Validation of an impedance-based phenotypic screening assay able to detect multiple mechanisms of chronic cardiotoxicity in human stem cell-derived cardiomyocytes

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Drug-induced cardiomyocyte toxicity

- **CiPA** is designed to address drug-induced cardiac arrhythmia (TdP)
  - Acute effects of drug discovery compounds (minutes – hours)
  - Focus on plasmalemmal ion channels that underlie the cardiac action potential

- **However, new and existing drugs can also cause structural cardiotoxicity**
  - Produced by a diverse set of chemical compounds and primary target mechanisms
  - Chronic effects can appear after days, weeks or months; not all are reversible
  - Classic examples include chemotherapy agents used for clinical oncology
    - Anthracyclines such as Doxorubicin (breast cancer)
    - Tyrosine kinase inhibitors (TKI) such as the ‘nibs
    - Proteosome inhibitors such as Bortezomib
    - HDAC inhibitors such as the ‘stats (some of which may also cause QTc prolongation)

- **Safety pharmacology testing needs a reliable & predictive assay for chronic cardiotox**
A wide and growing list of chemotherapy agents produce a variety of serious cardiac side-effects in cancer patients, only some of which reverse after drug washout.

- Non-traditional, non-CiPA related cardiac liabilities are a challenge for current in vitro assays.
- As well as safety, iPSC cardiomyocyte assays could enable cardiac disease modelling.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Indication</th>
<th>Possible cardiovascular damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>SM</td>
<td>Anthracycline - solid tumor (breast cancer)</td>
<td>Congestive heart failure, Acute myocarditis, left ventricular dysfunction</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>SM</td>
<td>Multi-targeted TKI (kidney cancer)</td>
<td>Arrhythmias</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>SM</td>
<td>Multi-targeted TKI</td>
<td>QT prolongation</td>
</tr>
<tr>
<td>Ponatinib</td>
<td>SM</td>
<td>TKI inhibitor</td>
<td>Myocardial infarction, congestive heart failure (HF), cardiac arrhythmias</td>
</tr>
<tr>
<td>Ibrutinib</td>
<td>SM</td>
<td>Bruton’s tyrosine kinase</td>
<td>AF + other arrhythmias</td>
</tr>
<tr>
<td>Trametinib</td>
<td>SM</td>
<td>MEK inhibitor (MEK1, MEK2)</td>
<td>Cardiomyopathy</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>SM</td>
<td>Proteasome inhibitor</td>
<td>Left ventricular dysfunction and atrioventricular block</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>Ab</td>
<td>Antineoplastic ErbB2-targeted therapies</td>
<td>Decline left ventricular ejection fraction</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>Ab</td>
<td>VEGFA monoclonal antibody</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Ab</td>
<td>Non-Hodgkin’s lymphoma</td>
<td>Arrhythmias (link to cytokine release by lymphocyte B?)</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>SM</td>
<td>HDAC inhibitor</td>
<td>QT prolongation, thromboembolism</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>SM</td>
<td>AF Class III antiarrhythmic</td>
<td>TdP, heart block, sinus bradycardia, CHF, VF</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>SM</td>
<td>Anti-microtubule agent</td>
<td>Arrhythmias (sinus bradycardia, ventricular tachycardia)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>SM</td>
<td>Antimetabolite</td>
<td>Myocardial ischemia + arrhythmias</td>
</tr>
</tbody>
</table>
Various cell-based screening assays and platforms can be used to monitor cardiomyocyte health and function as part of safety pharmacology testing
- High content image analysis (sarcomeres, nucleus, cell morphology)
- Functional imaging (Mitochondrial dyes, LDH, ATP, MTT, DNA damage)
- Gene expression profiling
- Secreted or expressed biomarkers are also gaining traction (translation to clinic)

However, many of these assays require fixation of cells (end-stage assays)
- Other assays require labelling of cells, which adds expense and potential artefacts
- Impedance is useful as it is cheap, label-free, and physiologic over long time-course
Nanion CardioExcyte 96: Chronic Impedance assay

**CiPA screening:**
- Dual mode platform: impedance & microelectrode array (MEA)

**Chronic cardiotox:**
- Long-term monitoring of beat rate (arrhythmia) and base impedance (growth and formation of a stable syncytium, cell viability)
- Beat rate and impedance stabilise after 1-5 days
- Chronic effects assayed over hours-days
Cardiotoxicity assay: Sunitinib

- Broad spectrum tyrosine kinase inhibitor (TKI) used to treat kidney cancer
- Known cardiac side-effect is mostly ventricular arrhythmia
  - Other TKIs also produce congestive heart failure, hypertension, and myocardial ischemia

Results:
- Complex dose-dependent increase (cell hypertrophy?) followed by profound decrease in base impedance (cardiotoxicity) over 24 hours
- Effects on beat rate occurred quickly and at lower concentrations than impedance (QT)
Cardiotoxicity assay: Trastuzumab (Herceptin)

- Monoclonal antibody against Her2/ErbB2 receptor, used to treat breast cancer
- Known cardiac side-effects are CHF and changes in left ventricular ejection fraction

Results:
- Very slow, dose-independent decrease in base impedance (cardiotoxicity) > 72 hours
- No effects on beat rate suggest little occurrence of arrhythmias or QT changes
Cardiotoxicity assay: Ion channel modulators

A. Lidocaine (Na\textsubscript{v}1.5)

- Compound effects on ion channels are usually only studied over short periods for acute toxicity
- We decided to see if CiPA drugs could also produce long-term effects in a chronic toxicity assay

B. Nifedipine (Ca\textsubscript{v}1.2)

- Along with beat rate and contraction amplitude, we could monitor base impedance over several days on the CE96 platform to assess effects of ion channel blockers on cell viability and sustained cardiotoxicity
  - Lidocaine (Na\textsubscript{v}1.5) had no effect on cell viability
  - Nifedipine (Ca\textsubscript{v}1.2) produced time- and dose-dependent decreases in iPSC-CM viability
  - High concentrations of Dofetilide (hERG) also decreased iPSC-CM base impedance
Cardiotoxicity assay: Doxorubicin

• Broad spectrum anti-cancer medication belonging to Anthracycline family

• DNA intercalating agent

• Known cardiac side effects:
  • Acute atrial and ventricular arrhythmia
  • Chronic cardiomyopathy
  • Congestive heart failure

• Concentration-dependent decrease of base impedance after 24 hours
Cardiotoxicity disease modelling: Doxorubicin

- Not all breast cancer patients show chronic cardiotoxicity in response to Doxorubicin.
- Stanford group explored this phenomena using patient-derived iPSC cardiomyocytes.
- Clear differences in cardiotoxicity measures in sensitive vs insensitive patients.
  - Cell damage markers, functional contractility and arrhythmia, gene expression profiles, etc
- Impedance-based cardiotoxicity personalised medicine assays could be used to:
  - Model other types of patient-specific drug cardiotoxicity.
  - Select patients for safest type of chemotherapy and other drug treatments.
• HL-1 cells are an immortalised mouse ‘atrial’ CM cell line
  • Exhibit immature physiology and de-differentiated morphology
  • A subset of cells demonstrate spontaneous contractility for a few passages
  • Express atrial-like ion channel expression profile (incl I_{K_{ACh}})

• We wanted to test them as a model for chronic atrial cardiotoxicity
  • Assess acute and long-term changes in cell impedance as a measure of cell toxicity
  • Assess any changes in spontaneous contractility (beat rate)

Results:
• HL-1 cells have similar sensitivity to a wide range of chronic cardiotoxicity agents as human ventricular iPS cardiomyocytes
  • Ion channel antagonists (esp mixed blockers like Amiodarone)
  • Doxorubicin
  • ‘nib kinase inhibitors
• HL-1 cells have greater sensitivity to drugs causing atrial-specific damage
  • Bortezomib
Bortezomib: Oncology agent with atrial cardiotoxicity

- Bortezomib is a proteasome inhibitor with known cardiotoxicity (incl AV block)
- Large time-dependent decrease in impedance, indicating significant cardiotoxicity
- We saw slower effects with lower sensitivity in ventricular human iPSC-CMs, suggesting that HL-1 atrial cells may be a useful model for chronic toxicity studies of agents such as Bortezomib with atrial-specific side-effects
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Cardiotoxicity assay: Amiodarone

- Class III antiarrhythmic widely used for treatment of atrial fibrillation
- Known cardiac side effects:
  - Arrhythmia: Torsade de Pointe, VF and sinus bradycardia
  - Congestive Heart Failure
- Base impedance decreased only after five hours drug exposure

A. 1 µM Amiodarone

B. Base Impedance (normalised)

C. Percent of control (%) vs Log [Amiodarone] (M)
Cardiotoxicity assay: Thapsigargin (Ca^{2+} pump)

- Tendency of increased basal impedance, suggestive of hypertrophy effect?
  - We have seen other compounds also produce chronic increases in impedance
- Decreased beat rate as expected with SR Ca^{2+} store depletion after SERCA block
- These data may support the contention that spontaneous beating in iPSC-CMs is due to Ca^{2+} leak from SR, and is a sign of Ca^{2+} signalling immaturity
Aspirin

Base Impedance

Beat Rate

Amplitude

0.1% DMSO
1 μM Aspirin
10 μM Aspirin
100 μM Aspirin
Doxorubicin: Breast cancer agent with cardiotoxicity

- Doxorubicin is a DNA alkylating agent used to treat breast cancer
- Large time- and dose-dependent decrease in human iPSC cardiomyocyte impedance, suggesting significant cardiotoxicity
- We saw similar effects and dose-dependency in ventricular human iPSC-CMs, suggesting that HL-1 atrial cells may be a useful model for chronic toxicity studies
Sunitinib: Kidney cancer agent with cardiotoxicity

- Sunitinib is a tyrosine kinase inhibitor used to treat kidney cancer
- Large time- and dose-dependent decrease in iPSC-CM impedance, indicating significant toxicity at high doses (steep dose-dependence)
- We saw similar time-course and dose-dependency in ventricular human iPSC-CMs, suggesting that HL-1 atrial cells may be a useful model for TKI toxicity studies
Amiodarone: Mixed $\text{Na}_V$, $\text{Ca}_V$ and $\text{K}_V$ inhibitor

- Amiodarone is a non-selective ion channel antagonist, class III anti-arrythmic
- Small but non-significant reduction in impedance, suggesting some toxicity at higher concentrations
- We saw larger effects in ventricular human iPSC-CMs, suggesting that either the expression levels or mixture of ion channels in HL-1 atrial cells is different