Use of hiPSC-derived Cardiomyocytes for Cardiac Safety Evaluation

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Disclaimer

- The speaker is an employee of Leidos Biomedical Research, Inc. (LBRI), a government contractor to operate Frederick National Laboratory for Cancer Research (FNLCR) in support of NIH/NIC initiatives
- No conflicts of interest to disclose
Outlines

- Definition of cardiotoxicity: functional vs. structural

- Use of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs):
  - In CiPA initiative as a translational model
  - As high/moderate-throughput assays to screen for both types of cardiotoxicity
    - Cellular impedance (+ biochemical/high content analysis)
    - Ca\(^{2+}\) transit (+ biochemical/high content analysis)
  - In mechanistic studies to investigate on-target cardiotoxicity of trastuzumab (Herceptin®)
    - Model potentiation of ErbB2 inhibition on anthracycline-cardiotoxicity

- Take-home messages
## Pathophysiological classification of cardiotoxicity

- **Cardiotoxicity**: “toxicity that affects the heart”

<table>
<thead>
<tr>
<th></th>
<th>Functional</th>
<th>Structural</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pathology</strong></td>
<td>(-)</td>
<td>(+)</td>
<td>(+)</td>
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<tr>
<td>(myocardial destruction)</td>
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<tr>
<td><strong>Physiology</strong></td>
<td>(+) primary</td>
<td>(+) secondary</td>
<td>(+) primary &amp; secondary</td>
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<td>(arrhythmia and/or contractility↓)</td>
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<tr>
<td><strong>Onset/progression rate</strong></td>
<td>fast, instantaneous/acute (minutes to hours)</td>
<td>delayed onset, slow, subacute/chronic (days to years)</td>
<td>both</td>
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<tr>
<td><strong>Reversibility</strong></td>
<td>yes</td>
<td>no</td>
<td>yes/no</td>
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<tr>
<td><strong>Mechanism(s)</strong></td>
<td>ion channels and/or receptors</td>
<td>key components in cell death/survival pathways</td>
<td>both</td>
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<td><strong>Therapeutic area(s)</strong></td>
<td>dofetilide, sotalol, terfenadine, nilotinib</td>
<td>anthracycline, trastuzumab</td>
<td>sunitinib, dasatinib</td>
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</table>

*National Cancer Institute NIH. NCI Dictionary of Cancer Terms <www.cancer.gov/dictionary>
hiPSC-CMs as a translational model

Cell-based in vitro models for screening and mechanistic study in drug development:

- **Pros:**
  - Human origin → eliminate concerns on species discrepancy
  - Functional with spontaneous beating → enable comprehensive test
  - Easy for long-term (> 3 months) culture → enable test on chronic effects
  - Easy to create CMs with specific genotype → model diseases

- **Cons:**
  - Fetal-like phenotype → impact on drug responses!!!
  - Mixture of nodal, atrial and ventricular cells → difficult to identify target cell type

“Fit-for-purpose” validation of endpoints is critical for translatability!
Endpoint selection for quantitative functional and structural testing in hiPSC-CMs

Functional
- Electrophysiology:
  - Action potential (AP)
  - Field potential (FP)
- Ca\(^{2+}\) cycling:
  - \([\text{Ca}^{2+}]_i\)
- Force generation
  - Transducer
  - Cell length
  - Movement

Structural
- Cellular morphology
- Membrane permeabilization
  - LDH, cTnI release
  - Nuclear stain
- Apoptosis and cell loss
  - Caspase 3/7, ATP
  - Nucleus count
- Mitochondrial damage
  - Mito membrane potential (JC-10 stain)

Excitation-Contraction Coupling

[Diagram showing the processes of excitation and contraction with various calcium and ion interactions, including action potential (AP), field potential (FP), Ca\(^{2+}\) cycling, force generation, and structural changes such as cellular morphology, membrane permeabilization, apoptosis, and mitochondrial damage.]
Update on hiPSC-CMs as a key component in CiPA initiative

- Comprehensive in Vitro Proarrhythmia Assay (CiPA)
  - ICH S7B and E14 guidelines: hERG inhibition and QT prolongation as a surrogate
  - CiPA goal: focus on “proarrhythmic” propensity with arrhythmia-like events as a predictor

- Four components:

- Work on myocytes:
  - 34 sites participated
  - 28 compounds tested
  - 2 platforms (MEA/VSD) evaluated
  - 2 manuscripts submitted

“These results demonstrate the utility of hiPSC-CMs to detect drug-induced proarrhythmic effects…”

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Cellular impedance platform as a screening tool

**Workflow**

Cardiac myocytes → 96-well E-plate Cardio → xCELLigence RTCA Cardio

- Real-time
- Non-invasive

**Technology**

- Single Well (side view)
- culture medium
- electron flow
- negative terminal
- well bottom (glass or PET)
- addition of cells
- positive terminal
- impeded electron flow
- Medium only
  - Cell Index=0
- Attached cell No. A
  - Cell Index A
- Attached cell No. B
  - Cell Index b1
  - Contraction
- Attached cell No. B
  - Cell Index b2
  - Relaxation

**Readout**

**Electrical impedance**
- Viability (CI)
- Contraction (ΔCI), Rhythm

**Biochemical measurement**
- Caspase 3/7, ATP, LDH, cTnI

**High-content analysis (optical plates)**
- Total/dead cell count
- MMP (JC-10 stain)
Validation of Cellular Impedance as a functional endpoint

- Impedance vs. Field Potentials:
  - Signals match with each other

- Impedance, not Field Potential, suppressed by a myosin ATPase inhibitor Blebbistatin

  Impedance waveforms represent contraction of cardiomyocytes!

Guo et al, 2015 CPiCB
Validation of impedance to predict functional and structural cardiotoxicity

E-4031 (functional)

Doxorubicin (structural)

Sunitinib (mixed)

Parameters:

Irregular Beat (IB) ratio = # irregular/total beats to predict proarrhythmic risk

Beat Rate (BR) reduction = Δ% in beat rate corrected by the time-matched vehicle control to predict QT prolongation liability
Validation of model performance to predict proarrhythmic liability

- Receiver operating characteristic (ROC) on ~120 compounds

Parameters to predict

1. Arrhythmia:
   - \( \text{IB}_{20} \): threshold conc. to induce \( \geq 20\% \) arrhythmic beats
   - \( \text{PPS-IB}_{20} \): \( \text{IB}_{20}/\text{Cmax} \)

2. QT prolongation:
   - \( \text{BR}_{20} \): threshold conc. to induce reduction in beat rate by \( \geq 20\% \)
   - \( \text{PPS-BR}_{20} \): \( \text{BR}_{20}/\text{Cmax} \)

Guo et al. 2013, Tox Sci.
Impedance and ATP predict structural cardiotoxicity

Validation with 40 compounds:

Parameter: Ratio of IC_{50}/C_{max}

Sequential measurement of Impedance & ATP at 72 hrs drug exposure

Impedance model is the first-line assay to test for cardiac liability
Ca^{2+} transit platform as a screening tool

<table>
<thead>
<tr>
<th>Cells plated</th>
<th>Replated</th>
<th>Functional Assays for Ca^{2+} transit</th>
<th>Structural DNA stain, ATP, Caspase 3/7, JC10, etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>1 week</td>
<td>30 min</td>
<td>72 hrs</td>
</tr>
</tbody>
</table>

- Readout with Tecan plate reader
- Imaging with IN-Cell Analyzer (HCA)
- Structural analysis
- Functional testing
- DNA dye JC-10 live-stain
- Caspase 3/7 ATP
- Temperature & CO2 control

Equipment:
- 6-well plate
- 384-well optic plate
- Nikon Eclipse Ti Fluorescence Microscope
- Filter plate reader

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Imaging \( \text{Ca}^{2+} \) transits

**1x objective lens**

**20x objective lens**

A \( \text{Ca}^{2+} \) transient trace taken from one well at 1x:

![Image of green cells with graph]  

Sampling at 51 fps

hiPSC-CMs from Stanford Cardiovascular Institute (SCVI) Biobank (Dr. Joseph Wu)
Multi-parameter analysis with CYBERnano i-Cardio software:

Ca^{2+} transit duration (CTD) as a surrogate of action potentiation duration (APD)

Beat rate: number of Ca^{2+} transit peaks/minute
[Ca^{2+}] baseline: Cal 520 intensity prior to a transit
[Ca^{2+}] amplitude: Cal 520 intensity between baseline and peak
Peak-Peak Interval (PPI): time between two transit peaks
[Ca^{2+}] rising rate (RR): the rate of Cal 520 intensity rising from 10 to 70% of peak
[Ca^{2+}] falling rate (FR): the rate of Cal 520 intensity falling from 70 to 10% of peak

Inter-Peak Interval (IPI): time between two transit peaks
Ca^{2+} transit duration 30 (CTD30): duration at 30% level from peak
Ca^{2+} transit duration 90 (CTD90): duration at 10% level from peak
Corrected CTD30: corrected by beat rate = CTD30(n)/IPI(n-1)
Corrected CTD90: corrected by beat rate = CTD90(n)/IPI(n-1)
Triangulation index: = CTD90c/CTD30c or Beat-to-beat variability
**Ca^{2+}** transit predicts proarrhythmic liability

Representative responses to hERG inhibition at 30 min post-dose

- **Pre-dose**
- **CTD* prolongation + ↓ beat rate**
- **CTD prolongation + EADs**
- **EAD**
- **Tachycardia**
- **Fibrillation**
- **Beat arrest**

* CTD, Ca^{2+} transit duration; **EADs, early-afterdepolarization-like events

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Nuclear stain detects cell death (membrane permeabilization)

Representative responses to structural cardiotoxicant at 72 hours post-dose

**A** 0.1% DMSO  
**B** Doxorubicin 3μM

![Images](A.png B.png)

**C**

![Graph](C.png)

**DAPI**: permeable; **DRAQ7**: impermeable

**Ca**²⁺ transit model was used to determine MOA and therapeutic index of compounds with anticancer activity but interruption of Ca²⁺ cycling

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The double-edged sword of ErbB2 inhibitor Trastuzumab (Herceptin®):

**The Good (↑ Survival rate)**

**The Bad (↑ Heart failure cardiomyopathy)**

Pre-clinical and clinical safety evaluation failed to predict cardiotoxicity at this level of severity!

How can we improve strategy to prevent the next “trastuzumab”?
Mechanism of trastuzumab liability: on-target toxicity

- HER2/ErbB2 expressed in Tumor Cell & Cardiomyocytes:

  Trastuzumab

  HER2/ErbB2(+) Tumor

  HER2/ErbB2 SIGNALING: NO LIGAND


  ErbB2 agonist: Neuregulin-1β (NRG)

  CARDIAC ErbB SIGNALING: LIGAND-INDUCED

Are hiPSC-CMs capable of modeling cardiotoxicity observed in the clinical?
ErbB2 is present and functional in hiPSC-CMs

**Expression**

- **A**
  - WES electropherogram showing ErbB2 and GAPDH bands for cardiomyocytes and H441 cells.

- **B**
  - WES electropherogram showing ErbB2 and GAPDH bands for cardiomyocytes and H441 cells.

- **C**
  - Area normalized to GAPDH for ERGFR, ErbB2, ErbB4, ErbB3, and ErbB3.

**Knockdown**

- **A**
  - Control siRNA and ErbB2 siRNA electropherograms for cardiomyocytes and H441 cells.

- **B**
  - Area normalized to GAPDH for Control siRNA and ErbB2 siRNA.

**Functional**

- **C**
  - Electropherogram showing pAKT levels with 0.01% DMSO, 20 ng/mL NRG, Lapatinib + NRG, and Trastuzumab + NRG.
Model modulation of Doxorubicin (Dox)-toxicity by ErbB2 signaling

Mean ± SE (n=3-6 wells); at 1μM (Dox, Trastuzumab) or at 100 ng/mL (NRG); *, # p < 0.05 compared to vehicle or Dox

Eldridge et al., Tox Sci 2014; Guo et al. CPiCB 2015
Model modulation of Doxorubicin (Dox)-toxicity by ErbB2 signaling

**Nucleus morphology**

Guo et al. CPiCB 2015

**Apoptosis**

Mean ± SE (n=3-6 wells); at 1μM (Dox, Trastuzumab) or at 100 ng/mL (NRG); *, # p < 0.05 compared to vehicle or Dox
Take-home message:

- hiPSC-CMs are a useful translational in vitro model system for cardiac safety evaluation
- Multiple platforms are available for assessing both functional and structural cardiotoxicity using screening and mechanistic approaches
- “Fit-for-purpose” qualification of model system is critical for translatability
- As demonstrated by our work with ErbB2 inhibitor trastuzumab, hiPSC-CMs can be a valuable model in chemotherapeutic development to assess on-target cardiac liability, since many of new anticancer targets play an important role in maintaining myocardial survival and functional integrity
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Thank you!