A new wave of arrhythmia assays

By C. Simone Fishburn, Senior Editor

Cardiotoxicity testing has plagued drug developers for years as the principal preclinical test, the hERG assay, and the primary clinical marker, prolonged QT, are relatively poor predictors of proarrhythmic potential. Now, information about the different ion channels present in the heart and how their interactions with drugs affect the cardiac action potential has opened the door for a wave of new preclinical assays to assess the arrhythmia risk of candidate therapeutics.

The FDA signaled its willingness last month to embrace a new suite of preclinical assays as replacements for both potassium channel Kv11.1 (KCNH2; hERG) testing and thorough QT (tQT) clinical studies. Key stakeholders from the FDA, industry, academia and the investment space met to discuss advances in research and technology that might form the basis of the new recommendations.¹

Since the existing guidelines were introduced in 2005, three particular developments have gained sufficient traction to be included in the new proposals: screening compounds for blockade of multiple cardiac ion channels rather than only for hERG, using cardiomyocytes rather than artificial cellular expression systems such as Chinese hamster ovary (CHO) cells and using *in silico* modeling to simulate how the preclinical findings will affect cardiac function in patients.

The FDA has set a timeline of three years to introduce the new guidelines for preclinical assessment. Although the race is on to select and validate the new assays, the field continues to make breakthroughs that could quickly supersede whatever assays are chosen.

Emerging technologies include patient-derived induced pluripotent stem (iPS) cells that give rise to organ cultures and improved *in silico* simulations with more sophisticated predictive algorithms.

Both sets of advances could ultimately lead to personalized cardiotoxicity testing suited to specific patient subpopulations.

Celling points

Perhaps the most active area in preclinical proarrhythmia research is the creation of improved cell-based systems for testing how candidate compounds affect cardiac function.

Currently, small molecules are routinely tested during preclinical safety studies for binding to hERG. hERG assays typically involve

radioligand binding studies or patch clamp recordings on hERG channels expressed either in CHO or human embryonic kidney 293 (HEK293) cells.

The focus on the hERG channel alone is a vast oversimplification of how drugs may predispose patients to arrhythmias because conductances from multiple channels contribute to the cardiac action potential, and drug effects on any of these channels can alter cardiac function.^{2,3}

The most well-validated research under consideration for the new FDA guidelines involves cardiomyocytes derived from human iPS cells that contain all the endogenous cardiac ion channels and spontaneously beat in culture with action potentials that mimic those of a human.⁴

Although iPS cell-derived cardiomyocytes are in use by several companies, they contain a mixture of cardiac cell types and have not yet been fully optimized.

One goal is to create cells identical to human adult ventricular cells because problems during the ventricular repolarization phase of the cardiac action potential are central to the development of arrhythmias.

However, generating a pure population of ventricular cells that can spontaneously beat in culture is a challenge, as the presence of some atrial and nodal cells appears to be necessary for continued generation of the ventricular action potential.

According to Chris Parker, VP and chief commercial officer at **Cellular Dynamics International Inc.**, there is some debate in the field as to whether a pure ventricular cell population is optimal or whether it is preferable to have a pan-population of majority

ventricular and minority atrial and nodal cells.

Because drugs can also act on nonventricular cells to interrupt heart function, a mixed population may be able to catch other possible liabilities. However, because ventricular cells represent the most likely target for proarrhythmia effects, diluting that population may reduce the probability of detecting subtle effects on the ventricular action potential.

CDI has developed a commercial supply of iPS cell-derived cardiomyocytes for use in screening candidate compounds for proarrhythmia potential. The cells are adult-like and produce atrial, nodal and ventricular action potentials that are similar to those of adult human cardiomyocytes.

More importantly, according to Parker, the CDI cells have been shown to respond to a set of ion channel-blocking drugs at the same concentrations that produce effects in human clinical trials, and their results translate more closely to the clinic than those from other existing preclinical models.⁵

"You can't make cardiac cells that just look like cardiomyocytes and act like them. They have to respond like a clinical patient would," he told *SciBX*.

Joseph Wu, director of the Stanford Cardiovascular Institute and a professor of medicine and radiology at the **Stanford University School of Medicine**, thinks the next frontier in the field is patient-derived iPS cells that will dominate future preclinical proarrhythmia assessments.

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The advantage of using patient-derived iPSC cells, according to Wu, is that drugs can be tested on cardiomyocytes representing normal and diseased hearts from multiple ethnic backgrounds, thus providing a window into the range of people likely to take a drug. This would give companies a better assessment of the benefit-risk ratio of each therapeutic candidate and could aid selection of compounds to take into the clinic.

"I would want to test the same drug on 1,000 different cell lines and know why my bottom and top 5% of responders and nonresponders behaved that way so that I could figure out what caused that. That is how I think drug discovery should be done in the future," said Wu.

Wu is a cofounder of **Stem Cell Theranostics Inc.**, a startup that has a high throughput screening platform for patient-specific cardiomyocytes. The company's goal is to partner with biotechs to aid preclinical cardiotoxicity safety testing.

Beyond iPSC cell-derived cardiomyocytes, early stage research on 3D organ cultures and heart-on-a-chip technologies are gaining momentum and could represent the next breakthrough in preclinical cardiotoxicity screening.⁶

3D organ cultures contain the different types of myocardial cells in an organized structure and have the advantage of being able to reproduce disease phenotypes that arise from nonventricular cells. Miniaturizing these on a chip could lead to a high throughput screening system that is affordable for smaller biotechs and could render preclinical personalized cardiotoxicity testing routine in the industry.

Mathematics of the heart

After creating the best cell type to screen in preclinical cardiotoxicity testing, the next step is optimizing the analysis of the data to predict arrhythmia risk.

Gary Mirams, a research fellow at the **University of Oxford**, has developed a computational algorithm that models arrhythmia risk based on the way that cardiomyocyte membrane potential is altered by drugs acting on hERG and other cardiac ion channels. As the membrane potential gets closer to the threshold for triggering an action potential, the likelihood of arrhythmia developing is thought to increase.

Because some drugs block multiple channels, their combined effects on the different channels contribute to their effect on the membrane potential.

For example, verapamil is known to block the hERG channel but does not carry a significant proarrhythmia risk. This is most likely due to the fact that it potently inhibits the calcium channel L-type and causes some blockade of the Nav1.5 (SCN5A) sodium channel.

Thus, its inhibition of the outward potassium flux through the hERG channel would be largely offset by its blockade of the inward calcium and sodium flux, creating a net minimum change for the membrane potential.

Rather than ask "can we predict what happens to a particular ion channel, we ask, 'Can we predict what happens to a whole heart cell?'" Mirams told *SciBX*.

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Mirams' mathematics-based cardiac electrophysiology model determines the individual channel conductances from IC₅₀ values of a compound measured at each channel. It then computes the membrane potential that would result.

Next, the program simulates the overall effect on the cardiac action potential and assesses the likelihood of arrhythmia based on the large amount of electrophysiological data available from human electrocardiogram studies over the last two decades, linking various action potential measurements to arrhythmia.

Mirams believes incorporating different ethnicities and disease conditions from

patient-derived iPSC cells will likely represent the next phase of modeling in this field. This could change the translational strategy in biotechs from assessing the general risk of a compound to assessing the risk of a compound—or even a combination of compounds—in a given patient population, he told *SciBX*.

Mirams' software is available as open source.

Certara L.P., a healthcare consulting company, recently announced its Cardiac Safety Simulator (CSS) software that incorporates population-based pharmacokinetics to help predict arrhythmia risk.

According to Sebastian Polak, principal scientist at Certara, CSS uses the pharmacokinetics data to account for individual variability in its arrhythmia assessment. It uses parameters from different cardiomyopathies, such as the volume and area of cardiomyocytes, heart wall thickness, heart rate and plasma concentration, to model how a drug might act in different disease backgrounds.

There is still a long way to go before preclinical data alone could be used to assess proarrhythmia risk. The current momentum for the revised FDA guidelines appears to favor a robust preclinical package combined with rigorous electrocardiogram testing in Phase I trials to replace Phase III tQT studies.

Nevertheless, if progress in the coming years continues at the pace of the last few years, there may be further revisions down the road as the technologies converge to improve cardiotoxicity assessment.

Fishburn, C.S. *SciBX* 6(30); doi:10.1038/scibx.2013.781
Published online Aug. 8, 2013

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COMPANIES AND INSTITUTIONS MENTIONED

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