Evaluating Cardiotoxicity Potential: Translational Approaches and Models

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www.fda.gov
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• 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 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

Cobalt Cardiomyopathy
A Critical Reappraisal in Light of a Recent Resurgence

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


Cardiotoxicity - Manifestations

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

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

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

O. Sirenko et al. / Toxicology and Applied Pharmacology 322 (2017) 60–74
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?

“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.”

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

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<th>7 day</th>
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<td>1.046766</td>
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Better specificity at 24h, better sensitivity at 7d

Ca\(^{2+}\) transient assays

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<th>24 h</th>
<th>7 day</th>
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<tbody>
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<td>False_P</td>
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<tr>
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*K. 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”

Patient-specific iPSC-CMs

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

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

Impedance-based beating profiles

Cell Index

Normalized cell index

sunitinib

vandetanib

gefitinib

Impedance-based beating profiles
Impact of Assay Conditions

Normal vs. Serum-Free Medium

### Vandetanib

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<thead>
<tr>
<th>Total Drug Conc. (Prepared)</th>
<th>Bound (%)</th>
<th>Free Drug Conc. (Measured)</th>
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<tbody>
<tr>
<td>1000 nM in NM</td>
<td>72.5 ± 0.7 %</td>
<td>309.0 ± 14.6 nM</td>
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<tr>
<td>1000 nM in SFM</td>
<td>60.6 ± 7.0 %</td>
<td>272.5 ± 23.1 nM</td>
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### Astemizole

<table>
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<th>Total Drug Conc. (Prepared)</th>
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<tr>
<td>30 nM in NM</td>
<td>95.1 ± 1.0 %</td>
<td>1.47 ± 0.12 nM</td>
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<tr>
<td>30 nM in SFM</td>
<td>79.2 ± 1.8 %</td>
<td>0.81 ± 0.02 nM***</td>
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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

Blood troponins (Myocardial injury)

miR-34a and miR-150 early biomarkers

DOX cumulative dose (mg/kg)

6 9 12 18 24

↑ miR-34a

Apoptosis

↓ miR-150

↓ miR-221, 208b

↓ miR-21 (fibrosis)

↓ miR-19b, 199a/b

↓ miR-149

↓ miR-24-2, 23b, 27b (fibrosis)

Cardiac hypertrophy

Cardiac lesion
## Circulating Protein Markers of Doxorubicin Cardiotoxicity

<table>
<thead>
<tr>
<th>SOMA ID</th>
<th>Target Full Name</th>
<th>UniProt</th>
<th>Fold ratio (Dox/Sal)</th>
<th>Drug exposure in weeks (cumulative dose in mg/kg)</th>
<th>Doxorubicin Effect</th>
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<td>SL005703</td>
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<td>SL000017</td>
<td>von Willebrand factor</td>
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<tr>
<td>SL016563</td>
<td>Mitochondrial glutamate carrier 2</td>
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<td>SL004652</td>
<td>Wnt inhibitory factor 1</td>
<td>Q9Y5W5</td>
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<td>SL008909</td>
<td>Legumain</td>
<td>Q99538</td>
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<td>SL011049</td>
<td>Mannan-binding lectin serine protease 1</td>
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### Early Injury Markers of Toxicity

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<th>SOMA ID</th>
<th>Target Full Name</th>
<th>UniProt</th>
<th>Fold ratio (Dox/Sal)</th>
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<td>Troponin I, cardiac muscle</td>
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<td>SL005233</td>
<td>Tumor necrosis factor receptor superfamily member 27</td>
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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 B6C3F₁ 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

\[ \text{Mean} \pm \text{SEM} \]

\(* p<0.05 \text{ Dox vs Sal} \)

\# 71 mg/m² HED

\sim 14\% - 20\% \text{ decline in FS at 4 -24 wk after the end of Dox treatment}
Clinical Cardiotoxicity

100 breast cancer patients receiving doxorubicin

60 mg/m² DOX + 600 mg/m² cyclophosphamide

2-3 weeks after 4th dose of DOX.

This study was approved by RIHSC

Measure LVEF
Multiple Gated Acquisition (MUGA) scan
Before 1st blood withdraw and 2-3 weeks after 4th dose of DOX.
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

Satin Doll

Duke Ellington

A great melody, but....
More Tools, Harmonized

Satin Doll
for Brass Quintet

Billy Strayhorn, Duke Ellington & Johnny Mercer

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.