Assessment of *in vitro* compound-induced pro-arrhythmia in human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes using multiple assay platforms

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Abstract

Introduction
hiPSC-derived cardiomyocytes hold great potential for safety pharmacology testing. Therefore, they are currently evaluated within the CiPA (Comprehensive *in vitro* Proarrythmia Assay) consortium as models for prediction of drug-induced arrhythmias.
Objective
Here, we assessed the effects of a set of low (verapamil, mexiletine, nifedipine, and diltiazem), intermediate (astemizole), and high (azimilide, dofetilide) risk CiPA compounds on the electrophysiology of hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes). Pluricyte® Cardiomyocytes were cultured in well-defined Pluricyte® Cardiomyocyte Medium, and compound-effects were assessed using MEA analysis and Ca^{2+}-flux assays, to evaluate them as a relevant *in vitro* model for preclinical cardiac safety assessment.
Results
Both MEA analysis and Ca^{2+}-flux assays are suitable to detect pharmacological responses in Pluricyte® Cardiomyocytes. As expected, high and intermediate risk compounds appeared to cause more dramatic effects (prolonged field potential durations and even Torsade-de-Pointes-like arrhythmias) than the low risk compounds at concentrations close to clinical plasma concentrations.
Conclusion
Our data support the use of Pluricyte® Cardiomyocytes to predict cardiac safety of pharmaceuticals in humans, thereby improving preclinical testing strategies for the assessment of cardiac safety, which is in line with the new regulatory approach embodied by the CiPA initiative.

MEA analysis and Ca^{2+}-flux assays for the assessment of electrophysiology and Ca^{2+}-transients of Pluricyte® Cardiomyocytes

Figure 1. A typical waveform of a Pluricyte® Cardiomyocyte monolayer acquired through MEA analysis. Cell Index (i.e. impedance) signal (top) and ECR (i.e. field potential) signal (bottom) obtained using the xCelligence® RTCA CardioECR instrument (ACEA Biosciences). The field potential shows a robust sodium spike amplitude and a well-pronounced repolarization peak.

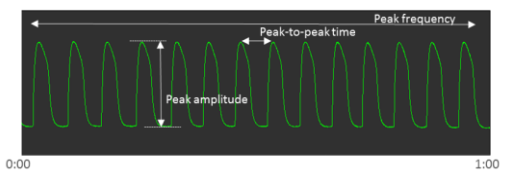


Figure 2. A typical baseline measurement of the fluorescent signal resulting from calcium transients in spontaneously contracting Pluricyte® Cardiomyocytes obtained with the Hamamatsu FDSS®/µCell instrument. Parameters that can be analysed are indicated. For this assay, the FLIPR Calcium 6 Assay Kit was used to detect calcium-transients (Molecular Devices).

Screening of compound-induced alterations of calcium transients in Pluricyte® Cardiomyocytes measured using the FDSS®/µCell system

Figure 3. Compound-induced alterations of calcium transients in Pluricyte® Cardiomyocytes. Blocking of the hERG channel by 100 nM **E4031** resulted in arrhythmic-like events (**top right panel**). By blocking L-type calcium channels, CiPA compounds **diltiazem** and **nifedipine** reduced the calcium transient peak amplitudes, eventually leading to complete diminishing of the signal (**middle panels**). The calcium channel agonist, **Bay K 8644**, caused increased peak amplitudes and increased peak widths (**lower left panel**). The β-adrenergic receptor agonist, **isoproterenol**, caused increased peak frequency, decreased peak width and increased peak amplitude (**lower right panel**).

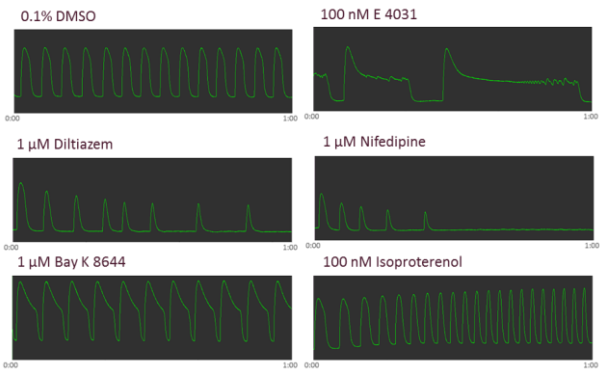


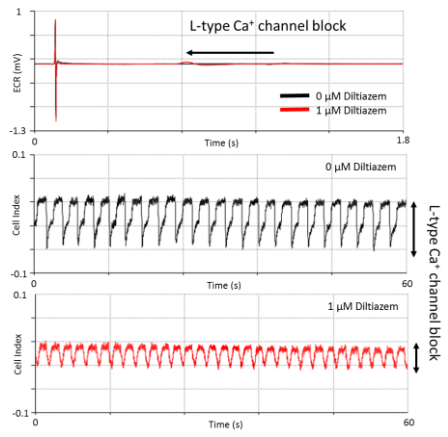
Figure 4. Calcium transient analysis of Pluricyte® Cardiomyocytes upon incubation with cardioactive compounds. Overview of different cardioactive compounds and their effects on beat rate (peak frequency) and peak amplitude, expressed as percentage change when compared to the baseline (DMSO control). In addition, the incidence of beating irregularity, arrhythmic-like events, and stopping of beating (within 5 minutes after compound addition) was analyzed. N=4 wells for each condition.

Acknowledgments

We greatly acknowledge Jean Marc D'Angelo from Hamamatsu Photonics for his contribution to this study. Parts of this work were supported by ECSEL J4 project InForMed (grant no 2014-2-662155), and the European Union's Horizon 2020 research and innovation programme under grant agreement No 726513.

Assessment of the effects of low, intermediate, and high risk CiPA compounds on Pluricyte® Cardiomyocyte Electrophysiology using MEA analysis

A. Example of low-risk CiPA compound: L-type Ca^{2+} blocker diltiazem



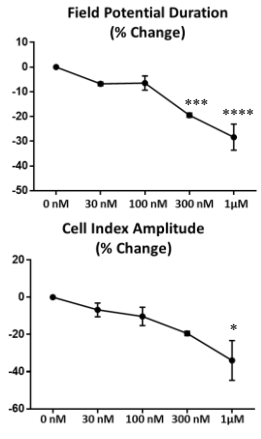
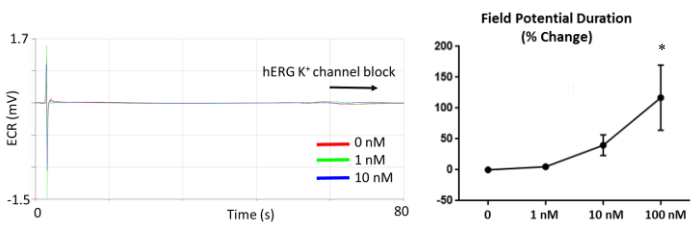


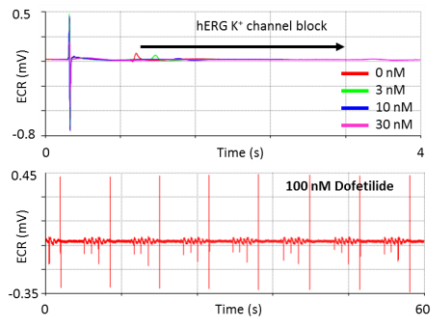
Figure 5. Pharmacological responses of Pluricyte® Cardiomyocytes to low-, intermediate-, and high-risk compounds determined with xCelligence® RTCA CardioECR field potential and Impedance analysis.
Diltiazem (A), determined as a low-risk CiPA compound, induced a concentration-dependent decrease in the field potential duration, and a decreased Cell Index Amplitude, which was in line with decreased calcium transient peak amplitudes (*Figure 3*). A one-way ANOVA test with post hoc Dunnett's multiple comparisons test was performed to determine significance at increasing compound concentrations. No arrhythmias were observed at any concentration tested.

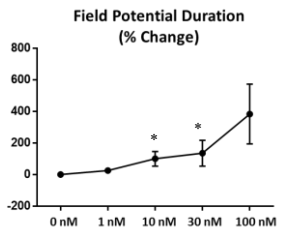
B. Example of intermediate-risk CiPA compound: hERG K^+ blocker astemizole

Astemizole (B), an intermediate-risk CiPA compound, induced a concentration-dependent increase in the Field Potential Duration. Arrhythmic-like events and cessation of beating were incidentally observed ≥ 100 nM (not shown). A one-way ANOVA test with post hoc Dunnett's multiple comparisons test was performed to determine significance at increasing compound concentrations.



C. Example of high-risk CiPA compound: hERG K^+ blocker dofetilide





Dofetilide (C), a high-risk CiPA compound, induced a concentration-dependent increase in the field potential duration. Arrhythmic-like events were observed in all wells ≥ 100 nM. A one-way ANOVA test with post hoc Dunnett's multiple comparisons test was performed to determine significance at increasing compound concentrations.

In vitro effects of CiPA compounds on Pluricyte® Cardiomyocyte electrophysiology – with respect to clinically relevant concentrations

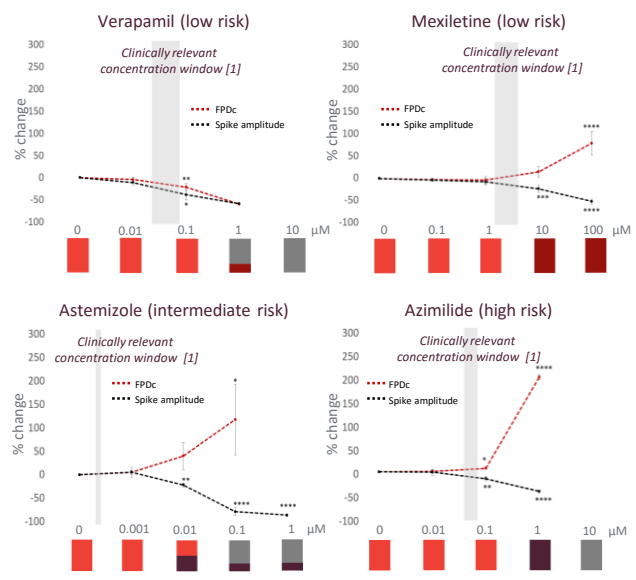


Figure 6. Overview of the effects of a set of CiPA-compounds on Pluricyte® Cardiomyocytes measured using MEA analysis, and shown with respect to their clinically relevant concentrations *in vivo*.
Verapamil, a dual inhibitor of the outward hERG potassium current and the L-type calcium channel, had minimal effects on FPDc and spike amplitude at clinically relevant concentrations. **Mexiletine,** a dual inhibitor of the inward sodium current and the outward hERG potassium current also had minimal effects on FPDc and spike amplitude at clinically relevant concentrations. Both compounds showed no TdP-like arrhythmic behavior at any of the concentrations tested.
Next, an intermediate (**Astemizole**) and a high-risk (**Azimilide**) compound were tested. In contrast to the low risk compounds, and especially for Azimilide, dose-dependent prolongations of the FPDc and arrhythmias at concentrations close to clinically relevant plasma levels were observed. For the high-risk compound Azimilide, all wells showed TdP-like arrhythmias at a concentration of 1 µM. In case of arrhythmias or stop of beating, FPDs were quantified prior to the onset these phenomena.

[1] Redfern et al., Cardiovascular Research 2003

Conclusive Remarks

- MEA analysis of Pluricyte® Cardiomyocytes shows monolayer field potentials with well-pronounced de- and repolarization peaks for easy detection and analysis of the field potential duration.
- Calcium-transient analysis in Pluricyte® Cardiomyocytes using the FDSS®/µCell system provides a robust medium- to high-throughput screening method for cardiac safety, which can be used already at an early stage of drug development.
- Pluricyte® Cardiomyocyte-based MEA assays show predictive and reproducible responses to low, intermediate, and high-risk level CiPA compounds.
- As expected, high and intermediate risk CiPA compounds appeared to cause more dramatic effects (prolonged field potential durations and even Torsade-de-Pointes-like arrhythmias) than the low risk compounds at concentrations close to clinical plasma concentrations.
- Our data support the use of hiPSC-derived cardiomyocytes to predict cardiac safety of pharmaceuticals in humans at an early stage of development, in line with the new regulatory approach embodied by the CiPA initiative.