

Physiologically relevant iPSC-derived cells and specialist services

In vitro cardiotoxicity: External validation of axoCells™ ventricular cardiomyocytes against CiPA cardiotoxicity compound panel

Axol Bioscience Limited
Roslin Innovation Centre, Charnock Bradley Building,
Easter Bush Campus,
Easter Bush, EH25 9RG, United Kingdom

Phone: +44-131 651 9710
www.axolbio.com
operations@axolbio.com

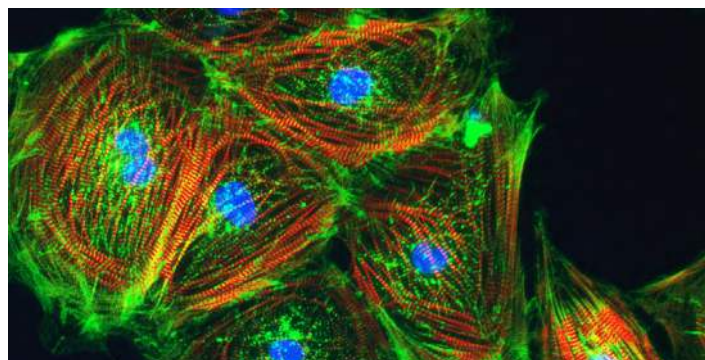
Background

Cardiotoxicity is responsible for one-third of regulatory clearance failures, placing it amongst the biggest challenges in drug development¹. axoCells human iPSC-derived ventricular cardiomyocytes have been developed to create a robust human-relevant model for cardiotoxicity testing.

Cardiac safety pharmacology has encountered well-characterized challenges, with fatal arrhythmias such as Torsades de Pointes (TdP) responsible for 14 major drug withdrawals^{2,3}. To screen for this, a range of cardiotoxicity models have been used including *in vivo* (animal) models, primary cell models (using cells taken directly from humans or animals), and *in vitro* immortalized cells transfected with ion channels. While these models have provided some value, there is still a translational gap between the “bench” and the “clinic”, highlighting the need for more human-relevant model systems that can better translate to humans.

In vivo cardiotoxicity models have been reported to offer only partial insights into how potential drugs interact⁴. Equally, *in vitro* models made from transfecting cell lines with a single ion channel gene also offer limited insights, as they can miss other important ion channel effects that may compensate for those single ion channel effects^{1,3}.

An improvement on *in vitro* single channel models may be found in “multi-channel” models, which better represent the complex ion channel activity driving action potentials in the human heart. These models can display the characteristic initial depolarization predominantly due to early sodium channels, plateau phase from a balance of calcium influx and potassium efflux, and rapid repolarization from potassium channels (primarily the hERG channel) with hERG blockade leading to action potential duration (APD) prolongation and arrhythmia. Recapitulating this interplay is essential for a physiologically relevant model.



About the Comprehensive Pro-Arrhythmia Assay (CiPA) initiative

The initiative was launched in 2013 to develop a more specific assay for the proarrhythmic potential of new drugs, compared to the hERG assay and Thorough QT study. The combination of human cardiomyocytes, *in silico* technologies and multichannel platforms enabled improved predictivity of TdP risk and has been used to inform regulatory requirements for measuring cardiotoxicity liabilities. Read more about it here: <https://cipaproject.org>

The importance of a multi-channel model is best illustrated with the drug verapamil, a widely used compound that causes hERG blockade, and therefore would fail a single-channel hERG block model. However, multi-channel effects (predominantly on calcium channels) compensate for the APD prolongation, preventing the appearance of arrhythmia.

Therefore, it is vital to screen compounds on more relevant model systems. The Comprehensive Pro-Arrhythmia Assay (CiPA) initiative was developed as a more specific test of the pro-arrhythmic potential of new drugs and employs multi-channel readings. The assay uses twenty-eight drugs of known TdP risk, outlined in the table below.

	High TdP risk	Intermediate TdP risk	Low TdP risk
Training set	Bepidil Dofetilide Quinidine Sotalol	Chlorpromazine Cisapride Terfenadine Ondansetron	tiazem Mexiletine Ranolazine Verapamil
Validation	Azimilide Ibutilide Vandetanib Disopyramide	Astemizole Clarithromycin Clozapine Domperidone Droperidol Pimozide Risperidone	Loratidine Metoprolol Nifedipine Nitrendipine Tamoxifen

Figure 1. The twenty-eight compounds that form the panel for CiPA testing of cardiomyocytes.

With this panel of compounds, iPSC-derived cardiomyocytes can be used on various platforms to assess compound cardiotoxicity liability. In this study, we sought a third-party provider to test axoCells ventricular cardiomyocytes against all 28 compounds as an external validation of the model for cardiotoxicity screening.

Materials and methods

axoCells iPSC-derived ventricular cardiomyocytes (ax2508) were delivered to Clyde Biosciences for an external assessment measuring the cell's response to the 28 CiPA compounds using their CelloPTIQ® platform system.

Briefly, cells were grown for 6 days, incubated with voltage-sensitive dyes (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.

Results

External validation demonstrated an expected response to all 28 compounds in the CiPA panel. Responses were recorded at several different concentrations for each compound and assessed for evidence of arrhythmia and quiescence (denoted Q on the traces).

Data for all 28 compounds is available on request, with the results from three key compounds shown below.

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

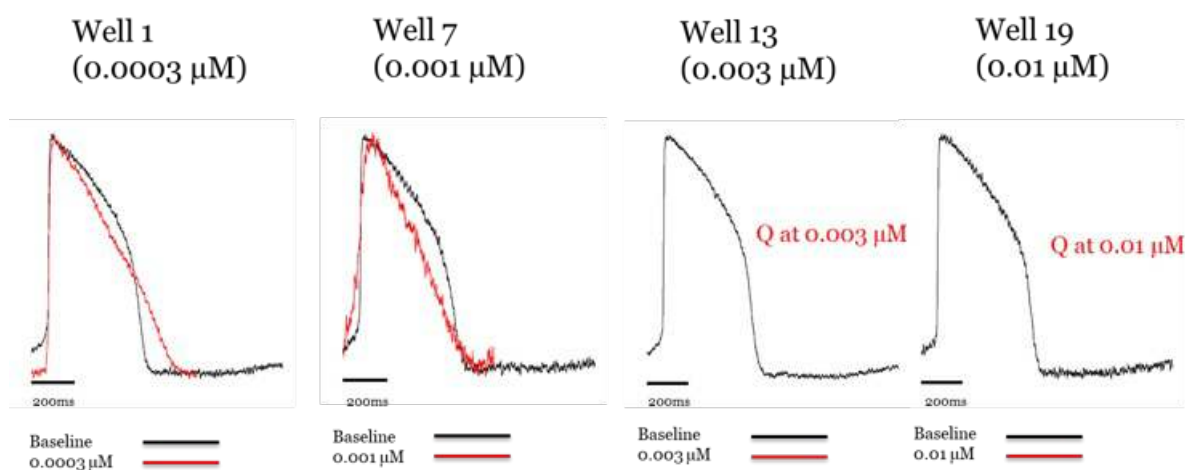


Figure 2. Addition of classic hERG blocker dofetilide to axoCells ventricular cardiomyocytes produces hERG blockade at low concentrations (demonstrated by triangulation of the action potential), with quiescence at high concentrations. Raw trace comparing control (black) to treatment with dofetilide (red).

Results: Strong hERG block demonstrated even at lowest concentrations. No early after depolarizations (EADs) but quiescent cells at higher concentrations (Figure 2).

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

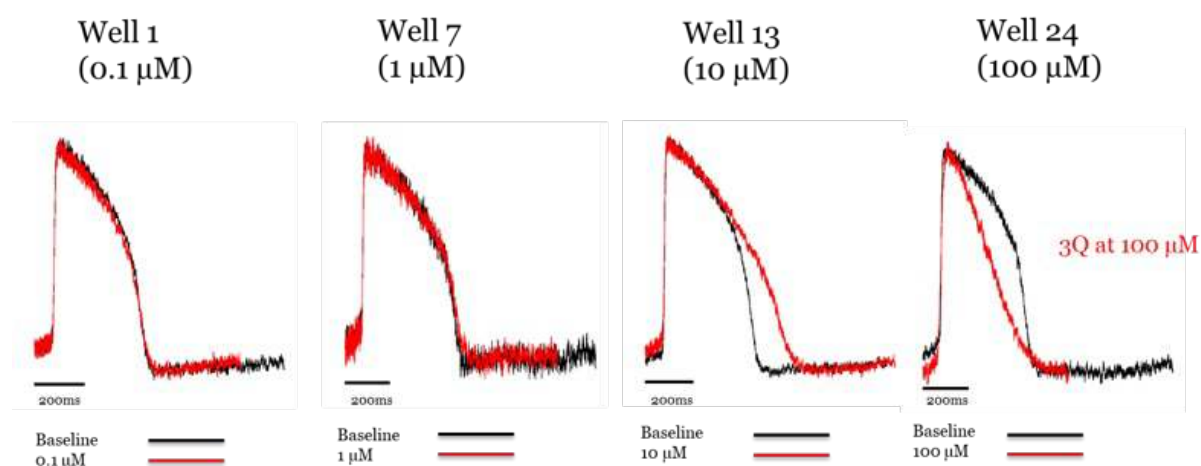


Figure 3. Addition of ranolazine to axoCells ventricular cardiomyocytes produces hERG blockade at 10 μM . Raw trace comparing control (black) to treatment with ranolazine (red).

Results: At 10 μM the APD is prolonged (hERG block). At 100 μM , there is evidence of triangulation with some wells showing quiescence (early Na^+ channel block) (Figure 3).

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

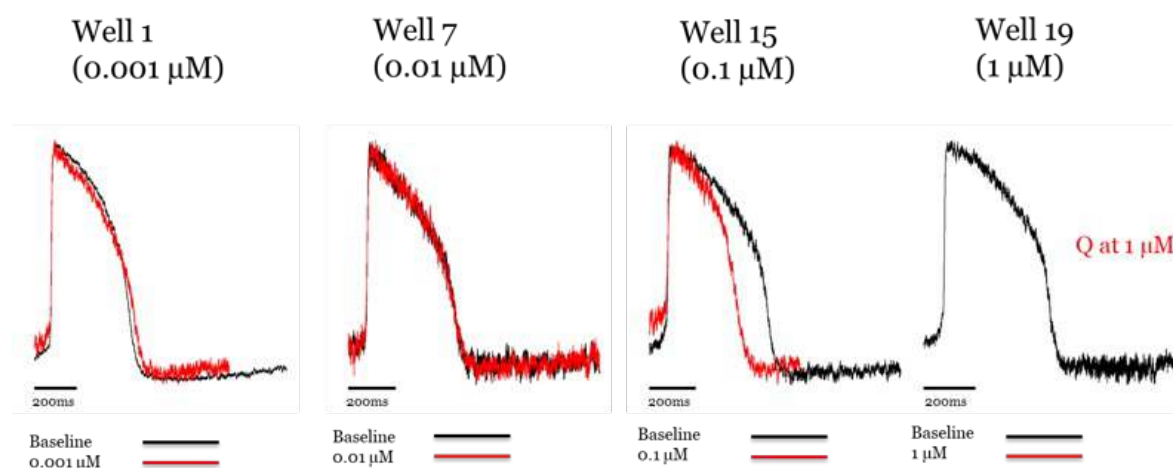


Figure 4. Addition of verapamil to axoCells ventricular cardiomyocytes produces hERG blockade at low concentrations, with L-type Ca^{2+} channel block at higher concentrations. Raw trace comparing control (black) to treatment with verapamil (red).

By assessing maximal change in action potential duration at 90% depolarization (APD_{90}), change in APD_{90} at therapeutic concentrations and evidence of arrhythmic events, all 28 CiPA compounds show a graded response according to their torsadogenic risk (Figure 5). That is to say, there is a greater change in action potential duration or evidence of early-afterdepolarizations and quiescent events in drugs with a higher known risk of causing TdP.

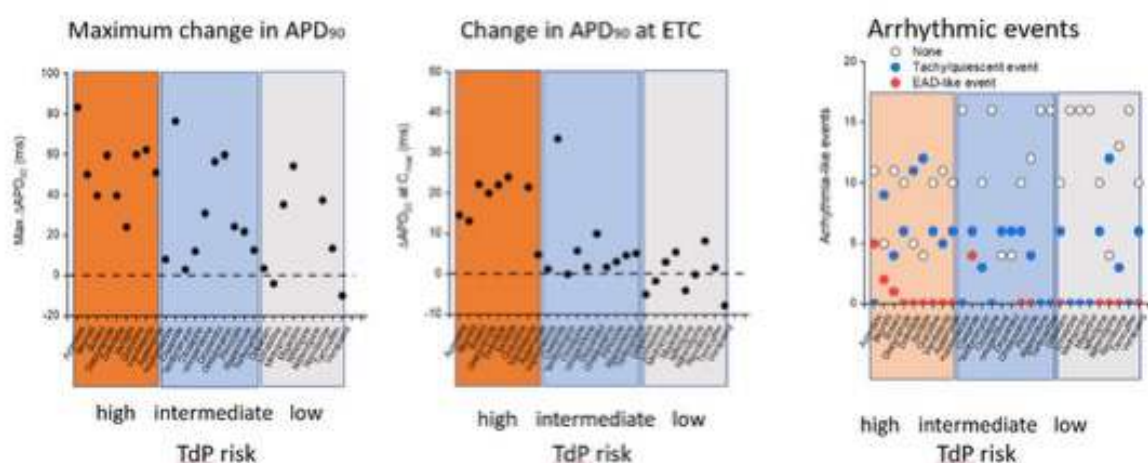


Figure 5. Summary data for all 28 CiPA compounds showing (left) maximal change in APD_{90} , (centre) change in APD_{90} at therapeutic concentrations, and (right) evidence of any arrhythmic events including EADs and quiescent events.

Discussion

An external partner was sought to provide an independent assessment of axoCells ventricular cardiomyocytes for *in vitro* cardiac safety screening. The cardiomyocytes were tested against the 28 compounds of known torsadogenic risk that make up the CiPA assay, with responses at increasing concentrations compared against the known compound effects.

axoCells ventricular cardiomyocytes demonstrated an expected pharmacological response in response to all 28 CiPA compounds, thereby validating them for use in cardiotoxicity screening models.

axoCells hiPSC-derived ventricular cardiomyocytes express a number of key ion channels and can respond to calcium channel blockers such as nifedipine and sodium channel blockers such as terfenadine. In particular, prolongation of the action potential duration, a proxy for QT prolongation, could be seen in response to hERG block.

Crucially, a graded response was seen according to the risk profile of known compounds. axoCells ventricular cardiomyocytes can therefore be used to screen for hERG block and associated TdP risk.

In conclusion, axoCells ventricular cardiomyocytes have applications for *in vitro* cardiac safety testing and detection of pro-arrhythmic risk of new compounds.

References

- 1 Francis Grafton, Jaclyn Ho, Sara Ranjbarvaziri, Farshad Farshidfar, Anastasiia Budan, Stephanie Steltzer, Mahnaz Maddah, Kevin E Loewke, Kristina Green, Snahel Patel, Tim Hoey, Mohammad Ali Mandegar (2021) Deep learning detects cardiotoxicity in a high-content screen with induced pluripotent stem cell-derived cardiomyocytes eLife 10:e68714 doi: <https://doi.org/10.7554/eLife.68714>
- 2 DiMasi JA, Grabowski HG, Hansen RW. Innovation in the pharmaceutical industry: New estimates of R&D costs. J Health Econ. 2016 May;47:20-33. doi: <https://doi.org/10.1016/j.jhealeco.2016.01.012>. Epub 2016 Feb 12. PMID: 26928437.
- 3 Blinova K, Dang Q, Millard D, Smith G, Pierson J, Guo L, Brock M, Lu HR, Kraushaar U, Zeng H, Shi H, Zhang X, Sawada K, Osada T, Kanda Y, Sekino Y, Pang L, Feaster TK, Kettenhofen R, Stockbridge N, Strauss DG, Gintant G. International Multisite Study of Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes for Drug Proarrhythmic Potential Assessment. Cell Rep. 2018 Sep 25;24(13):3582-3592. doi: <https://doi.org/10.1016/j.celrep.2018.08.079>. PMID: 30257217; PMCID: PMC6226030.
- 4 Pognan, F., Beilmann, M., Boonen, H.C.M. et al. The evolving role of investigative toxicology in the pharmaceutical industry. Nat Rev Drug Discov 22, 317–335 (2023). <https://doi.org/10.1038/s41573-022-00633-x>

Axol Bioscience

Axol Bioscience is a leading provider of human induced pluripotent stem cell (iPSC) technologies, specializing in the manufacture of high-performance iPSC-derived cells and the delivery of industry-leading laboratory services. With over a decade of expertise, Axol partners with global pharmaceutical companies, contract research organizations (CROs), biotechnology firms, start-ups, and academic institutions to advance the use of iPSC-based models in drug discovery and disease research.

The company operates across four key areas, neuroscience - including ALS, Alzheimer's, Huntington's, Parkinson's - neuroinflammation, ophthalmology dermatology and cardiovascular.

Axol offers scalable solutions including iPSC banking, reprogramming, gene editing, cell manufacturing, and quality control. The company is committed to closing the translational gap and developing physiologically relevant *in vitro* systems that improve consistency and relevance in human disease modeling and compound testing.

Axol is recognized for its scientific excellence, transparent collaboration and rigorous quality standards. Manufacturing operations are ISO 9001:2015 certified.

Axol Bioscience Limited
Roslin Innovation Centre, Charnock Bradley Building,
Easter Bush Campus,
Easter Bush, EH25 9RG, United Kingdom
UK & Europe phone: +44-131 651 9710



www.axolbio.com
operations@axolbio.com