Assessment of human induced pluripotent stem cell-derived cardiomyocytes for evaluating drug-induced arrhythmias with multi-electrode array

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Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) are a promising tool for assessment of drug-induced arrhythmias during non-clinical drug development. This technology is under evaluation by the FDA's Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative and the Japanese iPS Cardiac Safety Assessment consortium (JiCSA) to develop new cardiac safety assessment measures to refine current S7B and E14 guidelines. The CiPA myocyte working group utilises imaging and electrophysiology platforms and a toolbox of 28 clinical compounds with known arrhythmia risk to correlate *in vitro* iPSC-CM data with clinical QT prolongation and Torsade de Pointes (TdP) liabilities.

Here we demonstrated that proarrhythmic liability can be accurately predicted using iPSC-CM on a multi-electrode array (MEA) system:

- We tested the full CiPA toolbox of 28 compounds against two commercial iPSC-CM cell lines (Ncardia vCor.4U and Cellular Dynamics International iCell^2 cardiomyocytes) to compare their responses to reference drugs with known human clinical arrhythmia risk (low, medium, and high)
- We utilised recommended methodology from FDA iPSC-CM studies for cell culture and compound application
- We compared our data on Phase 1 and 2 CiPA compounds to similar datasets published by JiCSA and the FDA

Materials and Methods

MEA assay: Human iPSC-CM were obtained from Ncardia (vCor.4U) and Cellular Dynamics International (iCell^2 cardiomyocytes) and seeded according to manufacturer's instructions onto 96 well MEA plates (Axion Maestro). Plates were incubated at 37°C (5 % CO_2) for 7-10 days and 100 % medium exchanges were performed every 2-3 days. Compounds were serially diluted in DMSO followed by a 1000-fold dilution into media. The final DMSO concentration did not exceed 0.1 %. Our MEA experiments utilised serum-containing media for compound addition, as this has been shown to be important for correctly identifying high risk compounds[2]. Compounds were tested using a cumulative acute application paradigm (30 min/conc.; Figure 1) in accordance with FDA studies[3]. Parameters reported from Metrion's MEA assay were ≥ 10 % change in the corrected field potential duration (ΔFPDc) and incidence of arrhythmic events (≥ 1 well).

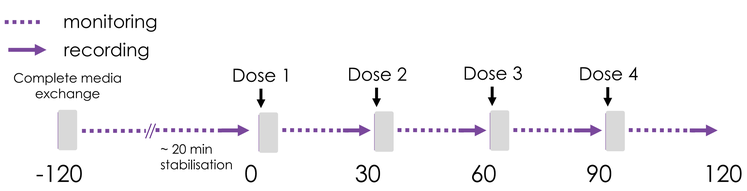
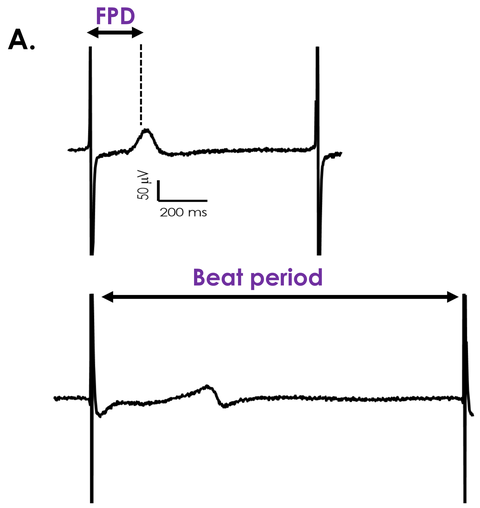
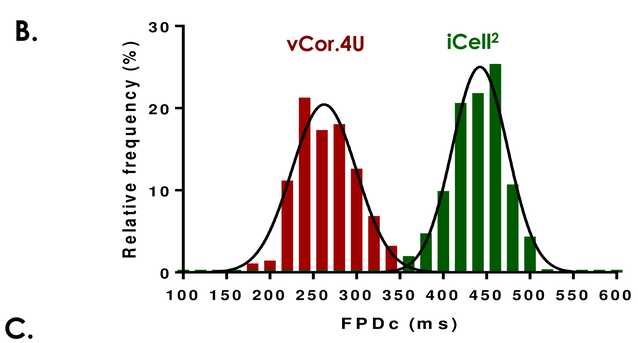


Figure 1: Experimental protocol for MEA recordings Schematic depicting the drug application and measurement schedule used for MEA recordings of hiPSC-CM field potential on the Axion Maestro system.

1. Control MEA parameters of iPSC cardiomyocyte cell lines





C.

Cell line	FPDc (ms)	Beat Period (ms)	Spike Amplitude (mV)	Conduction Velocity (mm.ms^{-1})
Ncardia vCor.4U	283.7 ± 60.3	880.9 ± 62.2	1.1 ± 0.56	0.23 ± 0.16
CDI iCell^2	454.6 ± 24.8	1433.4 ± 70.0	2.27 ± 0.94	0.35 ± 0.2

Figure 2: Baseline measurements characterising spontaneous beating in iPSC-CMs. A. Representative field potential recording from vCor.4U cell (top) and iCell^2 (bottom) showing the difference in field potential duration (FPD) and the beat period (BP). **B.** Corrected FPD distribution for vCor.4U and iCell^2 **C.** The mean Fridericia-corrected field potential duration, beat period and absolute value of the depolarisation spike amplitude during spontaneous beats from vCor.4U and iCell^2 cardiomyocytes, as well as the conduction velocity, were averaged for all control (no drug treatment) wells. Variability is shown as ± one standard deviation.

2. Prediction of proarrthymic risk using iPSC-CM on a MEA system

Metrion MEA assays align with CiPA risk classification and regulatory body publications

Compound	CiPA TdP Risk	ΔFPDc							Arrhythmia						
		Metrion vCor.4U	JiCSA Cor.4U (1)	FDA Cor.4U (3)	Metrion iCell^2	JiCSA iCell (4,5,6)	FDA iCell (3)	FDA iCell (2)	Metrion vCor.4U	JiCSA Cor.4U (1)	FDA Cor.4U (3)	Metrion iCell^2	JiCSA iCell (4,5,6)	FDA iCell (3)	FDA iCell (2)
Diltiazem	Low	↓		↓	↓	↓	↓	↓	-		-	-	-	-	-
Mexilitine	Low	↑	↑	=	↑	↑	=	=	-	-	-	-	-	-	-
Ranolazine	Low	↑	↑	↑	↑	↑	↑	↑	-	-	-	+ FP	+ FP	+ FP	+ FP
Verapamil	Low	↓	↓	↓	↓	↓	↓	↓	-	-	-	-	-	-	-
Chlorpromazine	Medium	↑	=	=	↑	=	=	↑	+		- FN	- FN	+	- FN	+
Cisapride	Medium	↑	↑	↑	↑	↑	↑	↑	+	+	- FN	+	+	+	+
Ondansetron	Medium	↑			↑	↑			+			+	+		
Terfenadine	Medium	↑	↑	↑	↑	↑	=	↑	+	- FN	- FN	- FN	- FN	- FN	+
Bepridil	High	↑	↑	↑	↑	↑	=	↑	- FN	- FN	- FN	+	- FN	- FN	- FN
D,L-Sotalol	High	↑			↑	↑			+			+	+		
Dofetilide	High	↑	↑	↑	↑	↑	↑	↑	+	+	+	+	+	+	+
Quinidine	High	↑	↑	↑	↑	↑	↑	↑	+	+	+	+	+	- FN	+

Table 1: Comparison of MEA data obtained at Metrion to published data for Phase 1 compounds
Compounds from each CiPA risk classification were screened against vCor.4U and iCell^2 cardiomyocytes on the Maestro MEA using a cumulative acute application protocol[3]. Overall, good correlation was observed between our data and published datasets[2-6]. Our MEA assay correctly identified all low risk compounds as non-arrhythmic, and all high risk compounds showed a significant FPDc prolongation. All high risk compounds resulted in arrhythmic events in both cell lines, with the exception of Bepridil for vCor.4U. For iCell^2 cardiomyocytes, both Chlorpromazine and Terfenadine failed to generate EAD/arrhythmic events. FP = False Positive; FN = False Negative

Compound	CiPA TdP Risk	ΔFPDc				Arrhythmia			
		Metrion vCor.4U	JiCSA Cor.4U (1)	Metrion iCell^2	JiCSA iCell (4,5,6)	Metrion vCor.4U	JiCSA Cor.4U (1)	Metrion iCell^2	JiCSA iCell (4,5,6)
Loratadine	Low	=	↓	=	=	-	-	-	-
Nifedipine	Low	↓		↓	↓	-		-	-
Nitrendipine	Low	↓		↓	↓	-		-	-
Tamoxifen	Low	↑		=	=	-		-	-
Metoprolol	Low	=	=	=	=	-	-	-	-
Astemizole	Medium	↑	↑	↑	↑	+	+	+	+
Droperidol	Medium	↑		↑	↑	+		+	+
Domperidone	Medium	↑	↑	↑	↑	+	- FN	+	+
Clarithromycin	Medium	↑		↑	↑	+	+	+	+
Risperidone	Medium	↑	=	↑	↑	+	- FN	+	+
Pimozide	Medium	↑	↑	↑	↑	+	- FN	+	+
Clozapine	Medium	=	↓	↓	↓	- FN	- FN	- FN	- FN
Azimilide	High	↑	↑	↑	↑	+	- FN	+	+
Ibutilide	High	↑	↑	↑	↑	+	+	+	+
Vandetanib	High	↑		↑	↑	+		+	+

Table 2: Comparison of MEA data obtained at Metrion to published data for Phase 2 compounds
Phase 2 compounds from each CiPA risk classification were screened against vCor.4U and iCell^2 on the Maestro MEA using a cumulative acute application protocol[3]. Overall, good correlation was observed between our data and published JiCSA datasets[2-6]. FN = False Negative

Our MEA assay correctly identified all low risk compounds as non-arrhythmic, and all high risk compounds showed a significant FPDc prolongation and arrhythmic events in both cell lines.

Metrion's data also correctly identified most medium risk Phase 2 compounds, with the exception of Clozapine. This compound showed a decrease or no effect on FPDc, and failed to generate EAD/arrhythmic events in either hiPSC-CM cell line.

Metrion's MEA assays show good risk prediction accuracy with both iPSC-CM cell lines

	TP	FN	FP	TN	Total	Sensitivity	Specificity	Accuracy
Metrion vCor.4U	**16**	**2**	**0**	**9**	**27**	**88.9**	**100.0**	**92.6**
JiCSA Cor.4U (1)	6	7	0	5	18	46.2	100.0	61.1
FDA Cor.4U (3)	2	4	0	4	10	33.3	100.0	60.0
Metrion CDI	**15**	**3**	**1**	**8**	**27**	**83.3**	**88.9**	**85.2**
JiCSA CDI (4,5,6)	15	3	1	8	27	83.3	88.9	85.2
FDA CDI (3)	2	4	1	3	10	33.3	75.0	50.0
FDA CDI (2)	5	1	1	3	10	83.3	75.0	80.0

Table 3: Analysis of risk categorisation against clinical information
Statistical evaluation of our MEA assay results compared to clinical proarrhythmic risk demonstrate excellent sensitivity, specificity, and predictive capacity. Only compounds included in CiPA Phase 1 and Phase 2 studies were included in the concordance analysis. TP=True Positive, FN=False Negative, FP=False Positive, TN=True Negative

Conclusions

We show that careful attention to optimisation and validation of *in vitro* methods is required for predictive CiPA assays.

- In our MEA experiments we use methods comparable to that developed by the FDA to validate two major iPSC-CM cell lines and generate data that aligned well to published findings
- Our data confirm previous observations[2] and highlight the importance of including serum during compound application to faithfully capture the pro-arrhythmic risk of test compounds

References

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