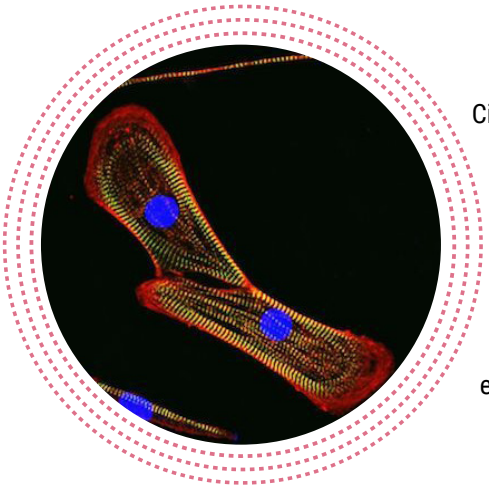
Human iPSC-Derived **Cardiomyocytes**



CiPA validation of Axol's human iPSC-derived ventricular cardiomyocytes now gives scientists added confidence in the use of these cells as *in vitro* screening tools for cardiotoxicity.

Axol's hiPSC-derived cardiomyocytes are capable of correctly detecting action potential duration (APD) prolongation and triangulation associated with hERG block and torsades de pointes (TdP) risk as validated against the CiPA panel of compounds and represents a robust and reliable human cellular model for use in cardiotoxicity testing, drug screening and electrophysiology applications.

Human iPSC-Derived Ventricular Cardiomyocytes

PRODUCT CODE	PRODUCT NAME	DONOR	STARTING MATERIAL	QUANTITY
ax2508	Human iPSC-Derived Ventricular Cardiomyocytes (Male)	74 years	Fibroblasts	1 million cells per vial
ax2500	Human iPSC-Derived Ventricular Cardiomyocyte Kit (Male)	74 years		1 kit (1 million cells)

Human iPSC-Derived Atrial Cardiomyocytes

PRODUCT CODE	PRODUCT NAME	DONOR	STARTING MATERIAL	QUANTITY
ax2518	Human iPSC-Derived Atrial Cardiomyocytes (Male)	74 years	Fibroblasts	1 million cells per vial

Human iPSC-Derived Cardiomyocytes Culture Media and Reagents

PRODUCT CODE	PRODUCT NAME	QUANTITY
ax2530-500	Cardiomyocytes Maintenance Media	500 mL
ax0049	Fibronectin	1 mL

Why CiPA?

The CiPA (Comprehensive *in vitro* Pro-Arrhythmia Assay) initiative, is a novel strategy for assessing the cardiac safety of compounds in vitro using hiPSC-derived cardiomyocytes and involves a standardized set of compounds (see table 1) and tests to enable better prediction of pro-arrhythmia risk of compounds using human iPSC-derived cardiomyocytes.

One such test, using voltage sensitive dyes (VSD) and performed by Clyde Biosciences (the only validated CRO for CiPA), was used with Axol's ventricular hiPSC-derived cardiomyocytes¥.

High TdP Risk	Intermediate TdP Risk	Low TdP Risk
Azimilide	Astemizole	Diltiazem
Bepridil	Chlorpromazine	Loratadine
Dofetilide	Cisapride	Metoprolol
Ibutilide	Clarithromycin	Mexiletine
Quinidine	Clozapine	Nifedipine
Vandetanib	Domperidone	Nitrendipine
Disopyramide	Droperidol	Ranolazine
D,l Sotalol	Terfenadine	Tamoxifen
	Pimozide	Verapamil
	Risperidone	
	Ondansetron	

Table 1. Proarrhythmia clinical risk categorization: Three tier ranking of TdP (CiPA 28 compounds)

Materials and Methods

Axol's iPSC-derived ventricular cardiomyocytes (ax2508) were grown for 6 days, inclubated with VSD in serum free media and treated with compounds at four concentrations (n=6). Vehicle/negative control was DMSO at 0.1%. Positive control was Dofetilide at 3nM. Recordings were obtained at 0.5h incubation time after drug addition. Data are presented as mean ± SEM. Statistical data are based on differences between absolute values and recorded as: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. 3Q, 6Q etc. refers to the number of quiescent wells. ¥ Data with all 28 compounds available on request.

Results

Test compound: Dofetilide (high TdP risk), 0.3nM – 10nM, classic hERG channel blocker.

Results: Strong hERG block demonstrated even at lowest concentrations. No early after deplorizations (EADs) but quiescent cells at higher concentrations (Figure 1).

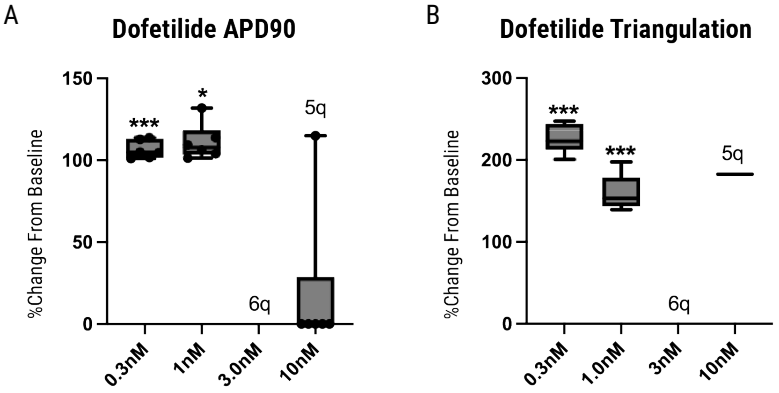


Figure 1. Dofetilide impacts iPSC-derived cardiomyocyte action potential kinetics. *A) percentage change in APD90 by increasing concentrations of Dofetilide. B) percentage change in waveform triangulation at increasing concentrations of Dofetilide.*

Test compound: Ranolazine (low TdP risk), 0.1µM - 100µM, multiple ion channel effects (early Na^+, late Na^+ and hERG ion channels).

Results: At 10µM the APD is prolongated (hERG block). At 100µM, TRise increases (early Na^+ channel block) (Figure 2).

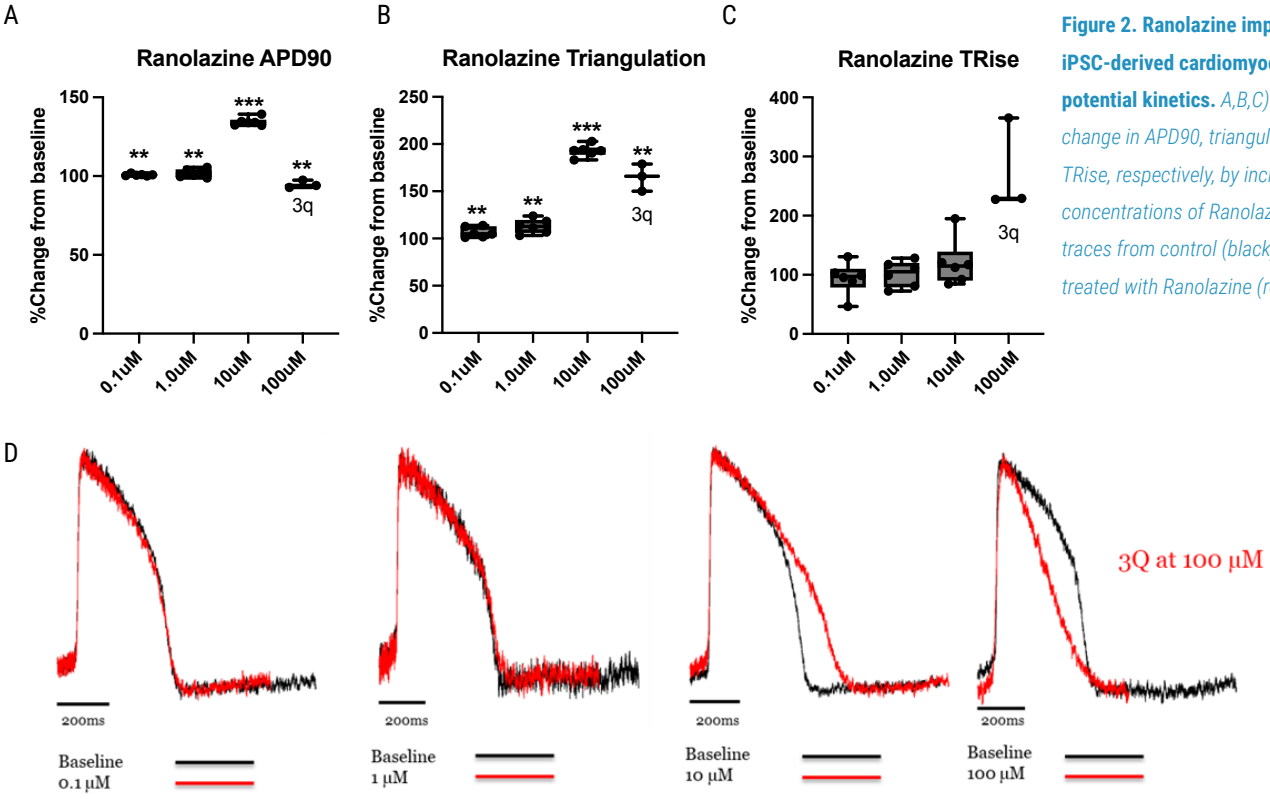


Figure 2. Ranolazine impacts iPSC-derived cardiomyocyte action potential kinetics. *A,B,C) percentage change in APD90, triangulation and TRise, respectively, by increasing concentrations of Ranolazine. D) raw traces from control (black) and cells treated with Ranolazine (red).*

Test compound: Verapamil (low TdP risk), 0.001 µM - 1 µM, calcium channel blocker.

Results: APD prolongation and increased triangulation at 0.001 µM (hERG block), APD shortening at 0.1 µM (L-type Ca^{2+} channel block) (Figure 3).

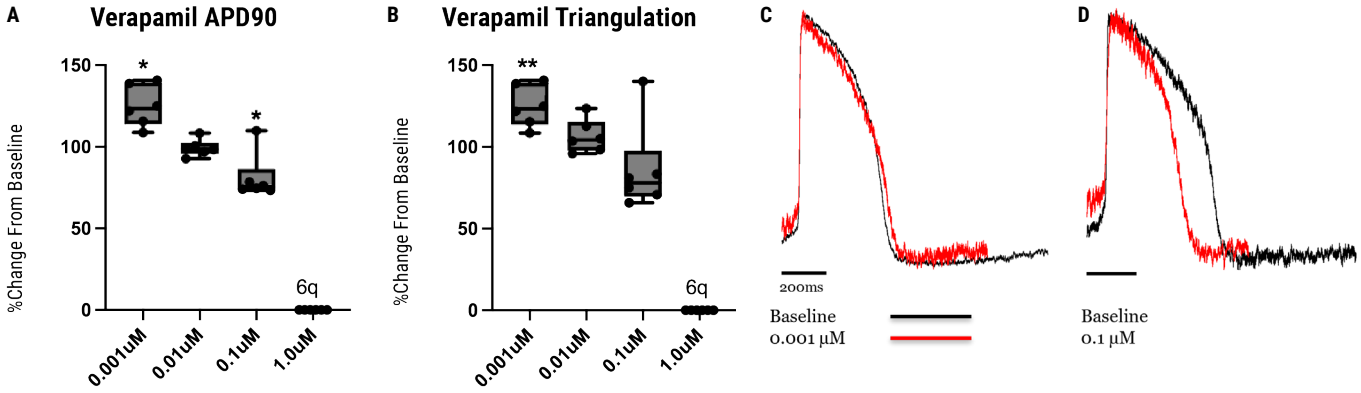


Figure 3. Verapimil impacts iPSC-derived cardiomyocyte action potential kinetics. *A) percentage change in APD90, and B) percentage change in triangulation, at increasing concentrations of Verapamil. C and D) raw traces from control (black) and cells treated with Verapamil (red).*

Transcriptomic Validation of Our Lines

Axol Bioscience has performed in-depth RNA-sequencing analysis and characterization of our ventricular (ax2508) and atrial (ax2518) cardiomyocytes and compared data to reference adult heart transcriptome (Heart-HPA, Heart-Gtex) as well as iPSCs (ax7018) and NSCs (ax0018) from the same donor (Figure 4).

Once matured (14 DIV), atrial cardiomyocytes express a greater amount of key atrial markers such as the KCNA5 (Kv1.5 channel) and SIRPA, compared to ventricular cardiomyocytes.

Likewise, our ventricular cardiomyocytes show higher expression of the ventricular isoform of myosin light chain 2 (MYL2) as well as myosin heavy chain 7 (MYH7), a sarcomeric protein found predominantly in the ventricle.

The pan-cardiac marker cardiac troponin T (TNNT2), encoding a key sarcomeric protein, is highly expressed across both hiPSC-derived cardiomyocyte products, highlighting the purity of our cardiomyocyte differentiation (>85% purity).

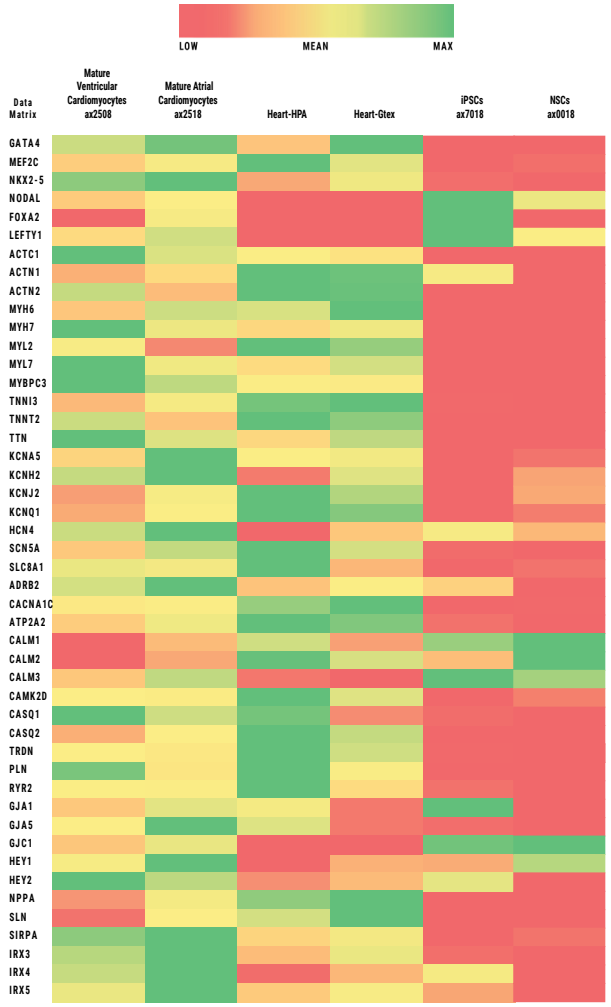


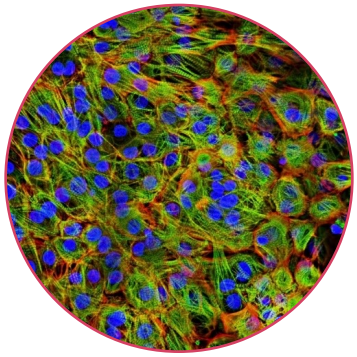
Figure 4. RNA-seq analysis of Axol's hiPSC–derived mature ventricular and atrial cardiomyocytes

Why hiPSC-Derived Cardiomyocytes?

The use of Axol's atrial cardiomyocytes (ax2518) and CiPA-validated iPSC-derived ventricular cardiomyocytes (ax2508) as *in vitro* research models present a major advantage over primary cells in that they provide a continuous source of cardiomyocytes from the same genetic background for use in multiple experiments.

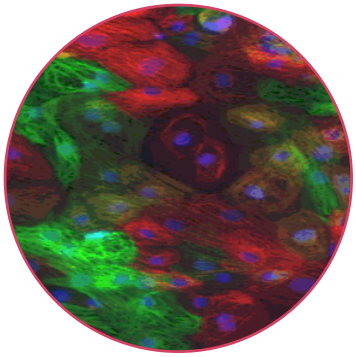
Axol's cardiomyocytes have been extensively characterized by immunocytochemistry of key markers, electrophysiological profiling of action potentials by microelectrode arrays (MEA) and transcriptomic validation by RNS sequencing (see figure 4).

Beta myosin heavy chain, Actin, DAPI



ax2508 Human iPSC-Derived Ventricular Cardiomyocytes (Male)

MCL2v, MCL2a, DAPI



ax2518 Human iPSC-Derived Atrial Cardiomyocytes (Male)

ax2530-500 Cardiomyocytes Maintenance Media (500 mL)

Quality Driven Production

Axol's ISO 9001 certification provides confidence that quality requirements in all our iPSC products are fulfilled. A minimum of 5 testing events are carried out on every product during QC testing, including sterility and mycoplasma testing by qPCR.

Structural markers specific to Axol's hIPSC ventricular and atrial cardiomyocytes are determined by immunocytochemistry and RNA sequencing, while functional responses on MEA and automated patch clamp may also be recorded.

Each cardiomyocyte production batch undergoes 14 separate tests, including morphology and cellular contraction studies before release, ensuring consistent quality and reproducibility for all drug screening and cardiotoxicity testing needs.

Figure 5. ***QC testing of iPSC-derived cardiomyocytes.*** *Population check by morphology and cellular contraction.*

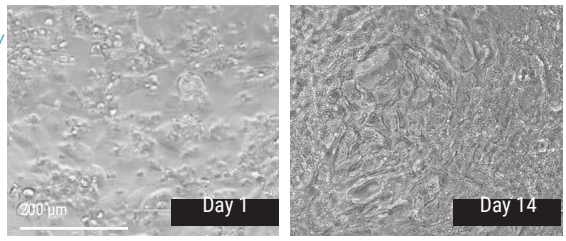


Figure 6. ***QC testing of iPSC-derived cardiomyocytes by Immunocytochemistry.*** *Cells are assessed for cardiac cell markers.*