activity monitoring: Impedance assay and multi-electrode array
- High throughput Ca^{2+} flux assays

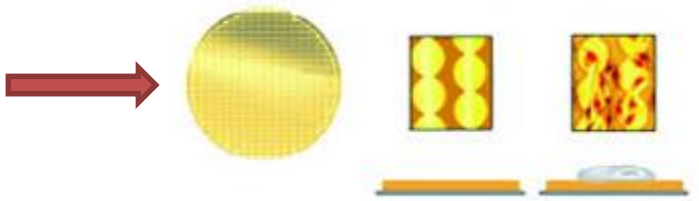
Derivation of human iPSC-CMs



From: "CDI: Providing True Human Biology in a Dish" DS-CDI17025 © 2017 CDI, Inc

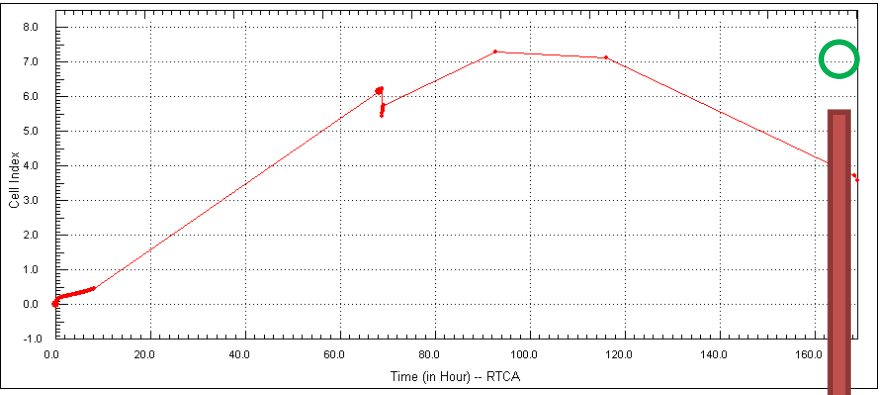
Non-invasive Impedance Assay

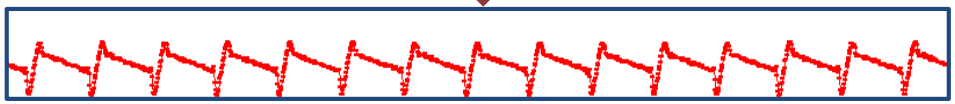
96-well



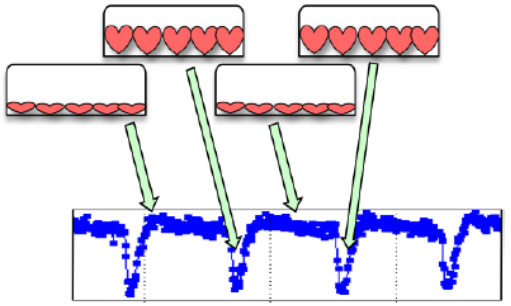
Real-time, label-free

- Morphology
- Cell-cell contact
- Adhesion





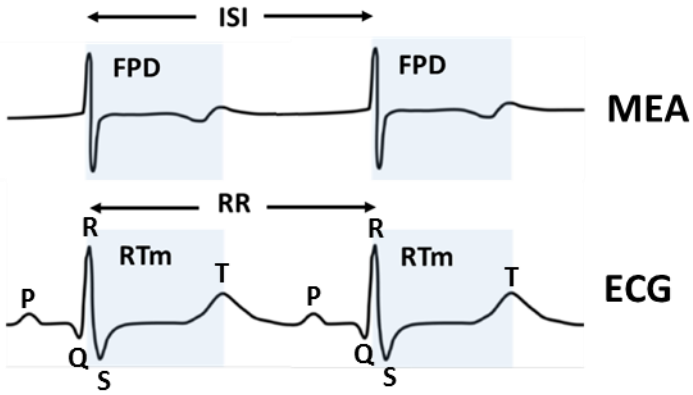
Contraction

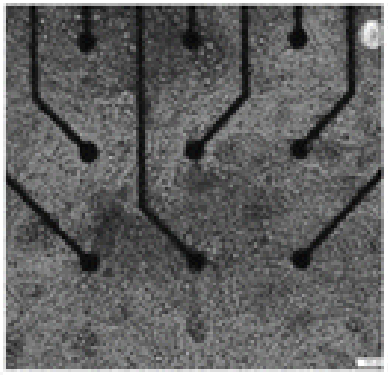


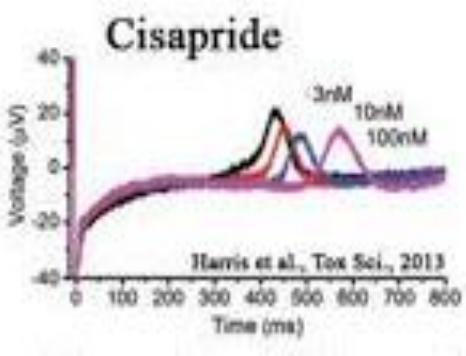
Sensitivity: Morphology change 1nm

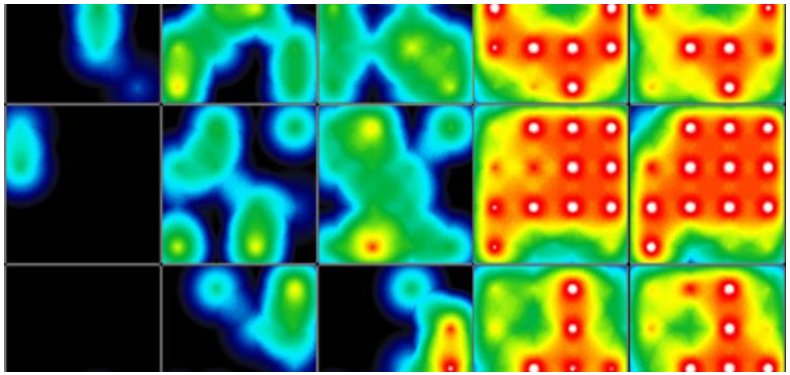
(Cell membrane 3nm; Light microscopy ~250 nm)

Micro-electrode Array (MEA)

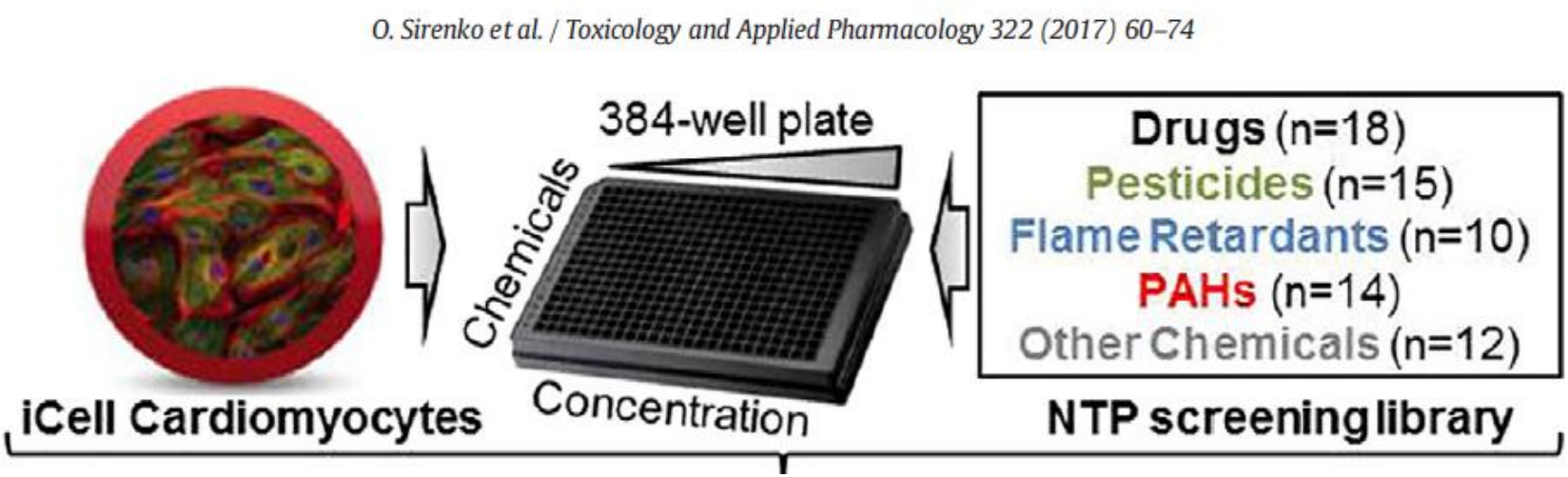








High-Throughput Screening



- 30’ / 24 hr time points
- Ca2+ flux measurements
- high-content imaging

Changing Qt Studies

The new CIPA paradigm will be driven by a suite of mechanistically based in vitro assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity, with verification of completeness through comparison of predicted and observed responses in human-derived cardiac myocytes.

What's Not To Like?

Acute (contractile) vs Chronic (structural) effects?

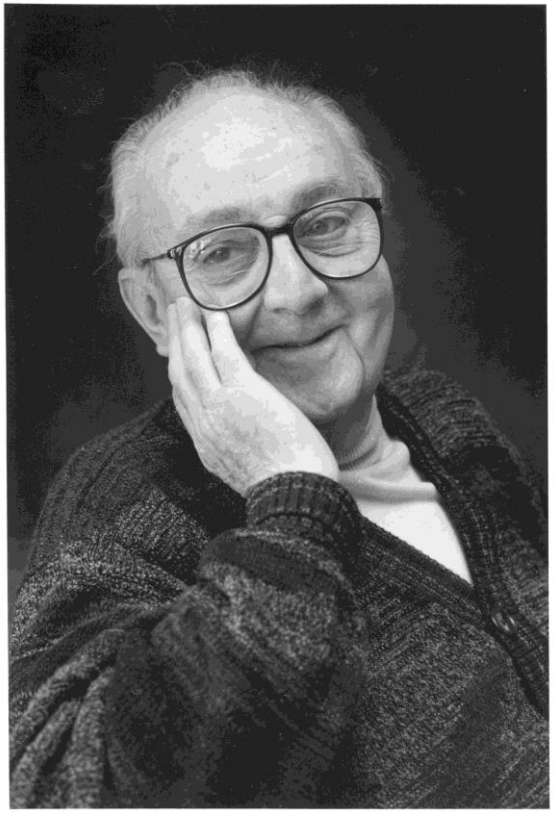
"However, QT prolongation and other arrhythmias are only one part of the iceberg, as they account for 23% and 4% of the cardiovascular issues, respectively. Therefore, to increase the likelihood of success, an effective de-risking strategy should not solely cover proarrhythmia liability, but also integrate hemodynamic and cardiac contractility assessment, and address both functional and structural aspects of cardiotoxicity."

- Atienzar, F., et al. (2016). Journal of Medicines Development Sciences **2:** 2

Basic Principle 1

All models are wrong;
some models are useful.

-George E. P. Box

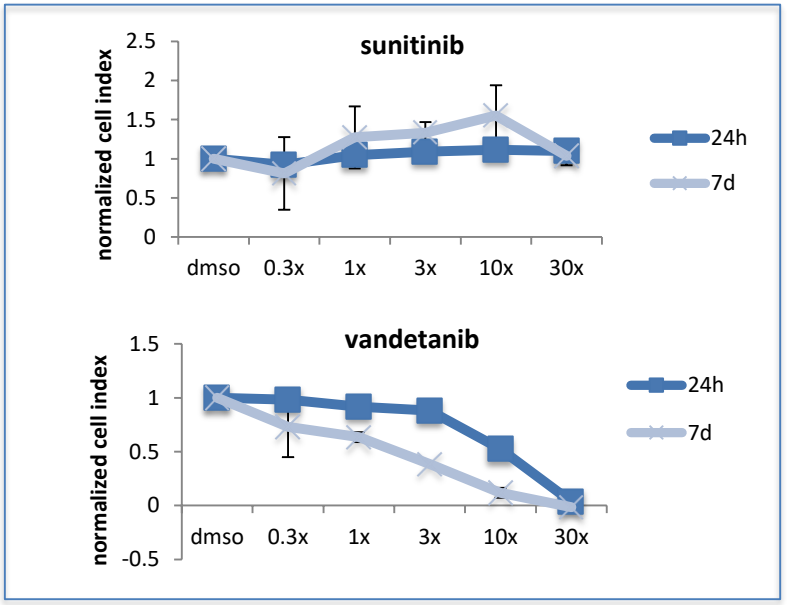


Model Improvement

- Can an *in vitro* system model chronic / structural type cardiotoxicity?
- What is the impact of different donors on cardiotoxicity?
- What impact do assay conditions have on results?
- How can an *in vitro* approach be informed from *in vivo* and clinical data*?*

How well do current iPSC-CMs model KI-induced cardiotoxicity? Are longer exposures more informative?

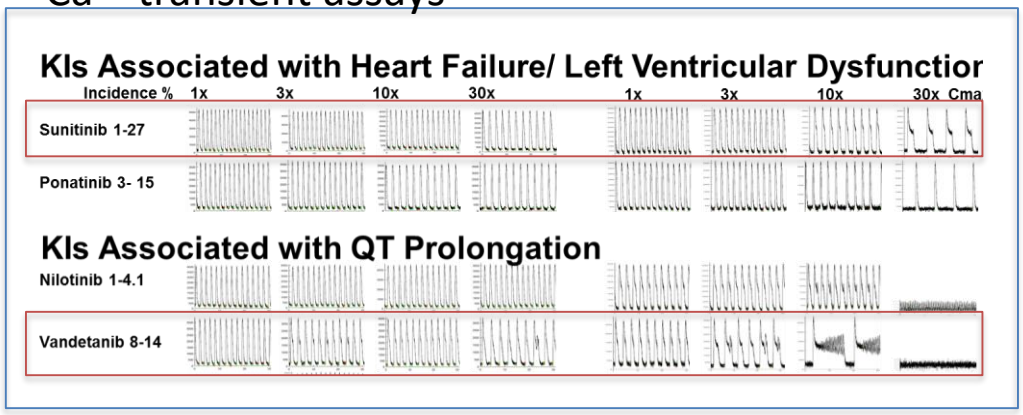
Impedance assays



	24 hour					7 day				
	dmso	1x	3x	10x	30x		1x	3x	10x	30x
CER	1	0.867735	0.411163	0.011234	0.003915	CER	0.405809	0.049479	0.004906	0.004572
VAN	1	0.917576	0.880684	0.523767	0.03512	VAN	0.634715	0.384564	0.117981	-0.01672
SOR	1	0.957793	0.936444	0.45242	0.045458	SOR	1.220704	1.142177	0.124714	-0.05206
CRI	1	0.933222	0.798612	0.377737	0.079433	CRI	0.873156	0.550844	0.137802	0.001654
NIL	1	1.075732	0.969591	0.870094	0.604992	NIL	1.185885	1.012054	0.287289	0.025311
PAZ	1	0.970546	1.010701	0.962083	0.669064	PAZ	0.939998	0.902113	0.684604	0.492077
PON	1	1.11435	1.119192	1.097897	0.872589	PON	1.121721	1.070031	0.720123	0.343855
TRA	1	1.026835	1.02134	1.032239	1.023473	TRA	0.89936	0.882507	0.810762	0.760366
IMA	1	1.024858	1.082229	1.118954	0.392564	IMA	0.899059	0.963713	0.812558	0.001878
AFA	1	0.997261	0.986966	0.942983	0.914418	AFA	0.967557	0.92243	0.882326	0.790572
GEF	1	1.000552	1.014942	1.014984	1.010355	GEF	1.004067	1.013952	0.982608	0.966714
SUN	1	1.046766	1.090492	1.115261	1.099914	SUN	1.273006	1.333866	1.554474	1.036261

normalized cell index

Ca^{2+} transient assays



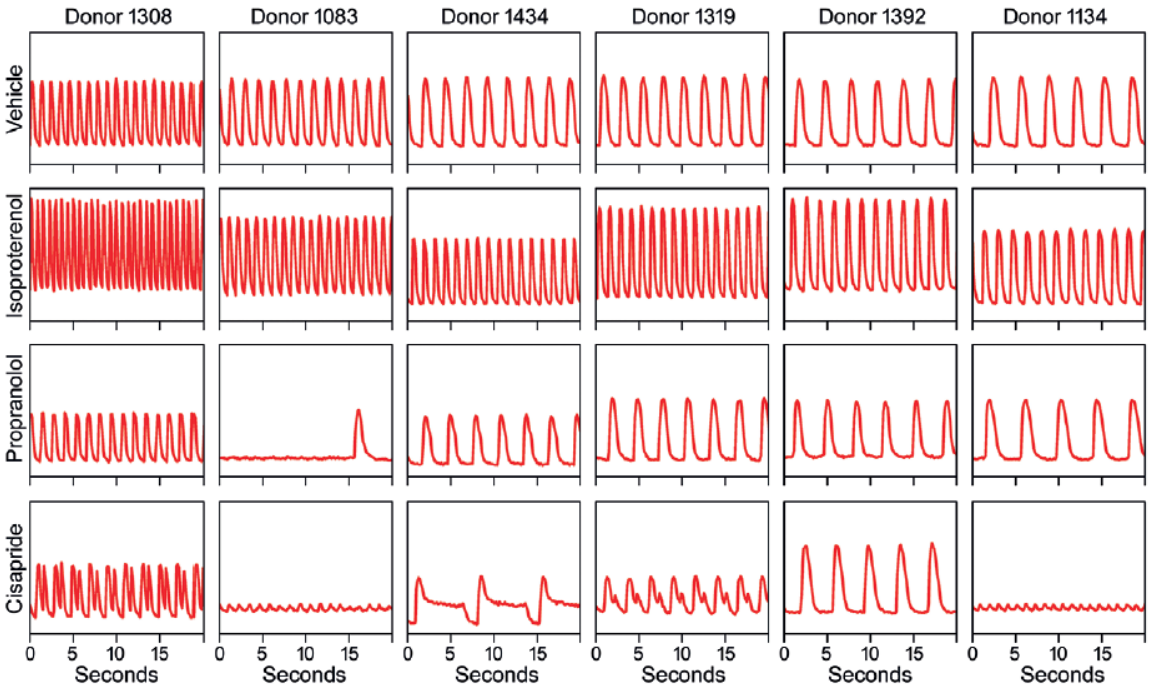
Better specificity at 24h, better sensitivity at 7d

		24 h			7 day		
		Amp	Mito	APD90	Amp	Mito	APD90
True_P		4	5	15	20	18	22
True_N		6	6	4	3	4	2
False_P		1	1	3	5	3	6
False_N		20	17	9	3	6	1
Sensitivity	TP/(TP+FN)	0.17	0.23	0.63	0.87	0.75	0.96
Specificity	TN/(TN+FP)	0.86	0.86	0.57	0.38	0.57	0.25
PPV	TP/(TP+FP)	0.80	0.83	0.83	0.80	0.86	0.79
NPV	TN/(TN+FN)	0.23	0.26	0.31	0.50	0.40	0.67

*X. Yang, SOT 2017

Impact of Donor on Derived iPSC-CM

Ca^{++} Flux Traces for Cells Derived from 6 Donors



iPSC-CMs:
27 healthy donors

"The degree of inter-individual variability in responses to treatment is reproducible, and depends on the chemical and phenotypic endpoint"

Grimm, F. A., et al. (2018). Altex **35:** 441-452.

Patient-specific iPSC-CMs

Medical College of Wisconsin and Cellular Dynamics Awarded NHLBI Grant Using Human Induced Pluripotent Stem Cells

Download PDF

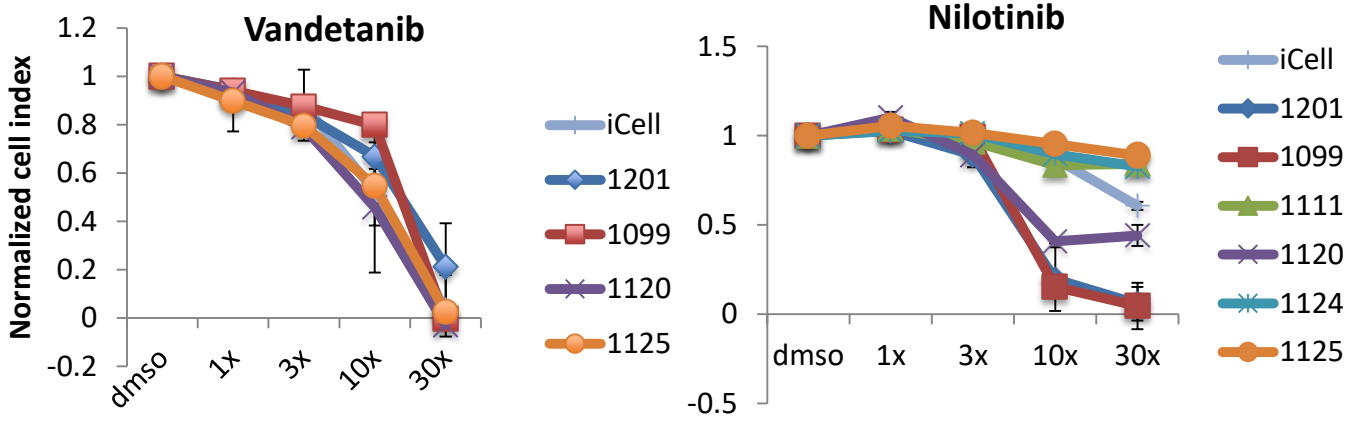
CDI Will Generate 250 Stem Cell Lines and Differentiate Them into Heart Cells to Investigate Mechanisms Underlying High Blood Pressure

HyperGEN – NHLBI Family Blood Pressure Program:

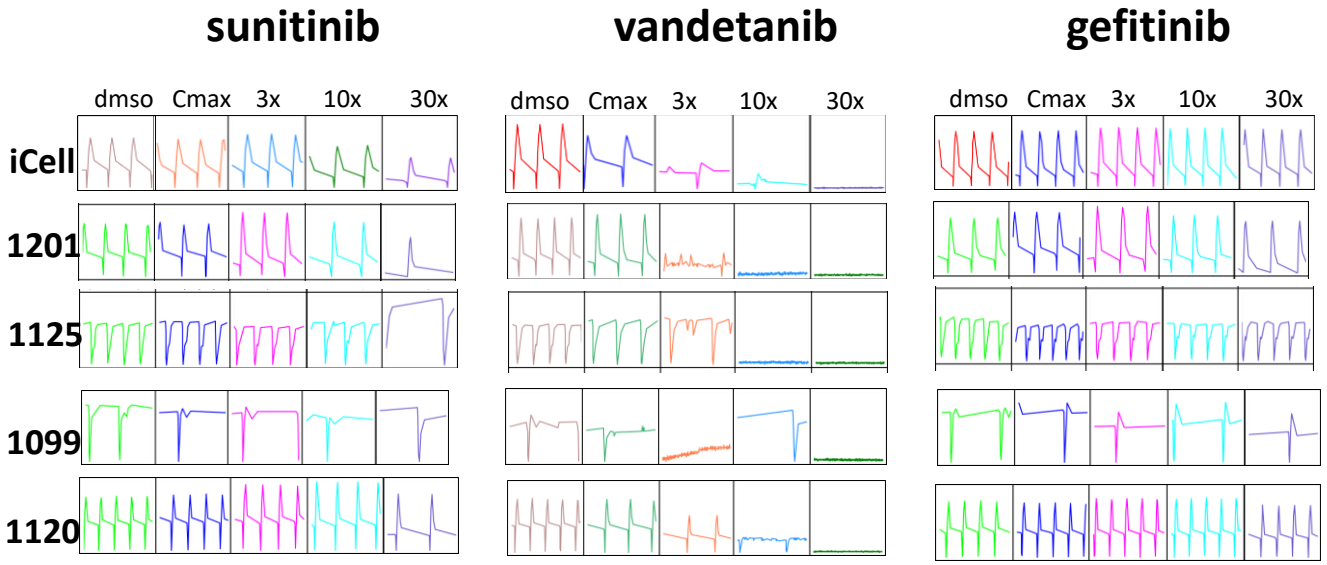
- African-American and Caucasian Cohort
- Phenotyping: Cardiovascular phenotypes and risk factors
- Family-based ascertainment
- GWAS performed in families
- WES data available + iPSC WGS grant submitted
- Generated from a peripheral blood sample
- Differentiated and cryopreserved
- Tested for pluripotency and chromosomal integrity

Impact Of Donor Variability On KI-induced Cardiotoxicity

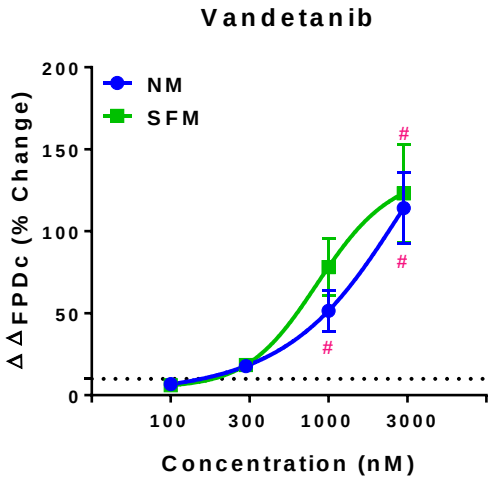
Cell Index



Impedance-based beating profiles

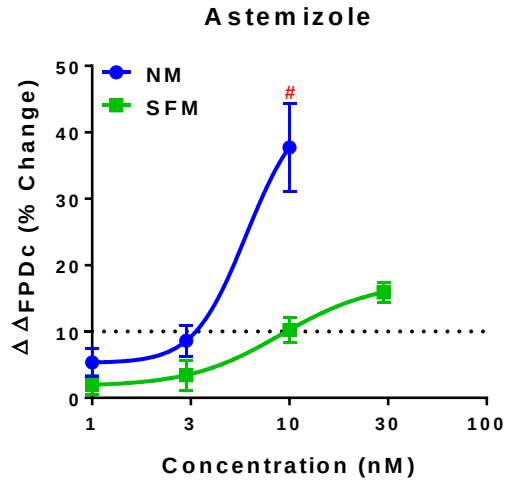


Impact of Assay Conditions



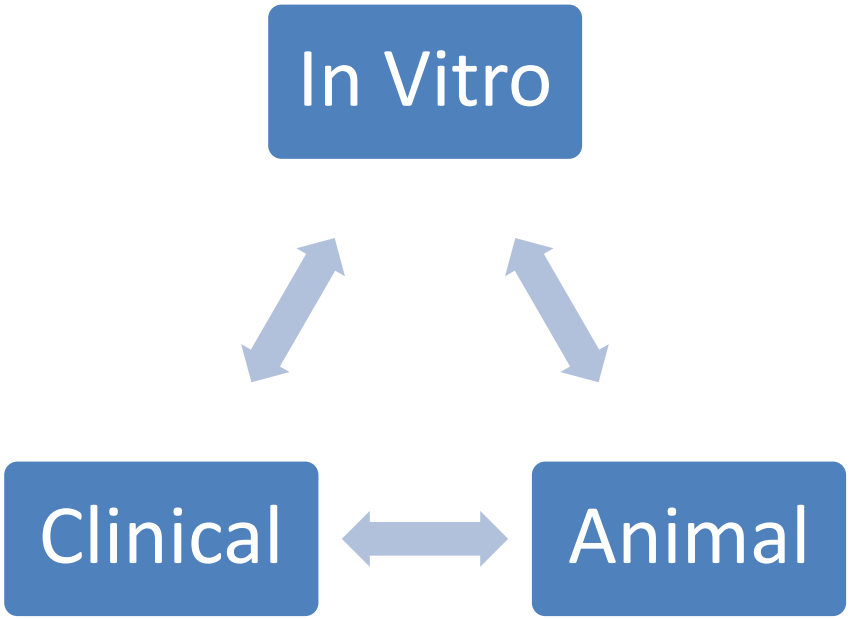
Normal vs. Serum-Free Medium

Total Drug Conc. (Prepared)	Bound (%)	Free Drug Conc. (Measured)
1000 nM in NM	72.5 ± 0.7 %	309.0 ± 14.6 nM
1000 nM in SFM	60.6 ± 7.0 %	272.5 ± 23.1 nM



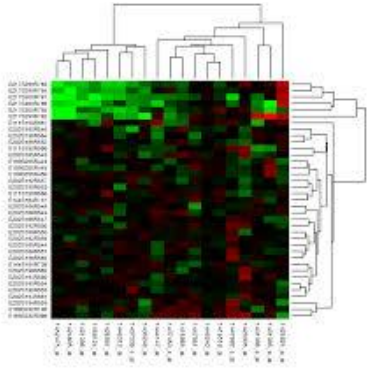
Total Drug Conc. (Prepared)	Bound (%)	Free Drug Conc. (Measured)
30 nM in NM	95.1 ± 1.0 %	1.47 ± 0.12 nM
30 nM in SFM	79.2 ± 1.8 %	0.81 ± 0.02 nM***

Translational Systems Biology

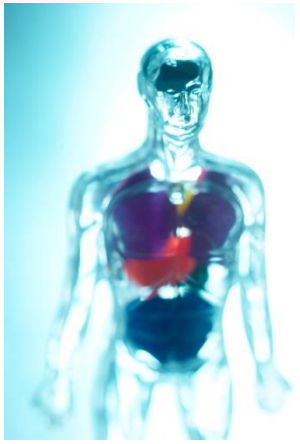


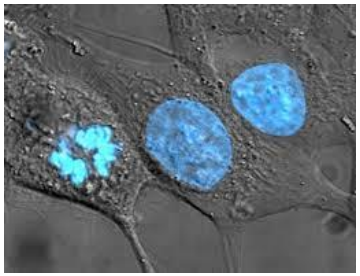
- Connect non-clinical studies with clinical investigations
  - Mechanism
  - Biomarkers
- Improve safety assessment tools

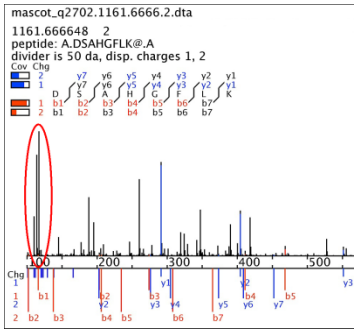
Systems Tools



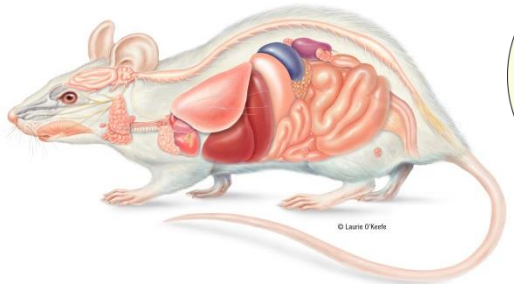
Transcriptomics

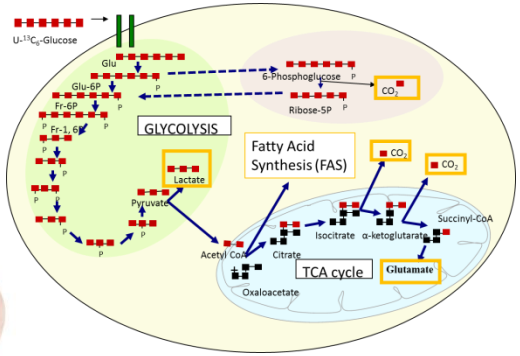






Proteomics



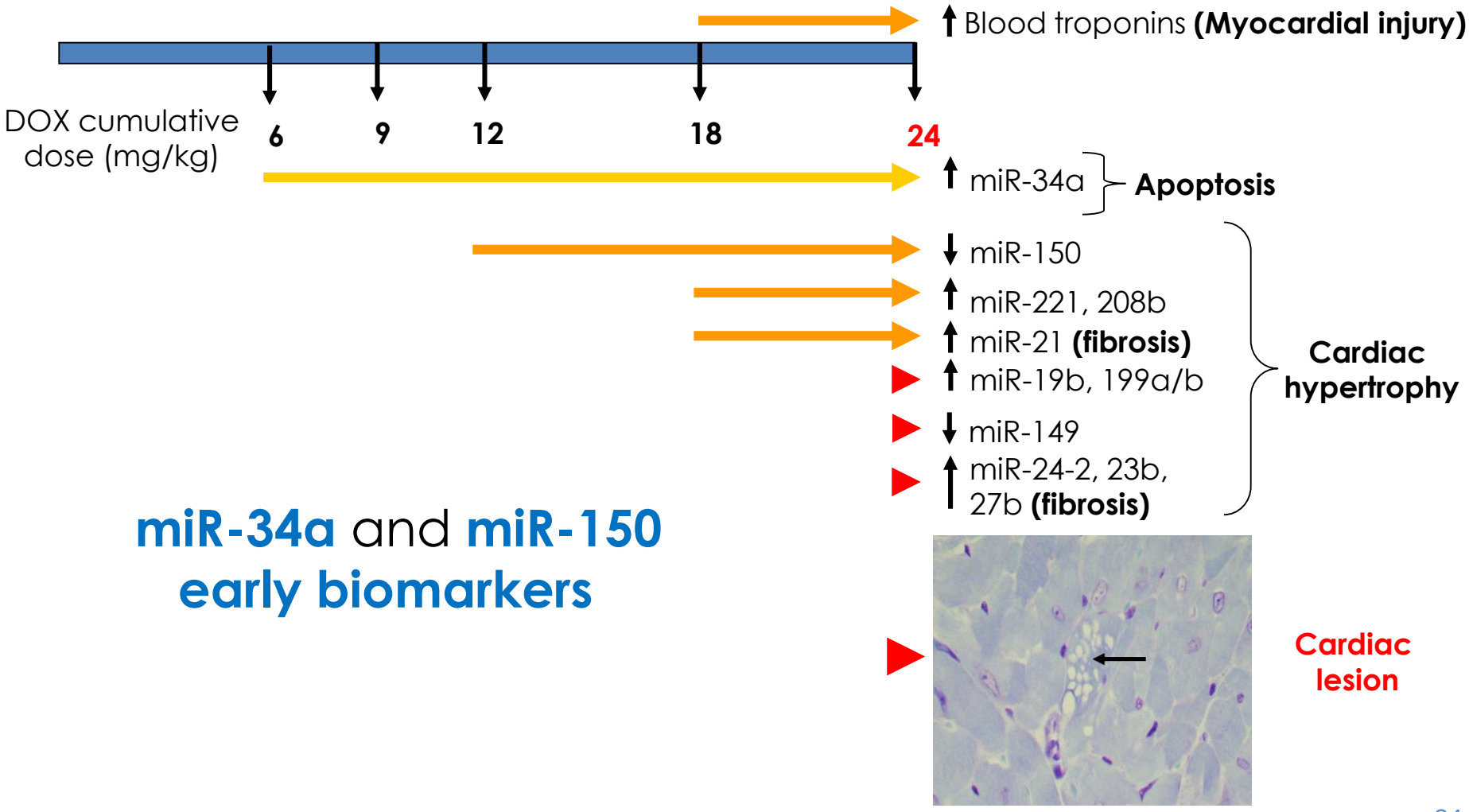


Metabolomics

Augmenting Progress

- *In vivo* and clinical studies to connect to *in vitro* models
  - *In vivo*
    - Mouse model of chronic cardiotoxicity
      - Doxorubicin, Sunitinib
    - Mouse model of delayed-onset cardiotoxicity
  - Clinical
    - Breast cancer patients treated with doxorubicin
    - Pediatric patients treated with doxorubicin

Mouse Model of Chronic Cardiotoxicity



miR-34a and **miR-150**
early biomarkers

24

Circulating Protein Markers of Doxorubicin Cardiotoxicity

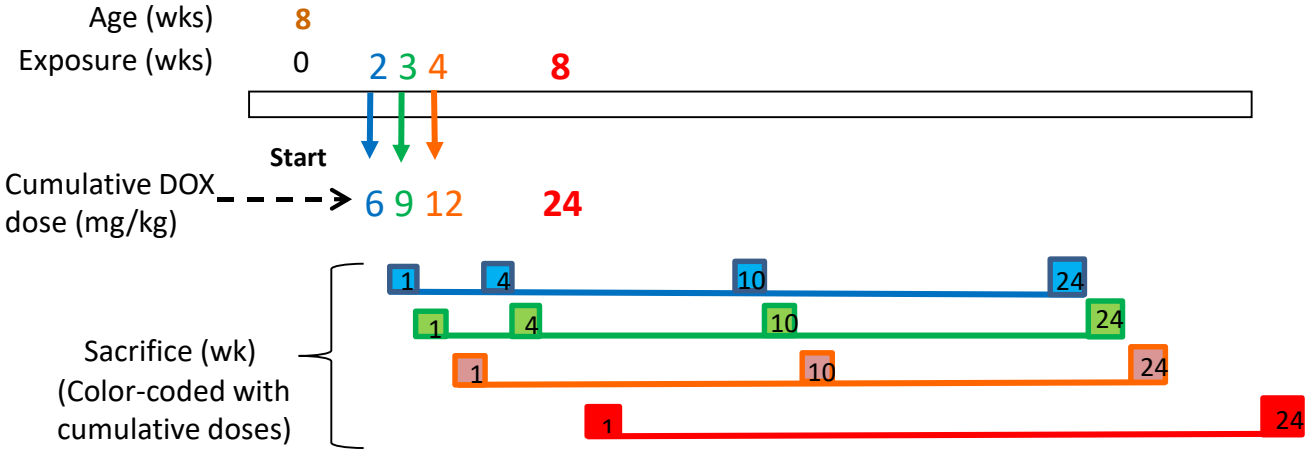
SOMA ID	Target Full Name	UniProt	Fold ratio (Dox/Sal)				
			Doxorubicin Effect				
			Drug expsoure in weeks (cumulative dose in mg/kg)				
			2 (6)	**3 (9)**	**4 (12)**	**6 (18)**	**8 (24)**
			No cardiotoxicity			**Myocardial Injury**	**Pathology**
Early Injury Markers of Toxicity							
SL005703	Neurogenic locus notch homolog protein 1	P46531	1.72	1.59	1.67	1.53	1.59
SL000017	von Willebrand factor	P04275	1.60	1.62	1.97	1.92	2.20
SL016563	Mitochondrial glutamate carrier 2	Q9H1K4	1.19	1.17	1.32	1.30	1.21
SL004652	Wnt inhibitory factor 1	Q9Y5W5	1.33	1.11	1.36	1.23	1.18
SL008909	Legumain	Q99538	1.30	1.02	1.20	1.23	1.24
SL011049	Mannan-binding lectin serine protease 1	P48740	1.35	1.17	1.30	1.23	1.24
Markers of Toxicity							
SL001761	Troponin I, cardiac muscle	P19429	1.61	1.52	1.95	3.50	3.59
SL005233	Tumor necrosis factor receptor superfamily member 27	Q9HAV5	1.21	1.20	1.39	1.50	1.65
SL003328	Complement factor I	P05156	0.96	0.88	0.86	0.82	0.83
SL007502	Carbohydrate sulfotransferase 15	Q7LFX5	0.94	0.81	0.75	0.78	0.72
SL003303	C-C motif chemokine 28	Q9NRJ3	0.73	1.10	0.79	0.68	0.54
SL004857	Desmoglein-2	Q14126	0.76	0.77	0.61	0.39	0.26
SL004791	Tumor necrosis factor receptor superfamily member 25	Q93038	0.80	0.87	0.74	0.55	0.45
SL007464	Anti-Muellerian hormone type-2 receptor	Q16671	0.87	0.84	0.65	0.44	0.41
SL010390	Coiled-coil domain-containing protein 80	Q76M96	1.03	0.83	0.91	0.89	0.69
SL008178	Dermatopontin	Q07507	0.99	0.83	0.88	0.85	0.72
SL002508	Interleukin-18-binding protein	O95998	1.16	0.98	1.12	1.23	1.38
SL000462	Insulin-like growth factor-binding protein 1	P08833	1.23	0.85	0.96	1.10	2.81
SL003679	Cation-independent mannose-6-phosphate receptor	P11717	1.13	0.95	0.91	0.85	0.79
SL009324	Follistatin-related protein 3	O95633	1.02	0.86	0.85	0.86	0.77
SL004676	Insulin-like growth factor-binding protein 5	P24593	1.13	0.94	0.94	0.96	0.83

Plasma protein measurements performed using aptamer-based technology by SOMALogic, Inc. **False Discovery Rate <0.1**

Mouse Model of Delayed-onset Cardiotoxicity

Study design

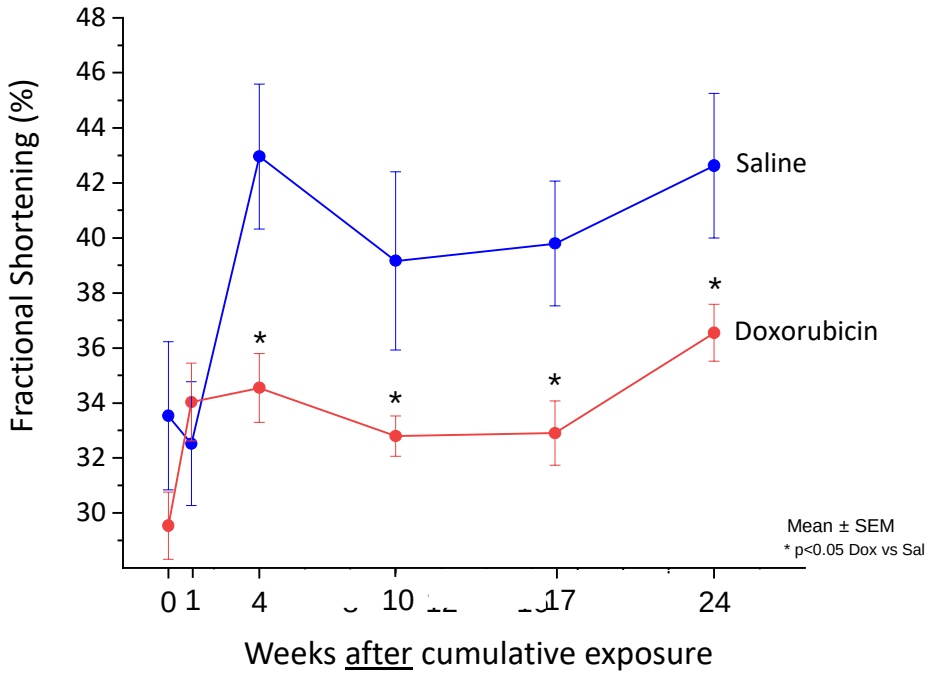
Animals:	Male B6C3F$_1$ mice
Treatment:	Doxorubicin or saline (i.v.)
Dose:	3 mg/kg body wt./week
Sacrifice:	1-, 4-, 10-, 24-week after each cumulative dose



Mouse Model of Delayed-onset Cardiotoxicity

Left Ventricular Fractional Shortening (FS)

24 mg/kg cumulative doxorubicin dose#

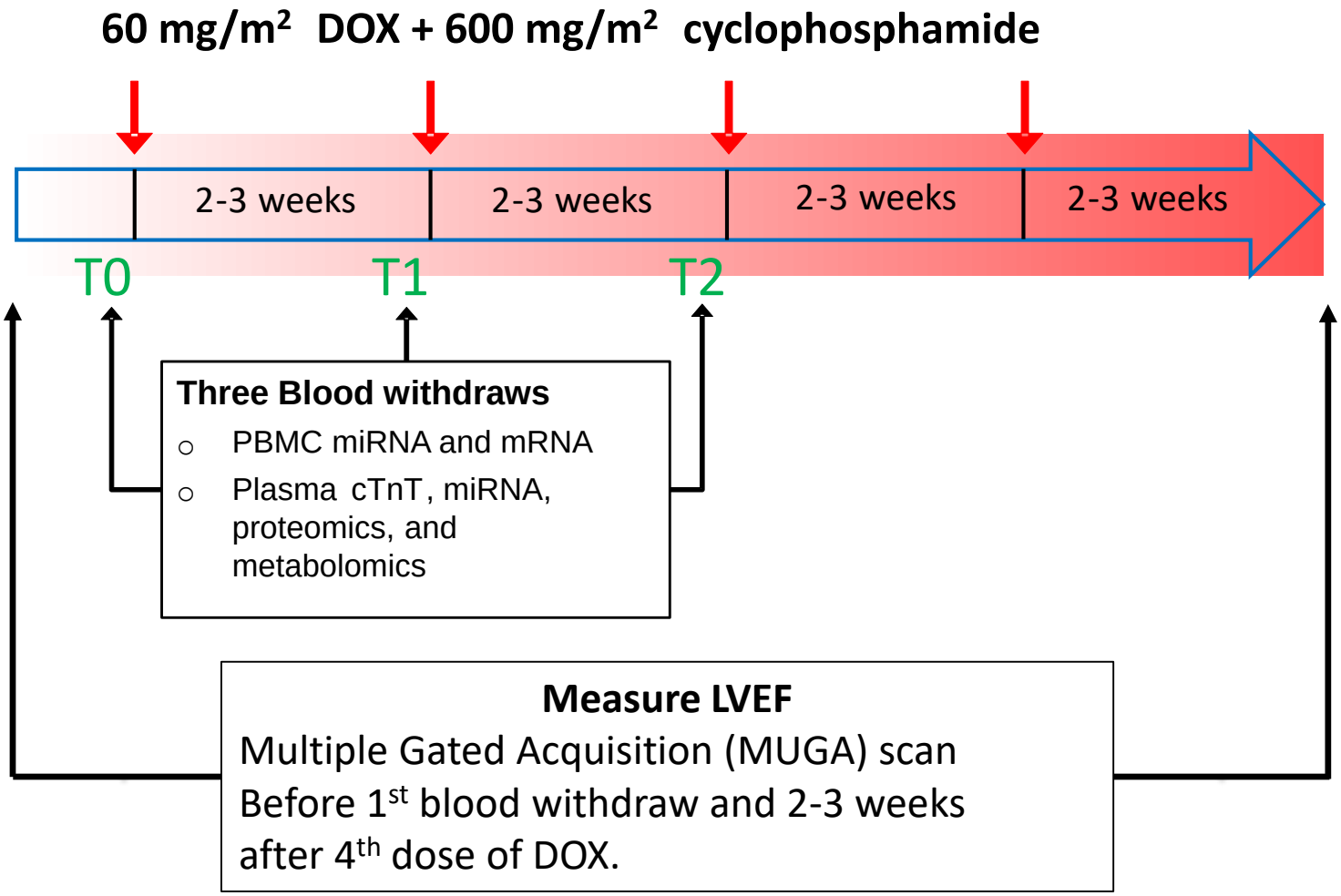


~14- 20% decline in FS at 4 -24 wk after the end of Dox treatment

71 mg/m^2 HED

Clinical Cardiotoxicity

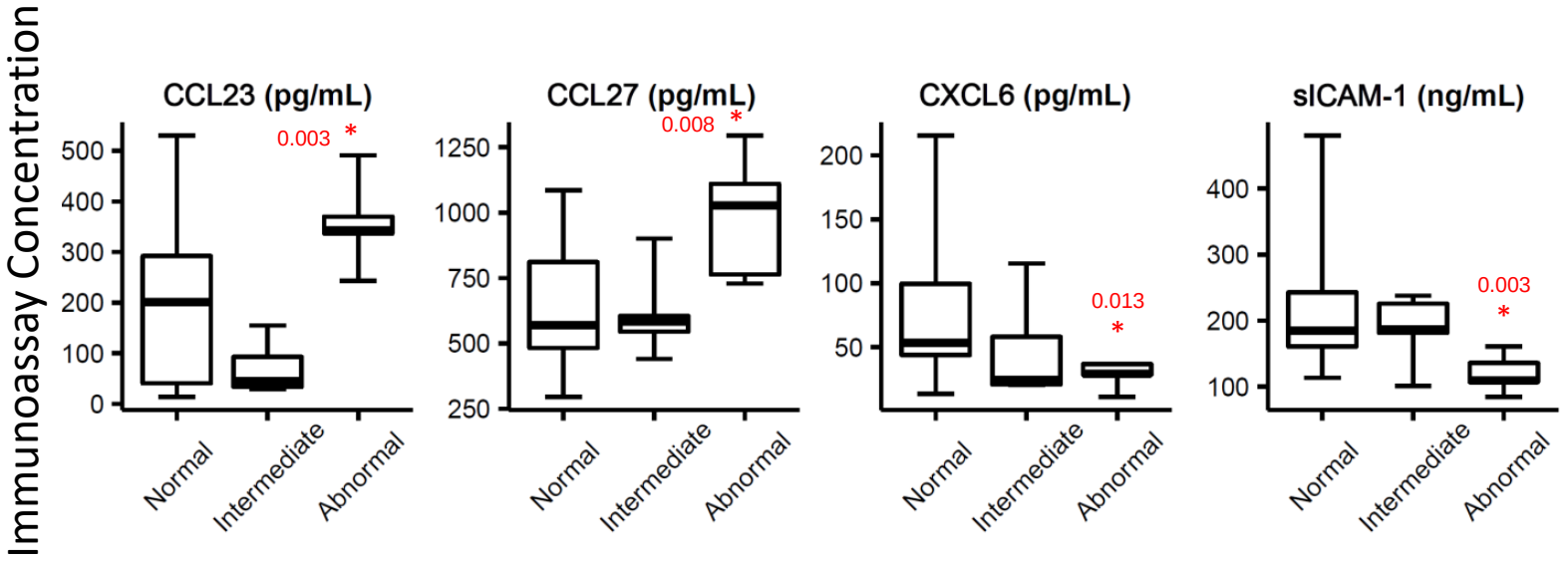
100 breast cancer patients receiving doxorubicin



This study was approved by RIHSC

Clinical Cardiotoxicity

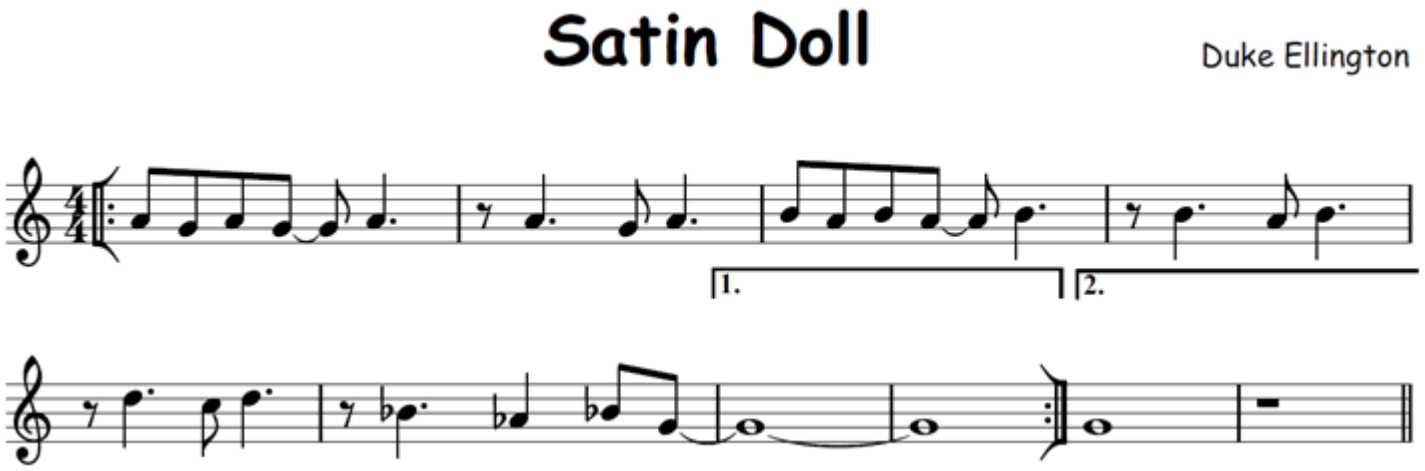
Differential Plasma Levels of Proteins in the Patient Groups before DOX Treatment (T0)



Next Steps

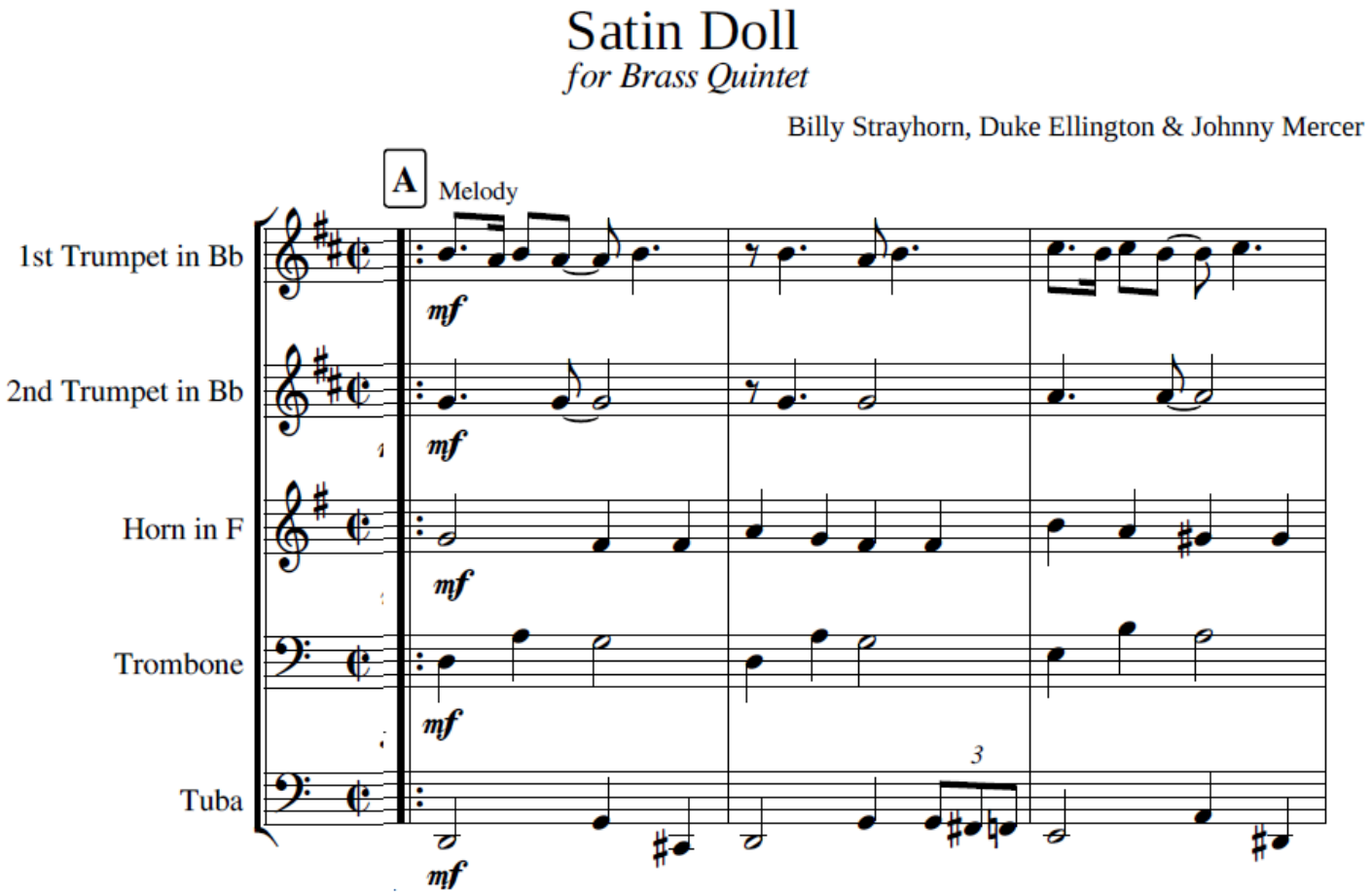
- Correlate *in vivo* with clinical endpoints
  - Protein / metabolomic biomarkers
- Examine *in vitro* model for correlative biomarkers
  - E.g. miRNA, metabolomic

One Tool by Itself



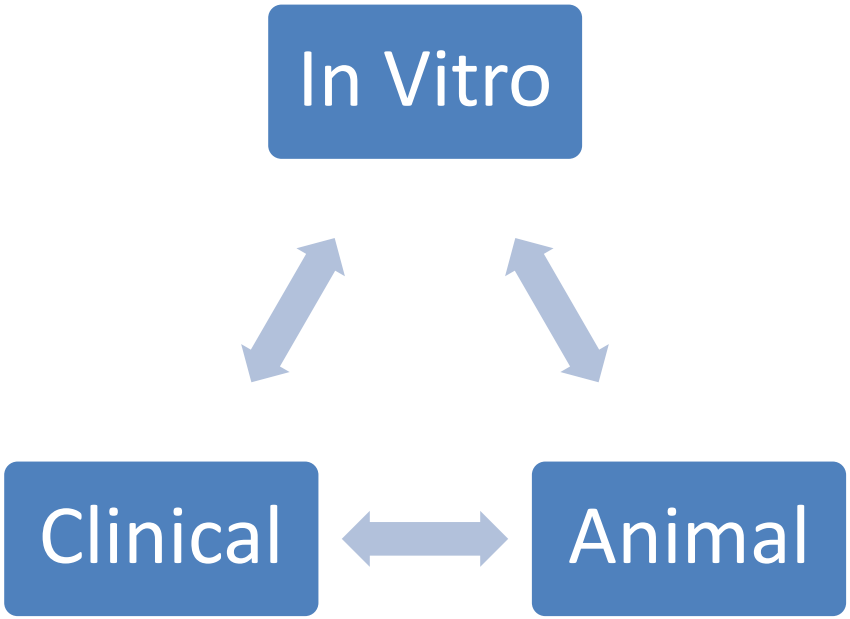
A great melody, but….

More Tools, Harmonized



Has greater impact with the whole band …

Translational Systems Biology



- Connect non-clinical studies with clinical investigations
  - Mechanism
  - Biomarkers
- Improve safety assessment tools
- Broaden the utility of *in vitro* screens
- An ongoing effort

The Band

- Li Pang
- Varsha Desai
- Tao Han
- Jim Fuscoe
- Matthew White
- Xi Yang
- Li-Rong Yu
- Rick Beger
- Laura Schnackenberg

Toxicity Assessment – 399 B.C.