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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⁺ transients, caspase 3/7 activity, and plasma membrane permeabilization sequentially in the same plate via a series of assay readouts. A comprehensive profiling of cardiotoxicity early in drug discovery and development should be included in preclinical safety assessment.

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 anthracycline 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 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, multiplex 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 the Stanford Cardiovascular Institute (SCVI) Biobank.

Reagents:
RPMI 1640, BD Matrigel (Fisher/Corning); B27-insulin, DMEM/F12 (Gibco/Life Sciences); Accutase (Sigma); Cal-520® Ca²⁺ dye (AAT Bioquest), DRAQ7® DNA dye (abcam), Caspase-Glo 3/Assay kit (Promega); Doxil®, motexafin, n福利ee, methylene blue, ethidium bromide (NEC Compound Repository)

Biomarkers:
Ca²⁺ transients: contractile function, repolarization-delay, arrhythmia DNA stain: permeabilization of plasma membrane (cell death) Caspase 3/7 activity: apoptosis activation

Workflow:
Cells plated
1 week
6-well plate
384-well optiplate
Assays for Ca²⁺ transients
DNA stain + Caspase 3/7

1 week
10 min and 72 hrs

Data analysis:
Measurement of beat-to-beat Ca²⁺ transients was performed by CYBERnano i-Cardo platform; treatment-related changes in each endpoint were shown as % of the baseline value. Cells stained positive with DRAQ7 were shown as % of total nuclear (DAP) counts and Caspase 3/7 activity was quantified as the luminescence intensity in each well. Statistical analysis was conducted with Student’s t-test.

Results

Ca²⁺ transients of beating cardiomyocytes:

- Figure 1: Ca²⁺ cycling imaged at 51 fps from a single view-field
- Figure 2: Ca²⁺ transients analyzed by CYBERnano i-Cardo

Caspase 3/7 activity:

- Figure 3: Representative traces of typical effects on Ca²⁺ transients

Discussion & Conclusion
- Ca²⁺ transients were sensitive to ion channel modifiers, with changes in beat rate and Ca²⁺ transients amplitude
- EADs, beat-to-beat variability and triangulation of CTD were more specific than CTD lengthening to predict arrhythmogenesis
- Caspase 3/7 activity was a sensitive indicator of insults to hPSC-CMs but increased nuclear stains of impermeable DNA dyes was more robust to label structural cardiotoxicity

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Figure 1. Ca²⁺ cycling imaged at 51 fps from a single view-field

Figure 2. Ca²⁺ transients analyzed by CYBERnano i-Cardo

Figure 3. Representative traces of typical effects on Ca²⁺ transients

Figure 4. Beat variability analysis on Poincaré plots of CTD90

Figure 5. Representative images of nuclear stains

Figure 6. Caspase 3/7 activity

Table 1. Summary of effects on Ca²⁺ transients parameters

<table>
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<th>Effect</th>
<th>Assay</th>
<th>CTD90</th>
<th>CTD60</th>
<th>CTD50</th>
<th>CTD30</th>
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<td>Pre-dose</td>
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In each well, the mean effect was calculated with the mean effect of the baseline (±SEM) and compared with vehicle (t-test; ND, not determined).