Calcium Transient Assay for Cardiac Arrhythmic Potential Evaluation: using human iPS-Derived Cardio Myocytes

Commonly used acronym: CTCM on hiPS-CMs


Contact person

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Organisation

Name of the organisation Janssen Pharma of JNJ
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SCOPE OF THE METHOD

<table>
<thead>
<tr>
<th>The Method relates to</th>
<th>Human health, Other</th>
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<tbody>
<tr>
<td>The Method is situated in</td>
<td>Regulatory use - Routine production, Translational - Applied Research, Other: Safety Pharmacology</td>
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<tr>
<td>Type of method</td>
<td>In vitro - Ex vivo</td>
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<tr>
<td>Specify the type of cells/tissues/organs</td>
<td>hiPSC-derived cardiomyocytes</td>
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</table>

DESCRIPTION

Method keywords

Calcium transient
Proarrhythmia
Stem cell
Fluorescent Dye Calcium-sensitive Imaging
Cardiomyocyte
hiPS-CMs
Human induced Pluripotent Stem Cell

Scientific area keywords
Safety Pharmacology
Side effects
medium-high throughput assay
cardiovascular derisking
regulatory

Method description
Cell Culture and Reagents hiPSC-CMs can be obtained commercially either as living pre-plated cells seeded onto fibronectin-coated 96-well mClear plates (Greiner Bio-One, No. 655090) or can be plated in house at a density (~25,000 cells/well) suited to forming a confluent synchronously beating mono-layer. Most commercially offered cardiomyocyte lines represent a mix of ventricular, atrial and nodal cells and derived from one human donor. Cells were cultured with commercial culture medium (optimized for the specific cell line) in a humidified incubator at 37°C and 5% CO2, with medium being changed once a day. On the day of the experiment, the culture medium was replaced with Tyrode’s solution (Sigma, No. T2397) supplemented with 10 mM HEPES together with KCl to represent isokalemic (4.2 mM K+) conditions. As a calcium-sensitive fluorescence dye Cal-520 AM (Cat. No. 36,338; AAT Bioquest) was used to capture the intracellular calcium transients. Accordingly, Cal-520 was incubated for 45 min followed by a washout and a 30-min recovery before starting the experiments. Calcium Transient Measurements Spontaneous beating activity of hiPSC-CMs was assessed through measurement of the Ca2+ fluorescence signal integrated over the whole well. Fluorescence signals were measured using the Hamamatsu FDSS/mCell platform and the records subsequently analyzed offline using NOTOCORD-hem software (version 4.3), containing EXT modules and an algorithm developed by XiTechniX to detect beat-by-beat Amp, BR, and CTD90 parameters. All wells within a plate were measured simultaneously using the
following FDSS/mCell settings: sampling frequency 66.7 Hz, exposure time 14.6 ms, excitation wavelength 480 nm, emission wavelength 540 nm, temperature controlled at 37°C. First, the experimental plates were put into the FDSS/mCell to stabilize for 10 min. Next, a baseline recording was run for 3 min followed by compound addition. The effect of a compound was recorded (5-min recording time) around 15 and 30 min after compound addition. CTD90, BR, and Amp were quantified for baseline and 30-min compound effects as the median value of all beats (calcium transients) measured within a 1-min interval of the recording. The recording around 15 min was used only for observation of Early-After-Depolarisation (EADs) or fibrillation-like events. EADs were manually monitored and evaluated. Cessation of beating was defined after 30 min in case BR was <5 beats/min. Wells that temporarily stopped beating during compound addition but recovered at the 30-min time point were not defined as beat stop.

**Lab equipment**

- Laminar flow hood;
- Cell Incubator;
- amamatsu FDSS/mCell platform;
- Analysis software.

**Method status**

- Internally validated
- Published in peer reviewed journal
- Validated by an external party (e.g. OECD, EURL ECVAM,...)

**PROS, CONS & FUTURE POTENTIAL**

**Advantages**

Early (fast) evaluation of arrhythmic potential using human cells

**Challenges**

- Maturation of cells remains debate;
- Cell-layer don’t reflect 3D-complexity of a (human) heart;
- Commercial cell line represents n = 1.
**Modifications**

Use of more mature cells (due to improved culture conditions?); Improved phenotypic appearance (obtaining closer cellular resemblance of the human heart).

**Future & Other applications**

Calcium transient method can potentially be applied for measuring activity of other cell types as well

**REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

**References**

