PC007 Cross-platform validation of axoCells™ hiPSC derived cardiomyocytes as a better human model for pre-clinical cardiotoxicity studies

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Overview and Introduction

Drug-induced arrhythmia has been a major cause of drug development failure and the market withdrawal of novel compounds. Of particular concern is the block of the ion channel IKr (hERG) by drugs which can result in *torsades de pointes* (TdP), a dangerous ventricular fibrillation. Unanticipated toxic effects on contractility such as with the tyrosine kinase inhibitors and anthracyclines are also of increasing concern.

Human iPSC-derived cardiomyocytes (hiPSC-CMs) offer the opportunity to screen drugs *in vitro* using a more physiologically relevant model that expresses multiple ion channels and spontaneously contracts.

Until recently, ICH guidelines have favored animal models and hERG overexpression lines to assess the cardiac safety of investigational new drugs. The Comprehensive in vitro Proarrhythmia (CiPA) initiative utilizes human iPSC-derived cardiomyocytes as a more complex physiologically relevant model to investigate compound effects on multiple ion channels in vitro.

Methods

Maestro Pro MEA – axoCells hiPSC-derived cardiomyocytes were plated onto 48wp Axion MEA plates at 50,000 cells/well following Axol's user guide. The cells were cultured until day 10 before being transferred to the Axion Maestro Pro for recording. The cells were allowed to stabilize for 30 minutes before a 5-minute baseline was recorded. Compound or vehicle control (0.1% DMSO) was added at 10x concentration before 5 minutes test recording was taken. Each condition was then compared and analyzed before and after drug application (min. n=5).

FLEXcyte 96 - The cells were cultured on fibronectin-coated FLEXcyte 96 well plates in 200 µL cardiac maintenance medium per well. Cells were seeded approximately 6 days before compound treatment at 65,000 cells/well. A final media change was conducted 4-6 hours before drug application. Compounds were added at 4x concentration, resulting in the desired final compound concentration. The FLEXcyte 96 enables the analysis of a number of contractility parameters including contractile force, beat rate, and beat duration.

CellOPTIQ – axoCells hiPSC-derived cardiomyocytes were plated on 96-well plastic bottom plates (50,000 cells/well) following Axol 's user guide. On day 6 the media was replaced with Fluorobrite serum-free (SFM) media and the cells were loaded with the Fluovolt voltage-sensitive dye (1:2000, 25min, 37°C). Cells were maintained in this media for the duration of the experiments. Recordings were made for up to 10 seconds/well on the CellOPTIQ® platform (Sampling Rate 10kHz). Dofetilide (3nM) and 0.1% DMSO were used as controls (min. of n=5) . Treatment recordings were obtained following 30mins after compound addition. Data was analyzed with CellOPTIQ® and plotted using Report software. Graphs are displayed as % change to vehicle control.

Characterization (ICC & RNAseq)

Once matured (7-14 days post-thaw), immunocytochemistry expression of key cardiac markers including cardiac troponin T (*TNNT2*), alpha-actinin (*ACTN2*), beta myosin heavy chain (*MYH7*) and cardiac alpha-actin (*ACTC1*)

RNA seq (data available but not shown) shows expression of hERG1 (*KCNH2*), the cardiac ion channel responsible for repolarization of the cardiac action potential. *KCNH2* was expressed four-fold higher compared to human protein atlas transcriptomic analysis of human heart samples.

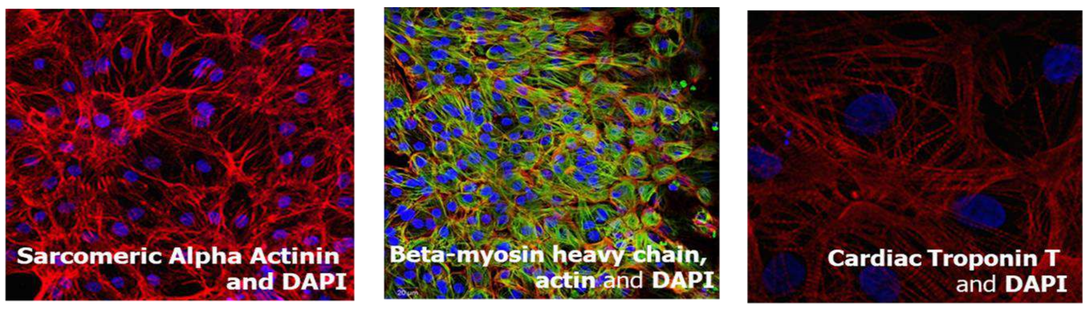

Figure 1. Immunocytochemistry for cardiac-specific markers.
axoCells cardiomyocytes fixed and stained 7 days post-thaw (d25). (**A**) Sarcomeric alpha-actinin (**red**), DAPI. 20x magnification. (**B**) Beta myosin heavy chain (**green**), actin (**red**), DAPI. 20x magnification. (**C**) Cardiac Troponin T (**red**), DAPI. 40x magnification.

MEA (Electrophysiology)

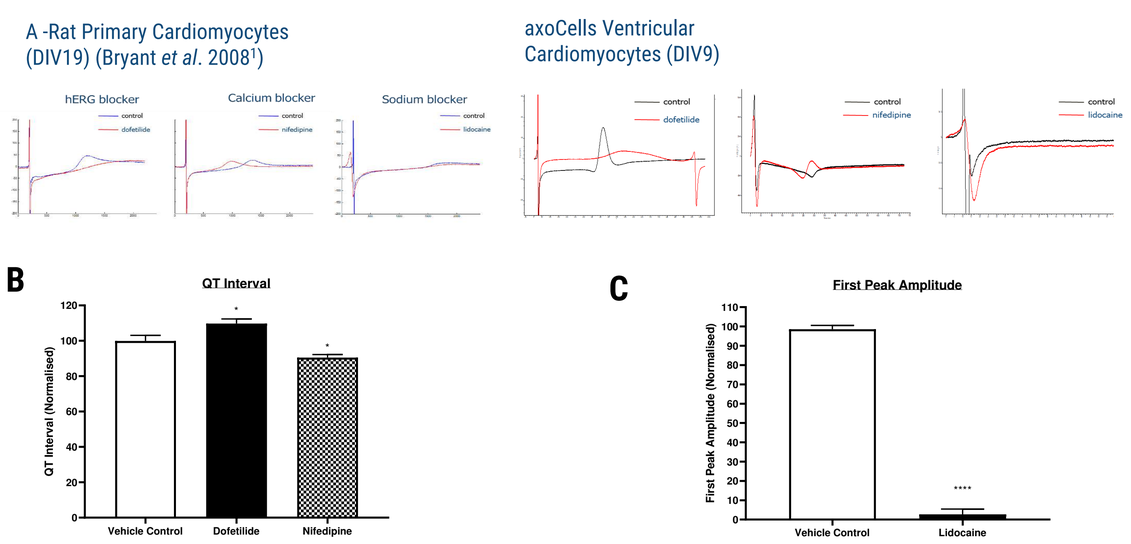

Figure 2. MEA recording demonstrates standard cardiac pharmacological responses.
axoCells ventricular cardiomyocytes (ax2508) produce the predicted responses to key cardiac blockers, comparable to reference data obtained from embryonic rat ventricular cardiomyocytes (**A**). Axol's cells produced the expected Field Potential Duration (FPD) prolongation (**B**) and arrhythmias with dofetilide (n=5), the expected FPD shortening with nifedipine (n=7), and the expected First Peak and Second Peak Amplitude reduction due to lidocaine block (n=8) (**C**) when compared to vehicle control (n=9).

CellOPTIQ (Voltage Sensitive Dye)

Data for all 28 CiPA compounds tested is available upon request.

Ranolazine

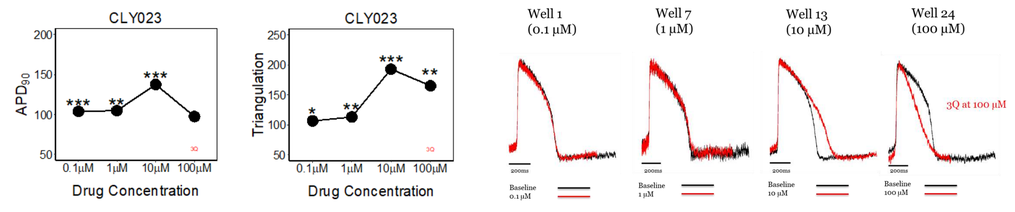

Figure 3. Ranolazine – multiple ion channel effects. Ranolazine, a low TdP risk, multiple ion channel effects compound at 10 µM prolonging the APD (hERG block) but at 100 µM, showed TRise increases (early Na^+ channel block) consistent with the documented ion channel IC50s for this drug.

Azimilide

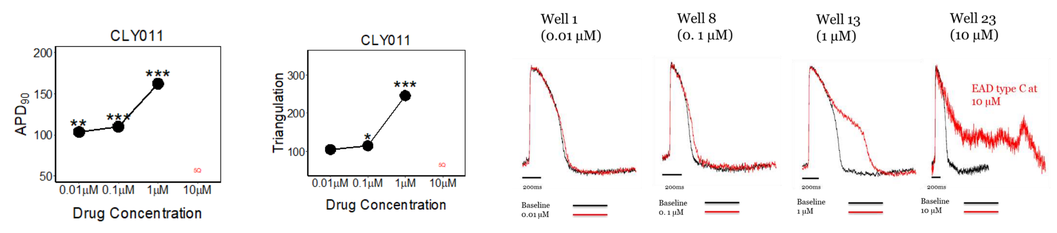

Figure 4. Azimilide – blocker of I_{kr} (hERG) and I_{ks} currents. 0.01 µM Azimilide addition induced APD_{90} prolongation. At 10 µM, this compound led to early afterdepolarizations, highlighting the high TdP risk of this compound.

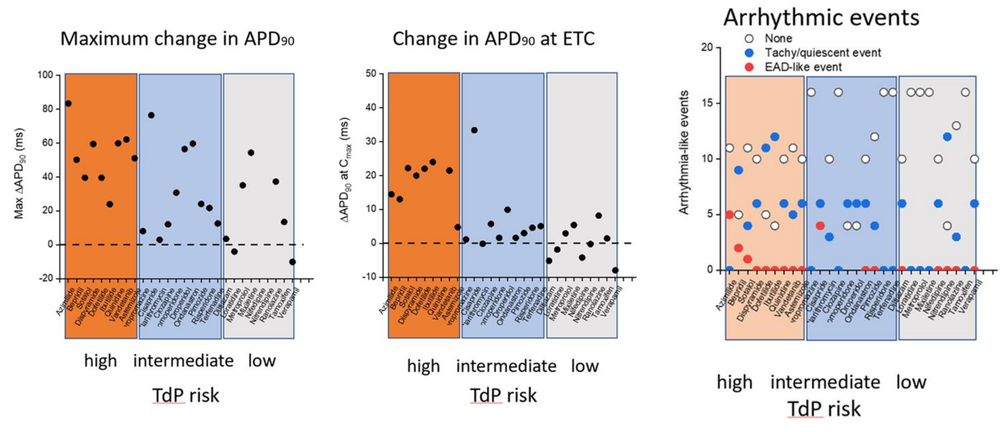

Figure 5. hiPSC-derived ventricular cardiomyocytes exhibit a graded response according to known proarrhythmic risk.
Taking into account three proarrhythmic predictors - APD_{90} change at maximal concentration, APD_{90} change at therapeutic concentration, and any occurrence of arrhythmic events, the hiPSC-derived cardiomyocytes provided a graded response to the 28 CiPA compounds according to known proarrhythmic risk

FLEXcyte 96 (Contractility)

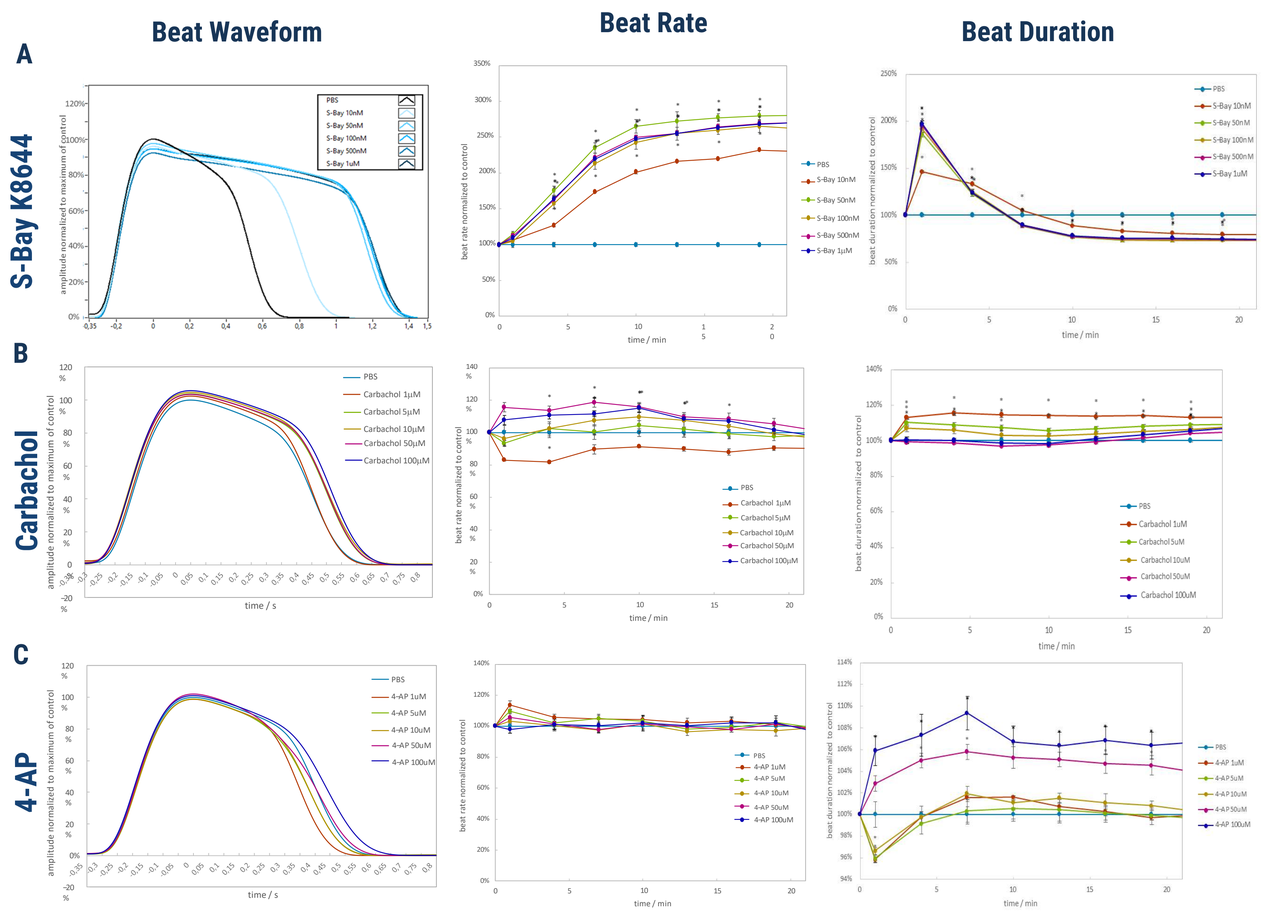

Figure 6. axoCells ventricular cardiomyocytes correctly reproduce the complex effects of standard compounds on primary cardiac cells.
The FLEXcyte 96 allows the measurement of the contractility of hiPSC-derived cardiomyocytes. (**A**) The L-type calcium channel agonist S-Bay K8644 is expected to increase beat duration and produce a positive inotropic effect by inducing calcium release from internal sarcoplasmic reticulum stores. The transiently increased beat duration is probably due to the depletion of internal calcium stores. (**B, C**) In contrast, carbachol, which activates the atrial-specific acetylcholine-activated inward-rectifying potassium current and 4-AP, an inhibitor of the atrial-specific ultra-rapid delayed rectifier potassium channels had only limited, transient effects on the axoCells ventricular cardiomyocytes highlighting how chamber-specific pharmacology can be modeled with hiPSC-derived cardiomyocytes.

Conclusion

Axol's axoCells ventricular cardiomyocytes have been demonstrated to express the correct markers by ICC and RNAseq and their appropriate electrophysiology, contractility and pharmacology has been demonstrated across three significant platforms, assessing a range of different cardiac functions.

These results show that axoCells ventricular cardiomyocytes can provide a reliable, human, physiologically-relevant and chamber-specific model to perform functionality, drug screening and cardiotoxicity studies over multiple platforms in a short time period and without using animal tissues.

References and further information

1. A novel analysis method for characterising in vitro cardioactive drug effects (2008) S. Bryant, S. Broadbent, C. Wyllie, W. Kotiadis, A. Parsons, R. Heal, J. Demmon & S. Nicol *Journal of Pharmacological and Toxicological Methods* Volume 58, Issue 2, Sept–Oct 2008, p.149

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