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Electrophysiological Characterisation of CDI iCell² iPSC-Derived Cardiomyocytes

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Introduction

To provide a more thorough and predictive cardiac safety profile of new chemical entities, the FDA is introducing the Comprehensive *in vitro* Proarrhythmia Assay (**CiPA**) initiative. To allow the successful integration of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) as a translational model of human cardiac tissue their physiology needs to be fully characterised.

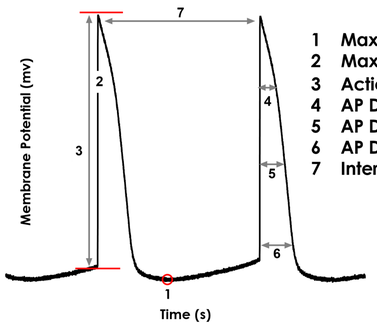
Here we highlight the work performed at Metrion Biosciences in collaboration with Cellular Dynamics International (CDI) to assess the utility of CDI iCell² ventricular iPSC-CM for cardiotoxicity screening. For this assessment we used manual patch clamp, the gold standard in electrophysiology, to determine the biophysical and pharmacological characteristics of iCell² iPSC-CM:

1. Whole-cell voltage clamp recordings to quantify key cardiac currents.
2. Determination of iCell² action potential (AP) parameters in control conditions.
3. Confirmation of the iCell² ventricular phenotype using pharmacological profiling.
4. Validation of iCell² AP physiology with four calibration compounds[1] targeting core cardiac channels.
5. Case study using a selection of low, intermediate, and high cardiac risk compounds from the CiPA toolbox[1].

Materials and Methods

CDI iCell² iPSC-CM were cultured on fibronectin-coated coverslips at 37 °C (5 % CO_2). All data were recorded 7-10 days post-seeding at RT. Voltage clamp recordings were obtained from single cells using whole-cell patch clamp with protocols and solutions designed to isolate the ionic current of interest[2]. AP recordings were made using perforated patch clamp (100 µg/ml gramicidin). Data were acquired with EPC10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Analog signals were low-pass filtered at 10 kHz before digitization at 20 kHz.

Data were analysed using CAPA software (SSCE UG, Germany) and FitMaster (HEKA). The AP parameters analysed in this study are shown in Figure 1. All spontaneous APD were rate corrected using Friderica's correction. Data are reported as mean ± SEM. Statistical comparisons were performed using a Paired Student's *t*-test or One-way ANOVA with post-hoc testing.



1 Maximum Diastolic Potential (MDP)
2 Maximum Upstroke Velocity (dV/dt_{max})
3 Action Potential Amplitude (APA)
4 AP Duration at 20 % Repolarisation (APD20)
5 AP Duration at 50 % Repolarisation (APD50)
6 AP Duration at 90 % Repolarisation (APD90)
7 Interspike Interval (ISI)

Figure 1: Action potential analysis parameters.
Example action potentials indicating the parameters that were quantified using FitMaster (evoked AP) and CAPA software (spontaneous AP).

1. Biophysics of key cardiac currents

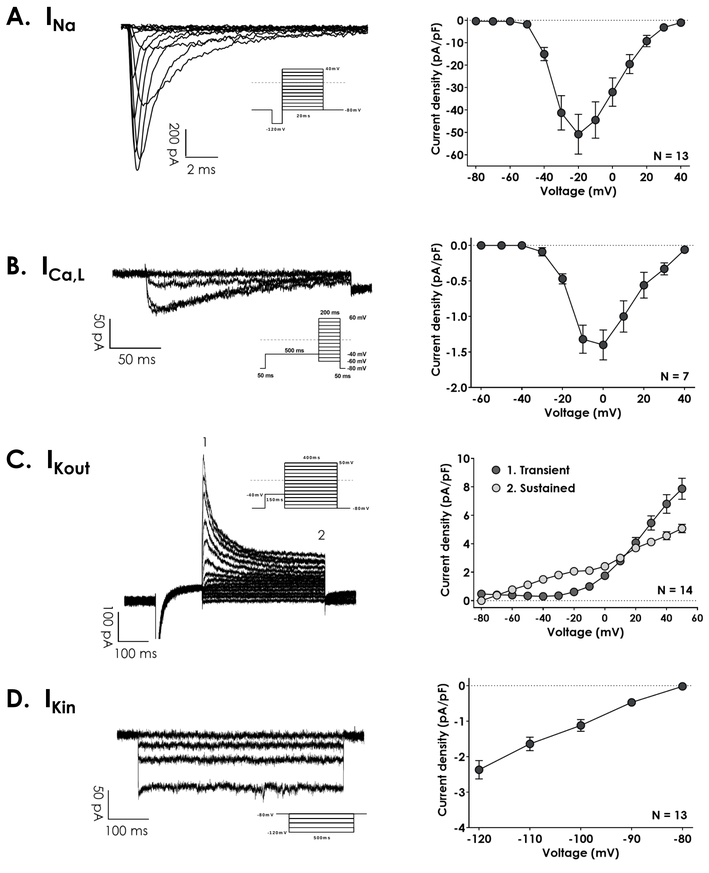
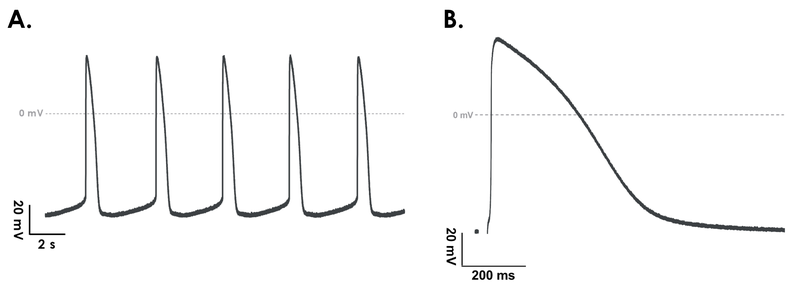


Figure 2: Quantification of key cardiac ionic currents.
Left: Representative traces for sodium (I_{Na}; **A**), L-type calcium ($I_{Ca,L}$; **B**), outward (I_{Kout}; **C**) and inward (I_{Kin}; **D**) potassium currents elicited by the protocols shown. Right: I-V relationships for I_{Na} (peak; **A**), $I_{Ca,L}$ (peak; **B**), transient and sustained I_{Kout} (peak and end, **C**), and I_{Kin} (end current, **D**).

2. AP properties in control conditions



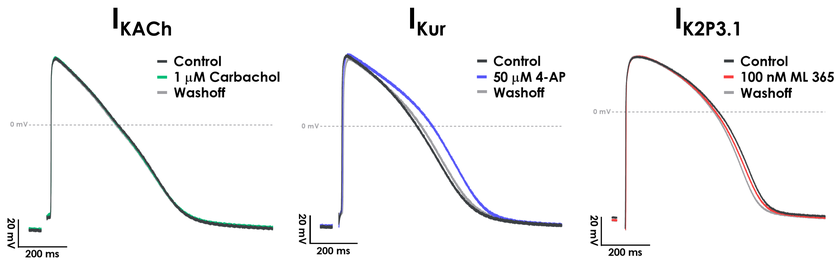
C.

AP Parameter	Spontaneous (N = 122)	Evoked (N = 36)
MDP (mV)	-68.1 ± 0.6	-67.1 ± 1.0
dV/dt_{max} (V/s)	23.6 ±1.52	23.6 ± 3.1
APA (mV)	98.4 ± 0.8	112.4 ± 2.0
APD20 (ms)	222.8 ± 4.1	164.9 ± 5.0
APD50 (ms)	361.6 ± 5.2	320.7 ± 6.6
APD90 (ms)	478.3 ± 7.2	507.9 ± 5.4
Frequency (Hz)	0.29 ± 0.01	1

Figure 3: Characteristics of spontaneous and evoked action potentials.
Representative traces of spontaneous (**A**) and evoked (1 Hz; **B**) AP recorded under control conditions at room temperature. **C**: Average AP parameters.

3. Confirmation of ventricular phenotype

A. Minimal effects of atrial-selective channel modulators



B. Average AP effects

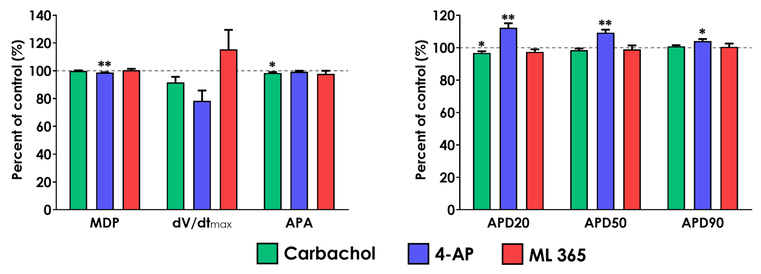
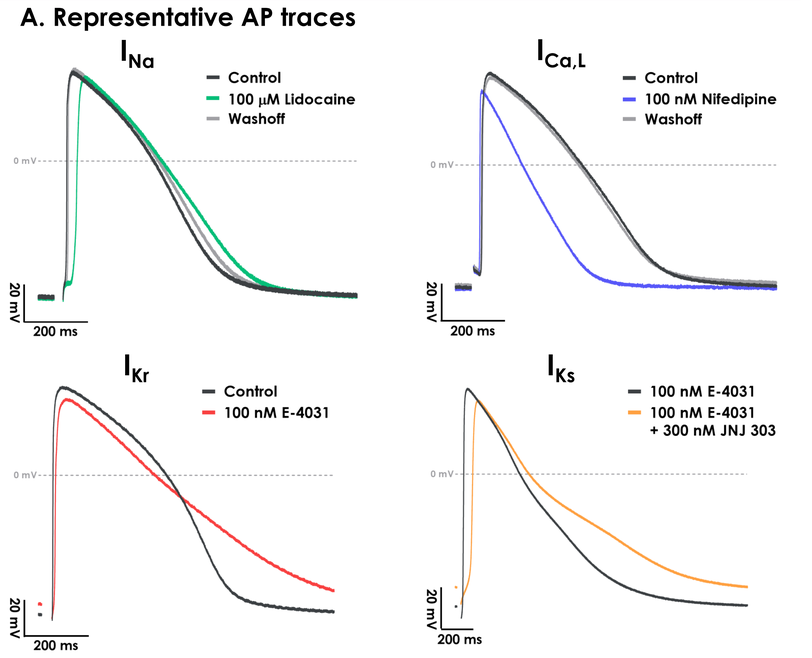


Figure 4: Confirmation of a predominately ventricular phenotype.
To confirm the iCell² phenotype, compounds which selectively modulate ion channels that are typically functional in the human atria were used: Carbachol (I_{KACh}), 4-AP (I_{Kur}), and ML 365 ($K_{2P}3.1$).
A: Representative evoked AP (1 Hz pacing) under control conditions (grey) and in the presence of 1 µM Carbachol (green), 50 µM 4-AP (blue), and 100 nM ML 365 (red). **B**: Average effect (% of control) on AP parameters. N ≥ 5. * p<0.05, ** p<0.01.

4. Expression of core cardiac channels

A. Representative AP traces



B. Average AP effects

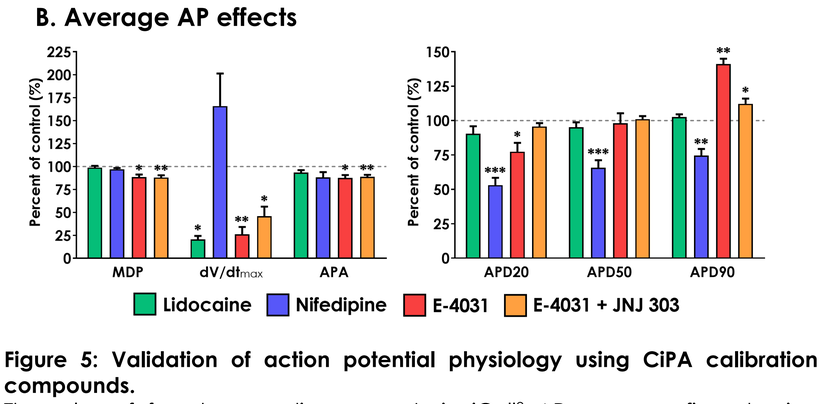
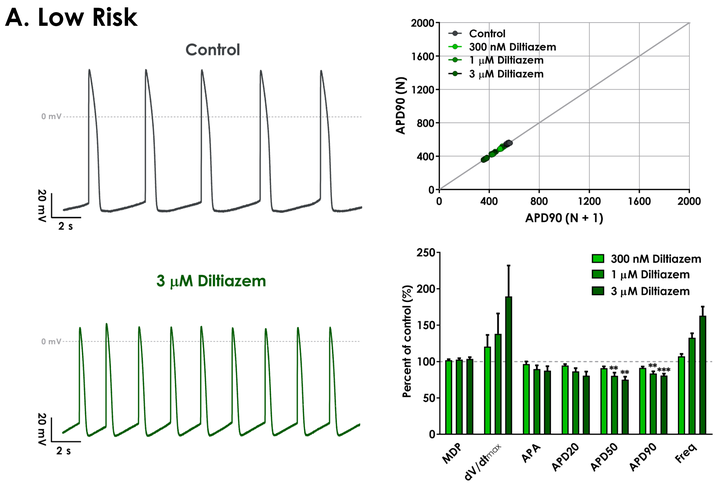


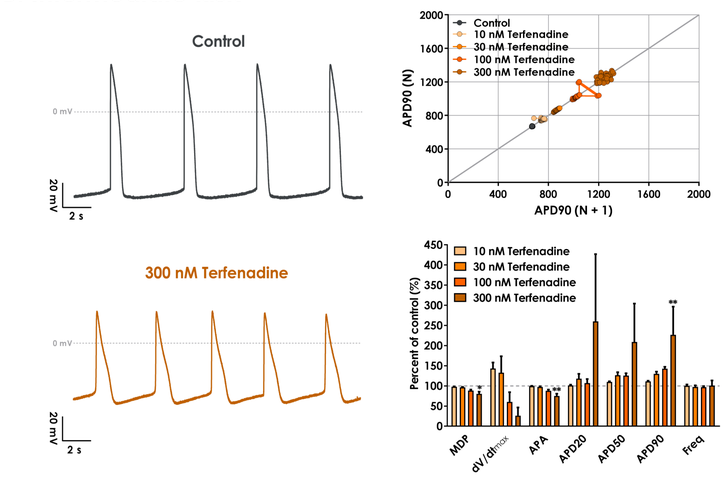
Figure 5: Validation of action potential physiology using CiPA calibration compounds.
The roles of four key cardiac currents in iCell² AP were confirmed using Lidocaine (I_{Na}), Nifedipine ($I_{Ca,L}$), E-4031 (I_{Kr}), and JNJ 303 (I_{Ks}). For isolation of I_{Ks} currents JNJ 303 was applied onto cells pre-treated with E-4031[3].
A: Representative evoked AP under control conditions (grey) and in the presence of 100 µM Lidocaine (green, 1 Hz), 100 nM Nifedipine (blue, 1 Hz), 100 nM E-4031 (red, 1 Hz), or 300 nM JNJ 303 (orange, 0.5 Hz). **B**: Average effect (% of control) on AP parameters. N ≥ 4. * p<0.05, ** p<0.01, *** p<0.001.

5. Assessment of CiPA compounds

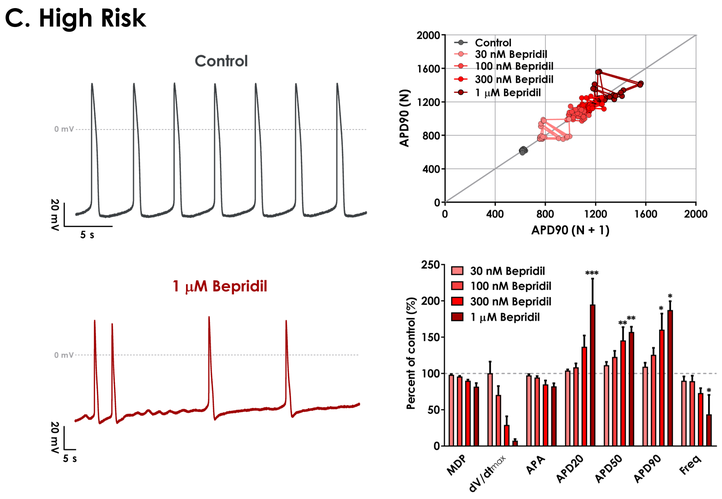
A. Low Risk



B. Intermediate Risk



C. High Risk



D. Average AP effects of CiPA compounds

Compound	CiPA risk level	Cmax (nM)	Test conc (µM)	Effects on spontaneous AP (highest test concentration)							
				MDP	dV/dt_{max}	APA	APD20	APD50	APD90	Freq	Arrhythmia
Diltiazem	Low	128	0.3-3	103.7	189.6	87.4	80.5	75.1	80.9	163.1	-
Ranolazine	Low	1948	10	101.2	105.7	101.2	102.7	118.4	130.1	76.9	-
Chlorpromazine	Intermediate	34.5	0.1-1	93.3	11.2	87.2	163.1	129.2	123.6	32.2	Qui (1/5)
Terfenadine	Intermediate	0.286	0.01-0.3	80.4	26.1	75.0	260.1	209.1	227.0	100.7	Qui (1/4)
Bepridil	High	31.5	0.03-1	81.9	7.4	82.3	195.1	157.1	187.3	43.8	Qui (2/5)
Dofetilide	High	2.14	0.05	81.0	18.4	87.1	121.8	140.7	245.1	91.8	EAD (5/5)
Quinidine	High	843	0.3-10	74.3	14.2	77.5	146.0	149.5	216.5	75.3	-
Sotalol	High	14,686	10-300	93.8	223.3	97.4	74.2	149.8	216.1	85.3	-

Percent of control: 0 % — 200 %

Figure 6: Determination of proarrhythmic risk using CiPA toolbox compounds.
Eight compounds from the CiPA toolbox with varying pro-arrhythmia risk levels were screened against iCell² iPSC-CM during spontaneous AP recordings.
A-C: *Left* panels; representative AP under control conditions (grey) and in the presence of 3 µM Diltiazem (green, **A**), 300 nM Terfenadine (orange, **B**), and 1 µM Bepridil (red, **C**). **A-C:** *Right* (top); representative Poincaré plots for APD90 over the final 2 min of each compound concentration. **A-C:** *Right* (bottom); average effect (% of control) on AP parameters for each compound. **D**: Average effect on AP parameters of the highest test concentration for each compound. The incidence of quiescence (Qui) or early after depolarisations (EAD) are shown. The reported clinical Cmax is also given for each drug[4,5]. N ≥ 3. * p<0.05, ** p<0.01, *** p<0.001.

Conclusions

Biophysical and pharmacological profiling enabled us to draw the following conclusions:

1. iCell² have a predominately ventricular phenotype.
2. Key cardiac currents (including I_{Ks}) are functionally expressed and encode the iCell² AP profile.
3. iCell² are able to generate arrhythmic events.
4. Effects of CiPA toolbox compounds on iPSC-CM APs correlate with their human clinical risk classification.

By undertaking a thorough electrophysiological profiling of CDI iCell² iPSC-CM at Metrion Biosciences we were able to confirm their utility for cardiac drug discovery and predictivity for toxicology screening under CiPA.

Acknowledgements

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References

1. Colatsky *et al.* (2016) J Pharmacol Toxicol Methods. **81**; 15-20.
2. Ma *et al.* (2011) Am J Physiol Heart Cir Physiol. **301**; H2006-H2017.
3. Biliczki *et al.* (2002) Br J Pharmacol. **137**(3); 361-368.
4. Crumb *et al.* (2016) J Pharmacol Toxicol Methods. **81**; 251-262.
5. Blinova *et al.* (2017) Toxicol Sci. **155**(1); 234-247.