UNBLINDED: RESPONSES TO CIPA 28 COMPOUNDS IN COR.4U® CARDIOMYOCYTES

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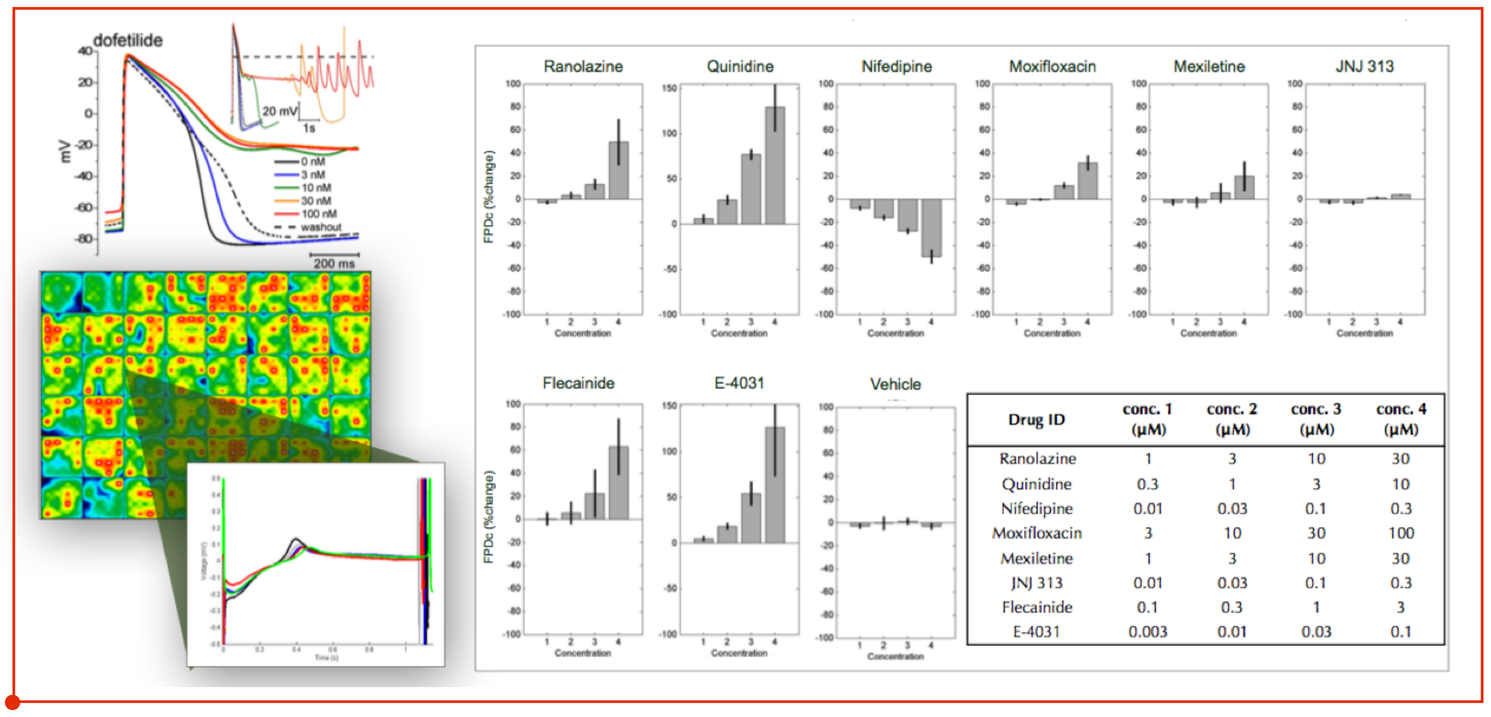
BACKGROUND

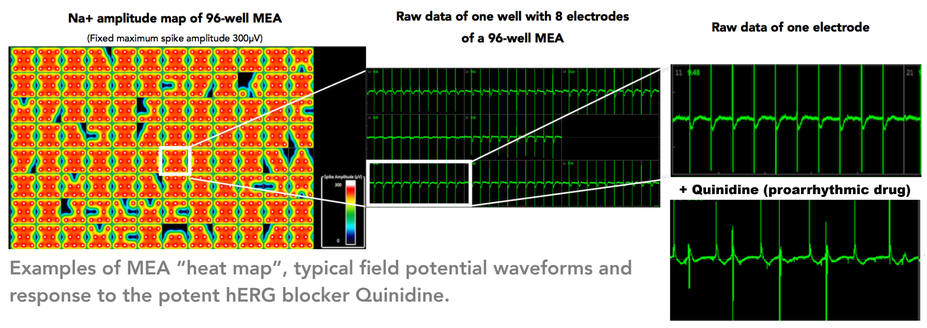
As part of the HESI/CSRC/FDA Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were supplied from two well-characterized vendors for the myocyte "core team" studies. These studies were performed to address the predictive responses of hiPSC-CMs using a 28 compounds dataset with known pro-arrhythmic potential (high, medium or low). Additionally, a number of non-core sites participated in this initiative using similar protocols and instrumentation (microelectrode arrays and voltage sensor technology). These data will be compared against the statistical models being constructed from CiPA myocyte core team data. Here we show our recently unblinded results from all 28 test compounds from low, medium, and high risk that were acquired on the Axion Maestro 96 well microelectrode array. In summary, the positive control, 3 nM Dofetilide, was strikingly reproducible as it produced a very consistent FPDc prolongation of 30% to 40% across all 7 MEA plates (with no EADs). Additionally we show data from all 28 compounds, including FPD prolongation and quantified the number of wells eliciting pro-arrhythmic responses (early afterdepolarizations, tachycardia, etc).

Review: CiPA Phase 1 Pilot Study Results (from 2015)



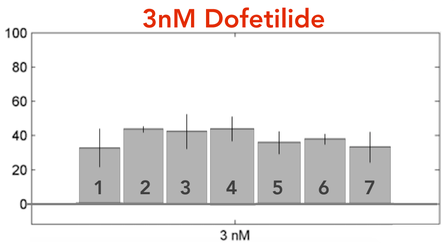
Drug ID	conc. 1 (µM)	conc. 2 (µM)	conc. 3 (µM)	conc. 4 (µM)
Ranolazine	1	3	10	30
Quinidine	0.3	1	3	10
Nifedipine	0.01	0.03	0.1	0.3
Moxifloxacin	3	10	30	100
Mexiletine	1	3	10	30
JNJ 313	0.01	0.03	0.1	0.3
Flecainide	0.1	0.3	1	3
E-4031	0.003	0.01	0.03	0.1

Top left panel: Manual patch clamp recordings detect changes in action potentials from Cor.4U® cardiomyocytes. A publication by Obejero-Paz et al. (2015) *Sci. Rep.* utilized Cor.4U® cardiomyocytes to support late-stage safety assessment that was "consistent with the emphasis on Multiple Ion Channel Effects (MICE) of the CiPA assay". **Bottom left,** Na+ Spike Amplitude Map and example of overlaid field potential recordings from Cor.4U® cultured on Axion Maestro 48 well MEAs. **Right Panels**, Cor.4U® cardiomyocytes were treated with the indicated (originally blinded) compounds. Shown is the % effect over baseline on FPDc, a surrogate marker of QTc interval.



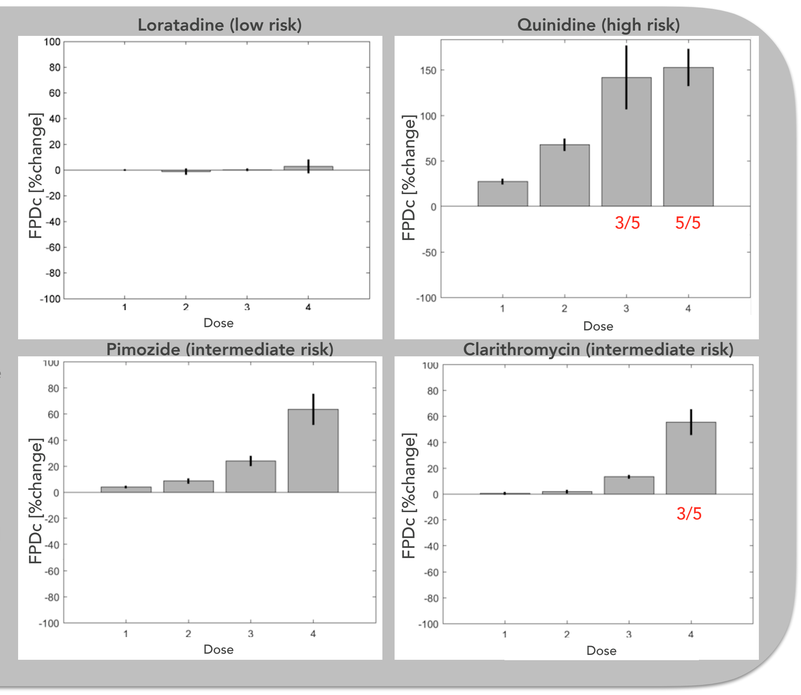
Examples of MEA "heat map", typical field potential waveforms and response to the potent hERG blocker Quinidine.

Plate to plate reproducibility: 3nM Dofetilide



The plate "quality control", Dofetilide, was applied at 3 nM across the 7 assay plates. Dofetilide induced prolongation, without proarrhythmic activity, to a similar extent across all plates, suggesting a reproducible and comparable cell preparation across experiments.

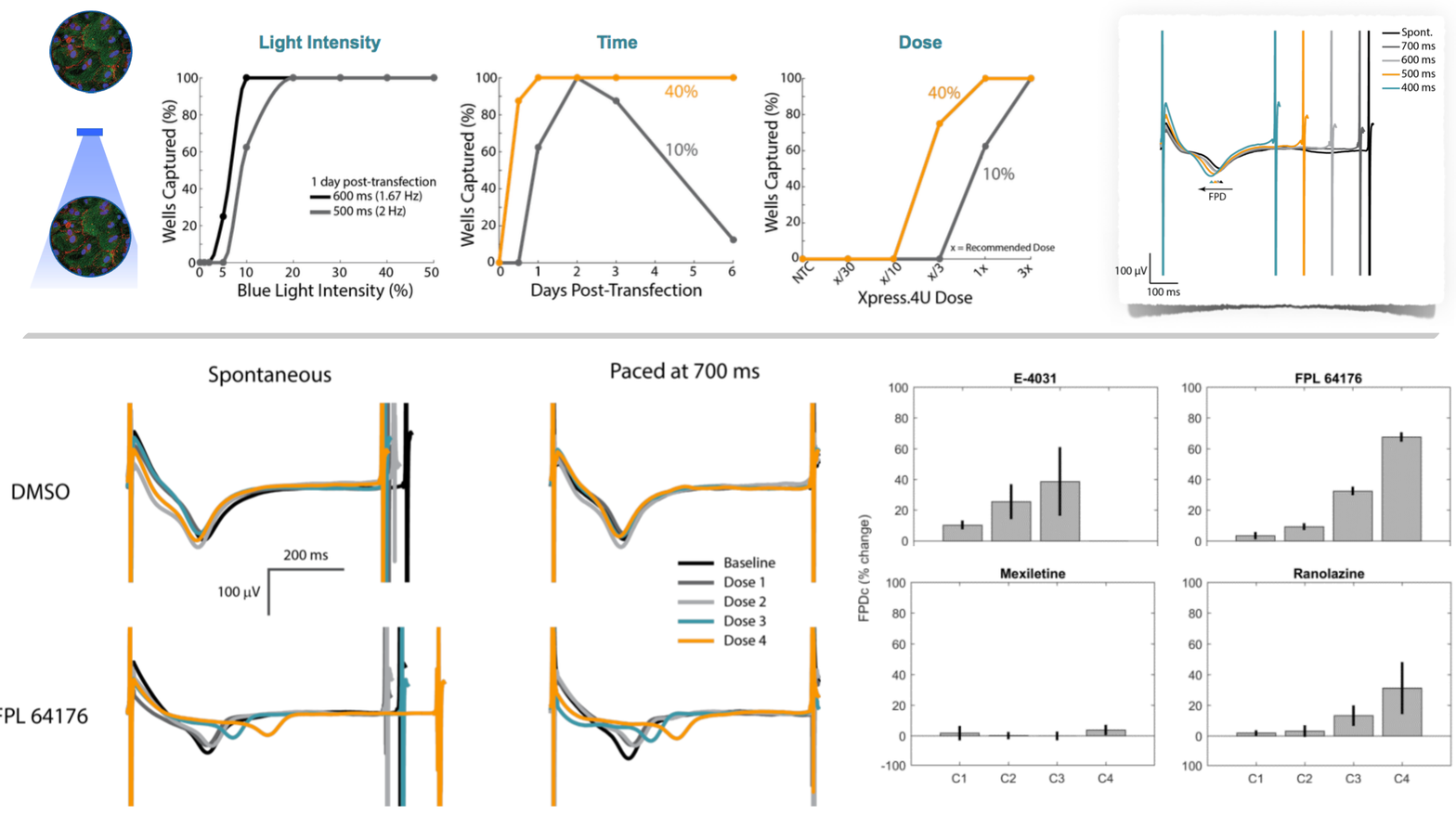
Charts to the right indicate the % change in (corrected) field potential duration for example compounds at low, intermediate, and high risk for *Torsade de Pointes*. Standard deviation of the mean % change is also denoted for each dose tested. For those doses eliciting a pro-arrhythmic response (early afterdepolarizations, arrhythmia, ectopic beats, etc), a red value is displayed denoting the number of wells (out of total wells tested) that the adverse event occurred. **Table to the far right** shows the summary of the 28 compounds, grouped according to perceived TdP risk, tested within the CiPA paradigm on the Axion Maestro. A good correlation between observed pro-arrhythmic activity and TdP risk was observed.



Drug	Dose 1	Dose 2	Dose 3	Dose 4	ETPC (free)	TdP Risk
Loratadine	0.95 nM	3 nM	9.49 nM	30 nM	0.45 nM	LOW
Metoprolol	3.17 µM	10 µM	31.7 µM	100 µM	1.8 µM	LOW
Mexilitine	0.1 µM	1 µM	10 µM	100 µM	2.5 µM	LOW
Nifedipine	1 nM	10 nM	100 nM	1000 nM	7.7 nM	LOW
Nitrendipine	9.5 nM	30 nM	95 nM	300 nM	3.0 nM	LOW
Diltiazem	0.01 µM	0.1 µM	1 µM	10 µM	0.128 µM	LOW
Ranolazine	0.1 µM	1 µM	10 µM	100 µM	1.948 µM	LOW
Tamoxifen	0.095 µM	0.3 µM	0.95 µM	3 µM	0.021 µM	LOW
Verapamil	0.01 µM	0.1 µM	1 µM	10 µM	0.07 µM	LOW
Droperidol	31.7 nM	100 nM	317 nM	100 nM	16 nM	INT
Dromperidone	0.003 µM	0.03 µM	0.3 µM	3 µM	0.02 µM	INT
Ondansetron	0.03 µM	0.3 µM	3 µM	30 µM	0.372 µM	INT
Pimozide	0.95 nM	3 nM	9.5 nM	30 nM	0.43 nM	INT
Chlorpromazine	0.095 µM	0.3 µM	0.95 µM	3 µM	0.0345 µM	INT
Clozapine	0.95 µM	3 µM	9.5 µM	30 µM	0.071 µM	INT
Clarithromycin	0.1 µM	1 µM	10 µM	100 µM	1.948	INT
Cisapride	3.17 nM	10 nM	31.7 nM	100 nM	2.58 nM	INT
Terfenadine	1 nM	10 nM	100 nM	1000 nM	0.286 nM	INT
Risperidone	0.01 µM	0.1 µM	1 µM	10 µM	0.032 µM	INT
Ibutilize	0.1 nM	1 nM	10 nM	100 nM	100 nM	HIGH
Dofetilide	0.3 nM	1 nM	3 nM	10 nM	2.0 nM	HIGH
Disopyramide	0.1 µM	1 µM	10 µM	100 µM	0.7 µM	HIGH
Quinidine	0.95 µM	3 µM	9.5 µM	30 µM	3 µM	HIGH
Vandetanib	0.01 µM	0.1 µM	1 µM	10 µM	0.3 µM	HIGH
d,l-Sotalol	0.1 µM	1 µM	10 µM	100 µM	15 µM	HIGH
Bepridil	0.01 µM	0.1 µM	1 µM	10 µM	0.032 µM	HIGH
Azimilide	0.01 µM	0.1 µM	1 µM	10 µM	0.07 µM	HIGH
Astemizole	0.1 nM	1 nM	10 nM	100 nM	0.3 nM	HIGH

KEY
arrhythmia or fibrillation
FPD or BP Prolongation > 20%, but no EAD/Arrhythmia
Signals below detectable threshold

Next step: optical pacing control via ChannelRhodopsin2 mRNA transfection (Xpress.4U)



Optical pacing of ChR2-YFP+ Cor.4U® on Axion Maestro MEA to isolate replorization effects. Top panels, Cor.4U® cardiomyocytes plated on Axion Maestro 48 well plates were transfected with Xpress.4U™ ChR2 mRNA (Ncardia) for artifact-free optical pacing with the Axion Lumos for (here) up to 6 days. Dose and light intensities were adjusted to determine an optimal functional window. **Bottom panels,** Application of FPL 64176 to non-paced cells induced a significant prolongation of BP and FPD relative to the vehicle control (DMSO); whereas pacing at 700 ms BP controls for the influence of beating rate on (and allowing for isolation of) repolarization effects.
Credit: Mike Clements, Daniel Millard, Anthony Nicolini @ Axion

METHODS

CiPA studies: All compounds were tested in blinded fashion using non-cumulative dosing with an incubation time of 30 min. Recordings were taken at baseline, 5, 15, and 30 min after compound addition. Analyses (MEA data) shown in graphs were from 30 min recordings only. For a detailed description of the plating protocols and experimental methods, please contact the author: Greg.Luerman@ncardia.com

ChannelRhodopsin2 Xpress.4U Studies: Studies were performed by Axion Biosystems using the LUMOS light delivery system according to Ncardia's optimized protocol. The Xpress.4U™ ChR2 iPSC transfection kit is available from Ncardia.

CONCLUSIONS

- Cor.4U® cardiomyocytes demonstrated highly predictive capabilities both the CiPA phase I pilot trials as well as the recent phase II validation experiments. Data from CiPA "core sites" are currently under statistical examination and will be presented in a publication to be released in early 2018.
- Pro-arrhythmic activity was identified in all of the "high risk" compounds, many of the "intermediate risk" compounds, and none of the "low risk" compounds.
- Optical pacing of iPSC-CMs using the Xpress.4U™-ChR2 kit presents a simple and easy solution to account for beat-rate dependent effects on FPD prolongation. This permits more mechanistic insight into drug induced effects on cardiomyocytes.

COOPERATION PARTNER

AXION BIOSYSTEMS

For more information please contact:

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