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Assessing functional and structural cardiotoxicity in cultured human iPSC-cardiomyocytes in a single plate format

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Abstract
A comprehensive profiling of cardiotoxicity early in drug discovery and development can aid in reducing late-stage attrition and establishing risk mitigation strategies during clinical development. In most cases, multiple assay platforms and instrument-specific plate formats are required for this type of approach. In this study, we evaluated both functional and structural endpoints associated with cardiotoxicity in human induced pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) cultured in a single 384-well plate. We measured intracellular Ca²⁺ transits, caspase 3/7 activity, permeabilization sequentially in the same plate via a series of assay readouts. A set of cardiac ion channel modulators (diastolic, systolic, nifedipine and mexiletine) and chemotherapeutics (tamoxifen, nitrofurantoin, and tetracycline) was tested at clinically relevant concentrations for effects on intracellular Ca²⁺ transits after a short-term (30 minutes) exposure, and plasma membrane permeabilization and caspase 3/7 activation after a long-term (72 hours) exposure. Intracellular Ca²⁺ transits were monitored by fluorescent images taken with a high-speed camera in beating cardiomyocytes loaded with CellRhoCa²⁺ dye, permeabilized plasma membrane (for dead-cell detection) was identified by live/necro staining and activation of caspase 3/7 was determined biochemically with the Caspase-Glo® assay kit. Multiple endpoints derived from Ca²⁺ transits, including beat rate, calcium transduction duration (CTD) measured at 30% or 90% from peak and corrected by inter-peak interval (IP), along with CTD triangulation, beat rhythm, short- or long-term variability of CTD90 and CTD30 Poincaré plots, were used to assess drug effects on intracellular Ca²⁺ cycling and arrhythmogenicity. Increases in positive and ryanodine receptor (RyR) calcium transduction levels (RR), falling rate (FR), transit duration 30 or 90 (CTD30 or CTD90), corrected CTD30 or CTD90 by RR (RR = CTD - (CTD × FR)/2), and CTD90/CTD30 ratio were robust indicators of functional effects. Positive nAChR staining for DRAG9™ was a robust indicator of structural effects. Accordingly, diastolic and systolic duration were identified as primary indicators of functional toxicity. Diastolic and systolic were both arrhythmogenic and structurally toxic. The use of these endpoints in a single plate format simplifies the cardiotoxicity assessment and does not require multiple cell plates for measurements.

Introduction
Cardiotoxicity is frequently a dose-limiting toxicity associated with many highly efficacious chemotherapeutics that include both classic cytotoxic or cytostatic agents, such as doxorubicin or other anti-mitotic analogs, and newly developed targeted anti-cancer molecules such as protein kinase inhibitors (i.e., sunitinib, dasatinib and nilotinib). As this adverse effect can be manifested by either structural damage (i.e., cardiomyopathy and heart failure) or functional alteration (i.e. arrhythmia and sudden cardiac death), evaluation on risk factors to induce both structural and functional cardiotoxicity should be included in preclinical safety profiling of new anti-cancer drug prior to the first dose in human.

Human induced pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) represent a novel cellular model system to test for cardiotoxicity and are being used increasingly with a wide variety of analytic platforms in study of cardiac biology and drug safety testing. In this study, we developed an image-based, multiplexed assay that enables interrogation of both functional and structural toxicity endpoints in a single plate format.

Methods & Materials

Cells:
Cryopreserved iPSC-cardiomyocytes were provided by Dr. Joseph C. Wu and Stanford Cardiovascular Institute (SVI) Biobank.

Reagents:
RPMI 1640, 20% Matrigel (Fisher/Corning), B27-insulin, DMEM/F12 (Gibco/Life Science), Accutase (Sigma), Cali-520™/Ca²⁺ dye (AAT Bioquest), DRAQ5™ DNA dye (abcam), Caspase-Glo® 3/assay kit (Promega), Doxil®, etoposide, nifedipine, mexiletine, tamoxifen, nitrofurantoin, and Tetracycline (NOCI Compound Repository).

Biomarkers:
Ca²⁺ transits: contractility measurement, depolarization-delay, arrhythmia
DNA stain: permeabilization of plasma membrane (cell death)
Caspase 3/7 activity: apoptosis activation

Table 1. Summary of effects on Ca²⁺ transit parameters

<table>
<thead>
<tr>
<th>Drug</th>
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Discussion & Conclusion

- Ca²⁺ transits were sensitive to ion channel modulators, with changes in beat rate and Ca²⁺ transit amplitude; EADs, beat-to-beat variability and triangulation of CTD were more specific than CTD lengthening to predict arrhythmogenicity
- Caspase 3/7 activity was a sensitive indicator of insult to hPSC-CMs but increased nuclear stains of impermeable DNA dye was more robust to label structural cardiotoxicity

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