Assessment of electrophysiology, contractility and viability effects of three UCB compounds on human induced pluripotent stem cell-derived (hiPSC) cardiomyocytes using the Real Time Cell Analyzer (RTCA) CardioECR platform

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Introduction

- The Comprehensive in vitro Proarrhythmia Assay (CiPA) is a novel cardiac safety screening paradigm intended to refine the current regulatory strategy based on the S7B and E14 guidelines (Sager et al. (2014) Am. Heart J.,167(3):292-300)
- One of the components of CiPA consists of evaluating the effects of candidate drugs on the action potential (AP) or field potential (FP) in human stem cell-derived cardiomyocytes

Materials and methods

Cells:

- Pluricyte® Cardiomyocytes seeded at 30.000/well on an RTCA xCelligence® CardioECR E-plate
- Cells cultured for 8 days and daily monitored using the CardioECR system

Platform: RTCA xCelligence® CardioECR

- Simultaneous recording of cellular impedance (presented as Cell Index [CI]) and electrical field potential (presented as extracellular recording [ECR]).
  - ✓ The ECR (extracellular recording) measurement captures changes in the extracellular field potential, generated by the electrophysiological processes across the cell membrane.
  - ✓ The Cell Index (CI) measurement (i.e. impedance measurement) can be used to study the overall cell viability over time and serves as a surrogate for contraction by measuring the alterations in impedance when the cardiomyocyte monolayer beats.

Study design:

- Compounds tested on day 8 post-thaw at 4 different concentrations (individual dosing per well, n=3). DMSO (0.03%) used as vehicle control.
- Both acute and long-term effects were tested (0, 5min, 10min, 30min, 1h, 2h 4h, 8h, 12h, 16h and 24h after compound addition).

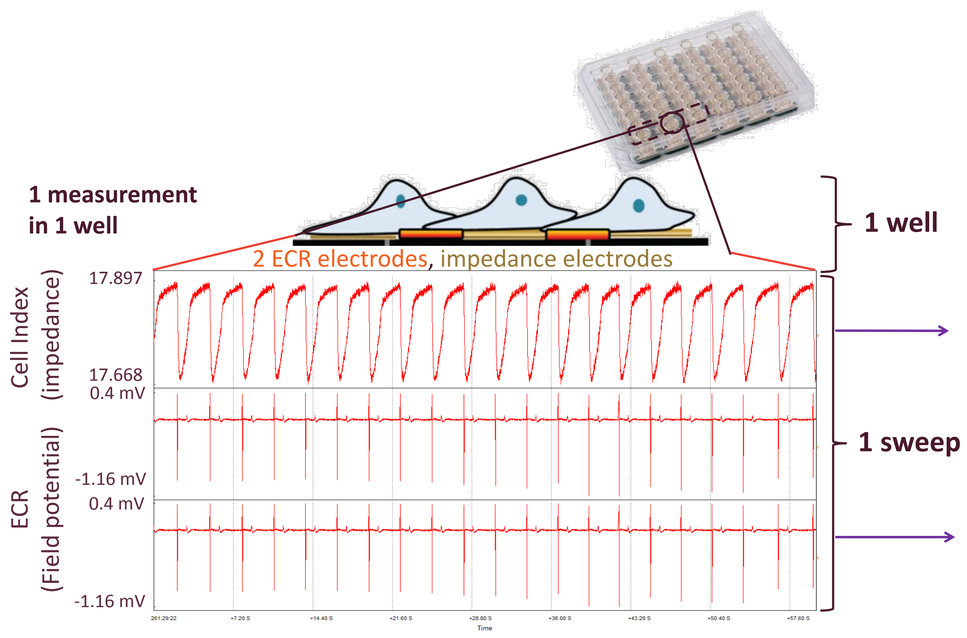
Data analysis: in 3 steps (*example below for UCB-B*)

Objectives of the work

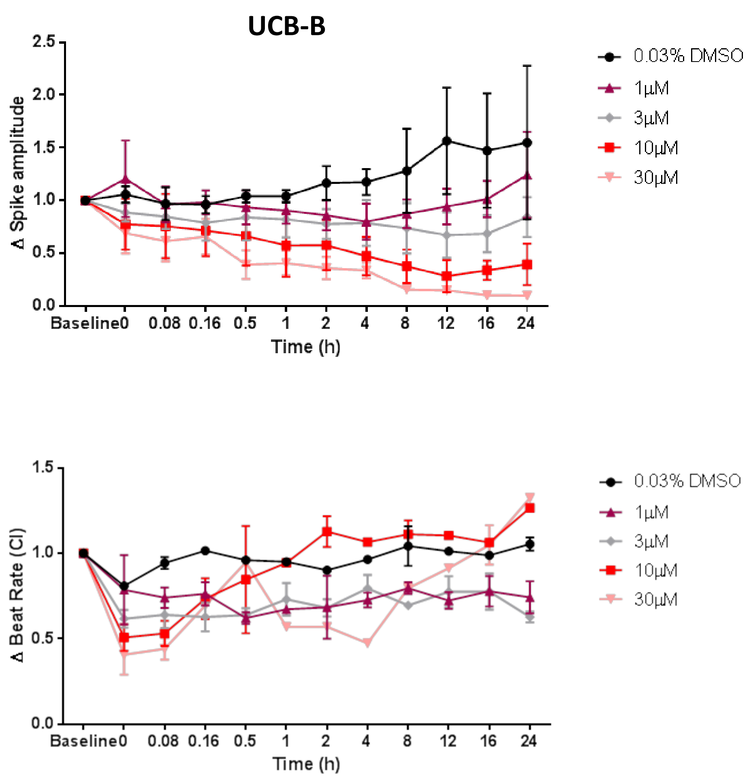
- The goal of the present work was to test in human induced pluripotent stem cell-derived (hiPSC) cardiomyocytes 3 UCB compounds (UCB-A, UCB-B and UCB-C) that failed in preclinical or clinical development due to cardiotoxicity.
- As an extended set of *in vitro* and *in vivo* data was available, it allowed verifying whether this assay would have been able to predict the cardiovascular effects observed with these compounds in preclinical studies or in the clinic.

Data collection

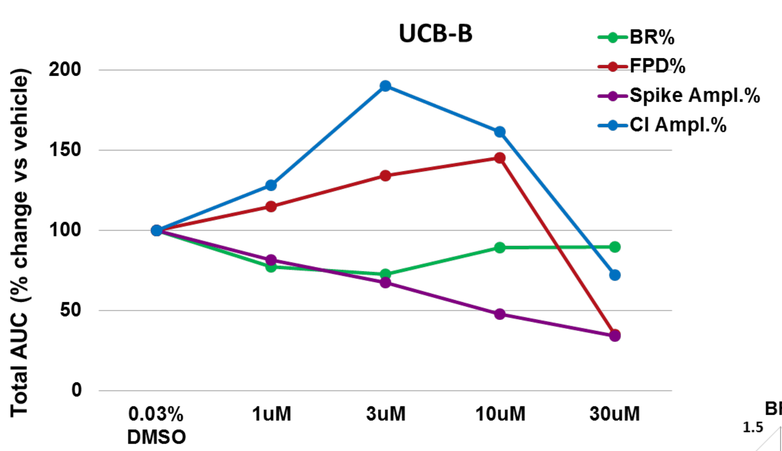
- Per condition → 3 wells
- Per well → impedance electrodes (1 readout) + 2 ECR electrodes (see figure below)
- Per electrode → measure 1 sweep = 60 seconds = ±25 measurements



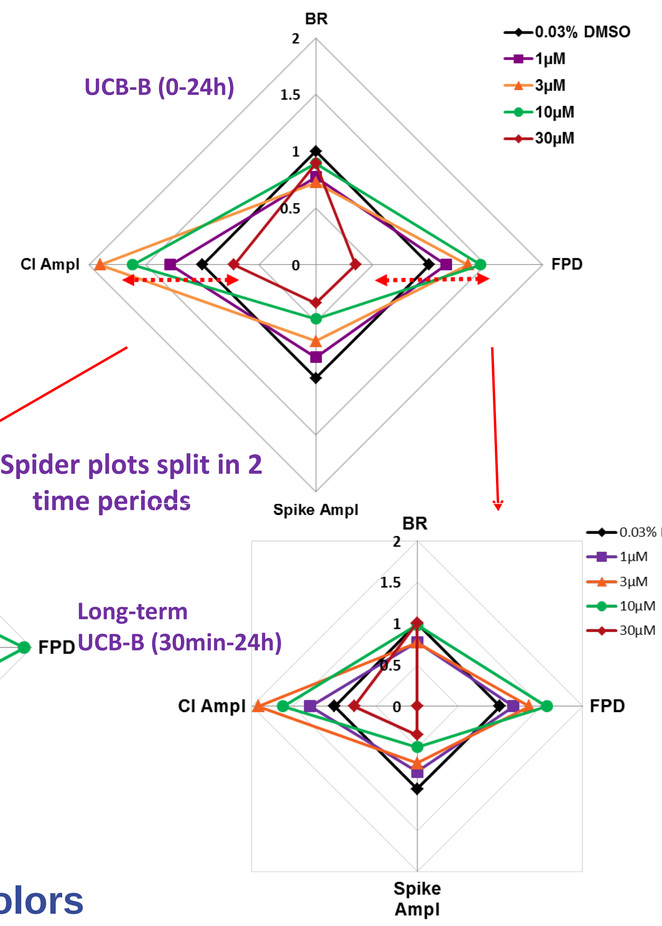
1) For each parameter: time-course graphs at each concentration



2) AUC calculated for each time-couse graph (1) leading to concentration-AUC response graphs for each parameter



3) Spider plots integrating AUC response of the 4 main parameters on a chosen period



Summary of identified hazard signals: Data correlating together across models are paired by colors

From previous data	UCB-A	UCB-B	UCB-C
hERG/Nav1.5/Cav1.2 IC_{50} (µM)	0.5/3.8/1	21/>30/>30	2.6/>30/>30
Purkinje fiber assay	at 10µM: **decrease in APD** at 100µM: **decrease in Vmax**, triangulation	Decrease in APD, RMP **Decrease in Vmax**	From 3µM: **increase in APD** From 10µM: EAD; **decrease in Vmax** at 30µM
Zebrafish	at 50µM: **decrease in BR**, type 2:1 **arrhythmia**	**Slight decrease in BR** at 50µM	Not tested
Guinea pig telemetry	at ~0.1µM: no relevant ECG, **increase in heart rate**	Not tested	At 2µM: increase in PR and QTc intervals, **decrease in HR**
Non-rodent telemetry	From 0.1µM: decrease in PR interval, no QTc changes	At 2.7µM: **prolonged QTc** after 4 weeks of treatment	up to 0.4µM: no ECG effects
Clinical data	at 0.015µM: **syncope, AV blocks** in 2 female healthy volunteers	Not tested	Not tested
From current study			
Pluricyte® hiPSC cardiomyocytes	*Concentrations tested: 0.01, 1, 3 and 10 µM* **Spike amplitude: decrease** from 1µM **BR**: **decrease** at 3µM, slight **increase** at other concentrations CI amplitude: decrease at 3µM (<1h) **FPD: decrease** at 10µM (8 to 24h) **Irregular beating** at 0.01 and 1µM (24h); transient **beating arrest** (10min to 2h) at 10µM Cell viability: acute decrease at 10µM, overall decrease at all concentrations	*Concentrations tested: 1, 3, 10 and 30 µM* **Spike amplitude** : conc-dependent **decrease** from 1µM **BR:** conc-dependent **decrease** from 1µM, biphasic effect at 10 and 30µM CI amplitude: increase at 3-10µM (4 to 24h), decrease at 30µM (10min-4h) **FPD**: **slight increase** at 10µM (up to 2h) Irregular beating from 3µM, arrhythmia-like events at 30µM Cell viability: acute increase at 30µM (<4h) followed by a clear conc-dependent decrease from 1µM	*Concentrations tested: 0.3, 1, 3 and 10 µM* **Spike amplitude: decrease** at 10µM (5min-2h) **BR**: slight increase at 1µM (<4h), **decrease** at 10µM (30min-4h), **decrease** at all conc. (12-16h) CI amplitude: increase at 10µM (30min-4h) **FPD: increase** at 10µM (30min-4h); **increase** at 3µM (16-24h); Irregular beating at 10µM; Cell viability: slight decrease at all concentrations

All concentrations given in the table are unbound; APD= action potential duration, RMP: resting membrane potential, HR=heart rate, BR=beat rate, FPD=field potential duration.

Conclusions

- The hiPSC cardiomyocyte model allowed to capture the relevant cardiovascular effects observed in multiple other *in vitro* and/or *in vivo* preclinical models at clinical relevant concentrations, suggesting that hiPSC cardiomyocytes could be a useful model to generate predictive data on multiple parameters in a single preclinical assay. Some (pre)clinical effects like PR interval changes could not be detected as Pluricyte® Cardiomyocytes are of pure ventricular origin. To capture these types of *in vivo* effects, more complex *in vitro* and/or *in vivo* models may be necessary.
- More experiments using reference compounds with well known clinical effects on the heart would be necessary to investigate the sensitivity of hiPSC cardiomyocytes and the potential of this model for implementation in early preclinical drug development for cardiac safety assessment.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under (Grant no. 726513).