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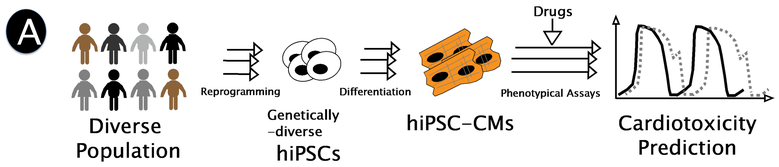
Inter-Individual Differences in Electrophysiology, Pharmacology and Drug Responses in a Cohort of hiPSC-derived Cardiomyocytes: Endorsing Clinical Trials-in-a-Dish

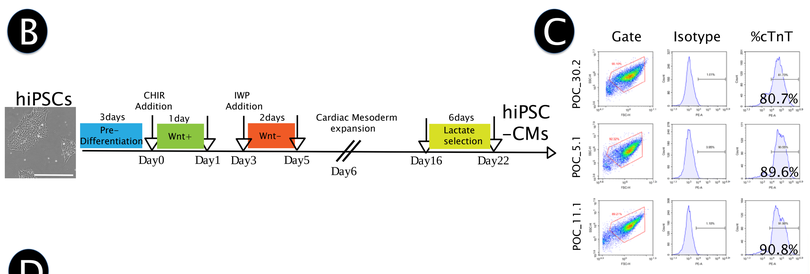
Bernard Fermini, Denise Sullivan, Jerome Chal, Eli Fine, Alexander Ulan Hernandez, Alexia King, Jessica Johnson, Shawn Coyne and Kevin P. Coyne
Coyne Scientific, Atlanta, GA, USA

The concept of "Clinical trials-in-a-dish" or *in vitro* clinical trials, has received significant attention lately because of emerging technologies that allow testing novel compounds on patient cells before moving into actual clinical trials. Moreover, recent studies have shown that individual serious adverse events (SAEs) susceptibility in a population of volunteers with unknown genetic background can be recapitulated in human inducible pluripotent stem cell cardiomyocytes (hiPSC-CMs) derived from these same individuals, providing a proof of concept for *in vitro* preclinical trials. Hence, leveraging genetic diversity in preclinical testing may hold the key to reducing clinical attrition, based on an increased ability to predict SAEs in a population.

In this study, we examined the electrophysiological and pharmacological properties of hiPSC-CMs derived from healthy donors. We first established the full transcriptome profiling of our cell lines, and found inter-line differences in the expression levels of important cardiac markers, and key ion channels. We then defined individual electrophysiological and pharmacological properties of a number of lines by measuring various parameters extracted from extracellular field potential (EFP) and Local Extracellular Action Potential (LEAP) recordings from spontaneously beating cells. Finally, we studied the effects of 3 different CiPA drugs (from all 3 risk categories for arrhythmia liability), on our hiPSC-CMs to establish a pharmacological profile.

Overview of the hiPSC-CM platform





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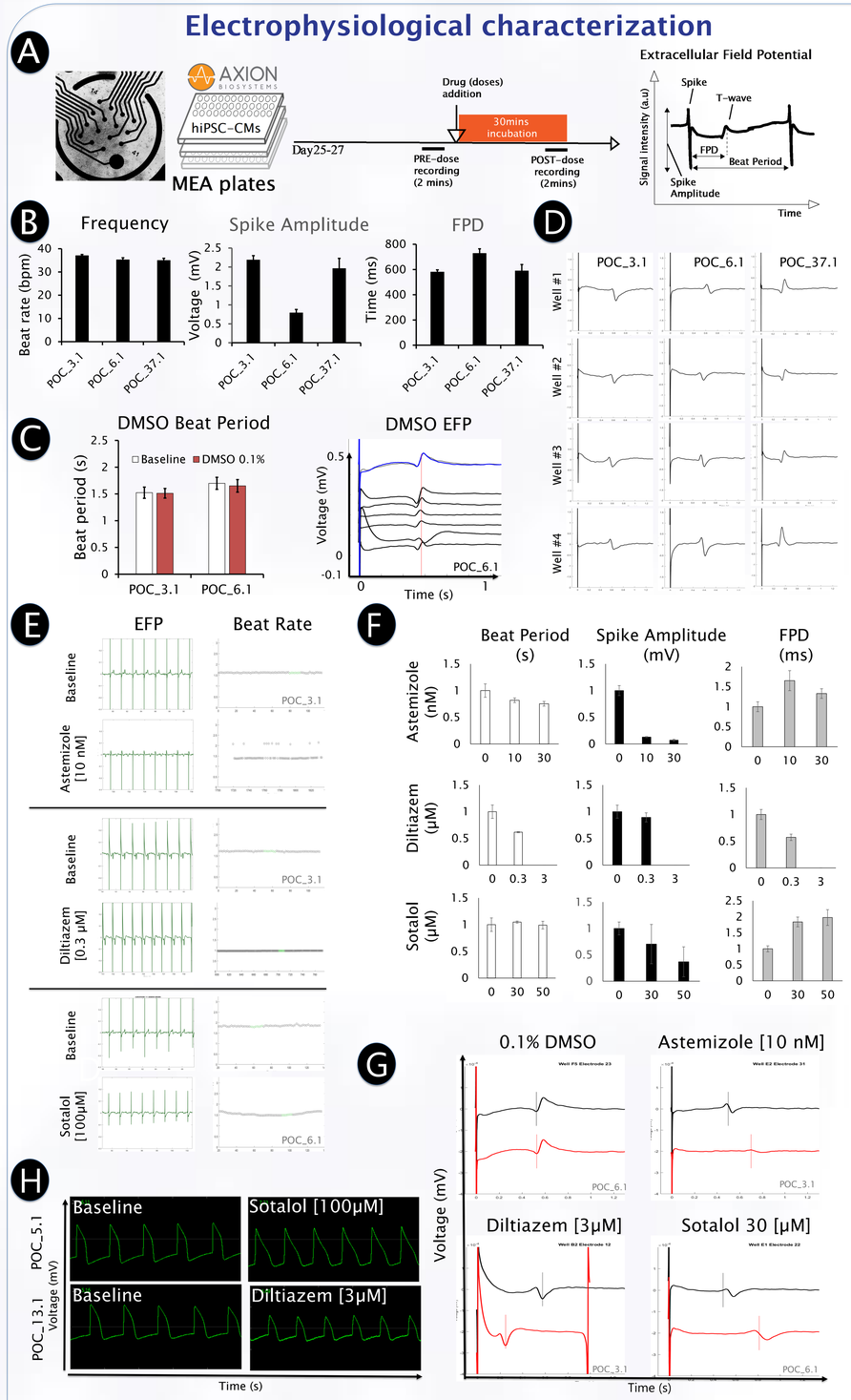
Compound	Action	IC_{50} (μM) *			Expected Effect on FPD	Concentration Tested (μM)
		hERG	Cav1.2	Nav1.5		
Diltiazem	Ca^{2+} channel blocker	13	0.8	22	⇓	0.3, 3
Astemizole	hERG blocker	.004	1.1	3.0	⇑	.01,.03, 0.1
Sotalol	β/K^{+} channel Blocker	111	193	7014	⇑	30, 50, 100

*From: Kramer, J.; Obejero-Paz, C. A.; Myatt, G.et al., MICE Models: Superior to the HERG Model in Predicting Torsade de Pointes. *Sci. Rep.* **2013**, *3*. FPD: Field potential duration.

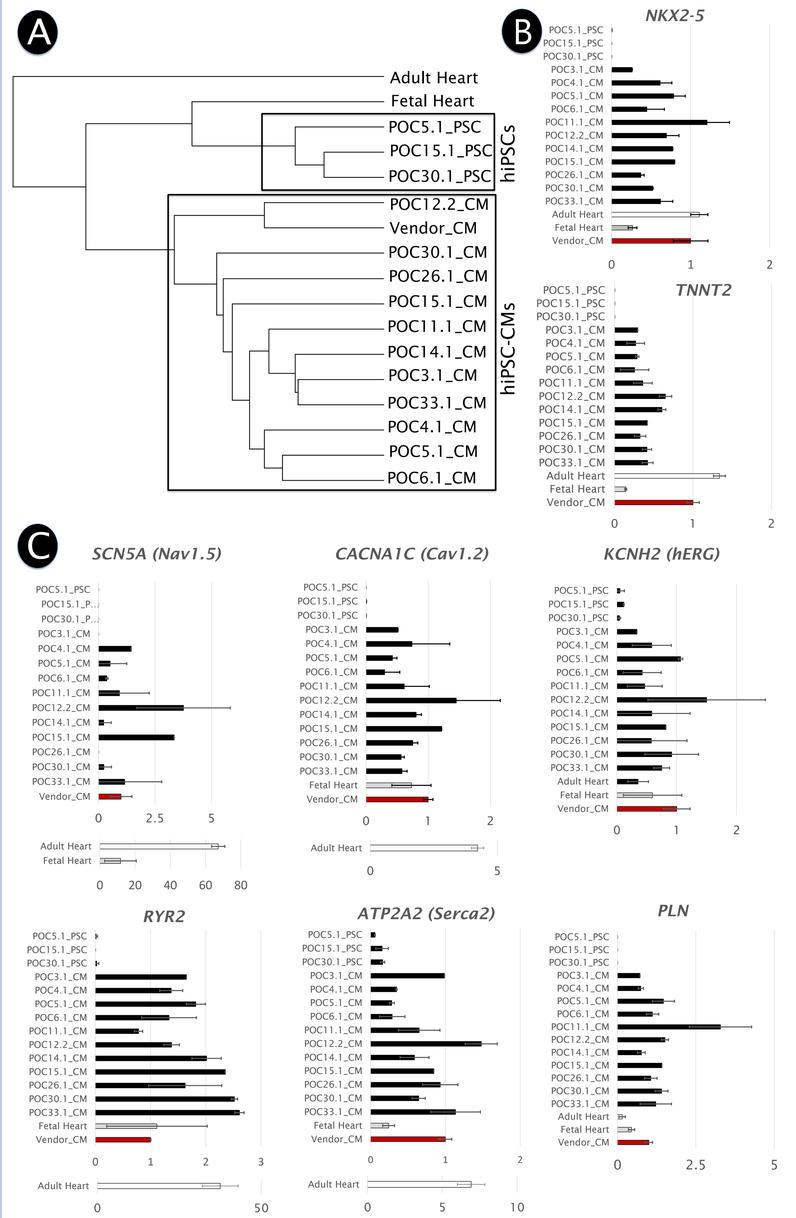
(A) Cohort of hiPSCs was generated from a genetically-diverse pool of donors, differentiated into hiPSC-CMs, and used to define their electrophysiological and pharmacological profile. (B) Directed differentiation protocol for hiPSC-CMs production, adapted from the GiWi protocol of Palecek and colleagues. (C) Representative flow cytometric analysis of cTnT for 3 lines of hiPSC-CMs.(D) Table of compound descriptions and concentrations used to dose hiPSC-CMs.

Electrophysiological characterization



(A) Multi-electrode assay (MEA) experimental timeline and parameters analyzed from the EFP. (B) Baseline beat period of 3 hiPSC-CM lines. (C) (Left) EFP stability of hiPSC-CMs following addition of 0.1% DMSO for 30 min and (right) comparison of EFP traces from 7 different electrodes within a single well (red horizontal line denote -wave landmark). (D) Baseline EFP signal from four different wells for 3 different hiPSC-CM lines. (E) Representative graphs demonstrating the changes in EFP signal and beat period for 2 different hiPSC-CM lines after 30 minute exposure to either Astemizole, Diltiazem or Sotalol. (F) Concentration-response graphs for POC_3.1 for Astemizole, Diltiazem, and Sotalol. (G) Representative EFP traces depicting baseline EFP signals (black) in control (black) and post-compound exposure for 30 minutes (red). (H) Representative LEAP traces of 2 hiPSC-CM lines treated with Sotalol or Diltiazem.

Transcriptome profiling of CoyneSci hiPSC-CM



(A) Whole transcriptome analysis of hiPSCs (PSC) and hiPSC-CMs in comparison to commercially available hiPSC-CMs (Vendor_CM) and human heart tissues. Data were generated by TempO-Seq (BioSpyder) whole transcriptome assay. Dendrogram was generated by maximum pairwise difference clustering. (B) Comparison of expression of cardiac markers and (C) various ion channels and regulators between hiPSCs and hiPSC-CMs. Values are normalized to Vendor CM sample.

Conclusions

Our data demonstrate that hiPSC-CMs derived from healthy donors exhibit electrophysiological and pharmacological profiles that are unique to each donor, resulting in a distribution of drug effects and potencies. Our results show line-specific baseline properties supporting validation and implementation of testing for safety and toxicity of drugs across a cohort of donors, rather than a single cell line, endorsing the concept of Clinical Trials-in-a-Dish.