An automated approach to solving Pharma's cardiac toxicity conundrum

The pharmaceutical industry is facing ever-growing difficulties in developing new drugs and bringing them to market[1,2]. Many factors stand in the way of R&D productivity, not least of which are shrinking budgets. Yet one of the most pressing challenges continues to be the issue of ensuring that new drug candidates have an acceptable safety profile.

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In an effort to avoid surprises far along the drug-development road, pharmaceutical companies are making it a priority earlier in the process to test for toxicity – especially for high-prevalence liabilities such as hERG cardiac toxicity[3]. But these tests are feasible only by using higher throughput methods that demand a small amount of compound[4]. The recent introduction of automated patch clamp platforms that deliver high-quality data has enabled this much-needed hERG profiling earlier in drug discovery during lead optimisation, helping pharmaceutical companies spend their resources wisely[5].

This review discusses the application of automated patch clamp platforms during lead optimisation and thoroughly explores advantages and disadvantages of various platforms in use today. Several examples from the evaluation of the automated patch clamp platform CytoPatch (**Figure 1**) at WIL Research, a global contract research organisation, are also shown.

The business risk of innovative drug candidates

Pharmaceutical companies spend tremendously on drug research and development, pouring resources into every phase: target finding, lead finding, lead optimisation, preclinical development, and Phase I-III clinical development. And that is before marketing costs are factored in.

Yet, despite a marked increase in spending over the past decade, the industry is putting forth fewer new drug candidates; the number of applications filed to FDA's Center of Drug Evaluation and Research declined to 23 in 2010 from 45 in 1996.

Analysis of a database with 28,000 R&D projects shows that the attrition rates in all phases of drug development have increased significantly[2].

The fall in R&D productivity corresponds with the pharmaceutical industry's focus on creating new therapeutic targets that will have less post-launch competition. The drawback of this approach, however, is the difficulty in developing such drug candidates with high efficacy and low safety risk. Due to this, the approach tends to come with lower success rates yet higher development costs.

Why aren't these innovative drug candidates meeting safety requirements? A review of drug development data[6] shows that cardiovascular toxicity is often to blame, accounting for approximately 27% of drug failures due to toxicity in the preclinical phase. Phase I clinical studies are relatively safer in terms of cardiovascular toxicity, with only 9% showing serious adverse drug reactions. The overall attrition rate due to cardiovascular events in clinical development is 21%, indicating that several cardiovascular effects occur in Phase II and III clinical trials which are not detected in the preclinical studies or earlier clinical trials[6]. There are several different types of cardiovascular toxicity; one major type is toxicity caused by drug effects on cardiac ion channels like hERG.

Figure 1
The automated patch clamp platform CytoPatch

It's all about hERG: the human Ether-à-go-go Related Gene (hERG) ion channel

The sum of the action potentials of the different parts of the heart is clinically monitored using surface electrodes and results in an electrocardiogram, or ECG. Drugs that affect ion channels in the heart can change ECG parameters such as the QT-interval, which represents the time from the depolarisation of the ventricles to the repolarisation of the ventricles. Many drugs showing cardiac toxicity prolong the QT-interval in the ECG[6,7]. Drug-induced prolongation of the QT-interval can lead to *torsade de pointes*, a life-threatening ventricular arrhythmia that can cause sudden cardiac death.

Drugs prolonging the QT interval appear to consistently inhibit the outward, rapid-delayed rectifier K+ current (IKr) conveyed by the hERG (human Ether-à-go-go Related Gene, or the KCNH2 gene in the modern nomenclature) channel. Therefore an *in vitro* study to assess a compound's potential to inhibit this channel is an essential part of the non-clinical regulatory testing battery. The hERG gene encodes for the pore-forming α subunit of the voltage-gated potassium (K+) channel that controls the outward potassium current during a cardiac contraction. The hERG ion channel has long been known to be the target of class III anti-arrhythmic drugs such as amiodarone. Unfortunately, the hERG channel also interacts with a variety of non-cardiovascular drugs. Several drugs, in fact, have been withdrawn from the market due to adverse cardiac effects. Examples include astemizole, an antihistamine, and cisapride, a gastroprokinetic drug[7]. As a result, a direct assay of hERG channel inhibition is now an expected part of the safety pharmacology package conducted to support initiation of First-in-Man clinical trials.

Regulatory studies for non-clinical cardiovascular safety testing are described in the ICH guidelines S7A and S7B. ICH S7A describes safety Pharmacology Studies for Human Pharmaceuticals. ICH S7B extends and complements this guideline and describes studies for the assessment of QT-interval prolongation. Studies described in the ICH S7B guideline identify the potential of a test substance and its metabolites to delay ventricular repolarisation (QT-prolongation), and assess the dose relationship between the compound concentration and the effect.

The manual patch clamp technique: the current standard

Today's standard for measuring hERG inhibition is the manual patch clamp technique. Through this technique, a cell present in a bath is approached with a glass pipette containing an electrode by using a micromanipulator. The principle of the manual patch clamp technique is shown in the upper part of **Figure 2**. During this

technique, positive pressure is applied to the glass pipette to keep the tip of the pipette clean. Subsequently, mild suction is applied when the tip of the pipette touches the cell. As a result, the membrane of the cell enters the pipette and a tight seal forms between the cell membrane and the inner surface of the pipette. Finally, more suction is applied to disrupt the membrane and an electric circuit is established between the electrode in the micropipette and the cytoplasm. In this way, the potential difference between a bath electrode and the electrode in the pipette directly reflects the membrane potential. By measuring cells that overexpress the hERG ion channel (such as HEK-293 cells stably transfected with hERG-1 cDNA), it is possible to accurately assess the effect that a drug candidate has on the current IKr that is conducted through the hERG ion channel.

An estimated 25-40% of all drug candidates show some level of hERG-related inhibition, which results in a high level of attrition due to QT-prolongation in the preclinical phase[8]. The early detection of hERG cardiac toxicity and use of the data for compound development with better safety profiles has therefore been proposed by the pharmaceutical industry as a useful strategy[9]. Toxicity screening in the lead optimisation phase requires assays and techniques that have a relative high throughput and need a very small amount of test sample[4]. Yet, the manual patch clamp technique is low throughput and requires a high amount of compound, which makes this standard technique less suitable during the lead optimisation phase of drug development.

A new option: automated patch clamping

There are several automated patch clamp platforms on the market[9-12] that are designed to address the drawbacks of manual methods. All have their own characteristics, throughput and data quality.

Automated systems suspend cells that are injected into a recording chamber containing a planar substrate with one or more small apertures. In this chamber, the cells are captured by negative pressure and directed on to the patch aperture. The negative pressure is then increased to help form a tight seal between the cell membrane and the chamber[10]. The formation of a tight seal is prerequisite for high-quality patch clamp recordings (good voltage control of the cell as well as a stable measurement). An electrical circuit is established after disrupting the membrane either by membrane rupture or the application of pore forming chemicals. By using a specific voltage protocol and cells with an overexpression of the hERG ion channel, researchers can determine a drug candidate's

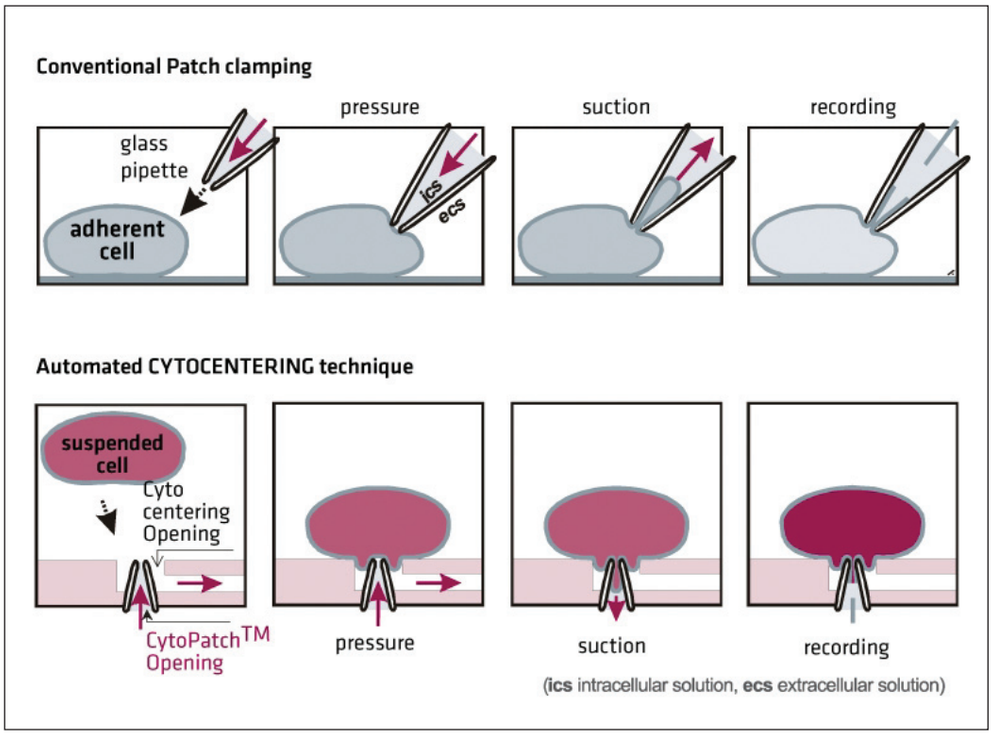

Figure 2
The principle of the automated cytocentring technique mimics conventional (manual) patch clamping. After catching the cell the principle of both techniques is equal. Positive pressure is applied to the pipette to keep it clean. Suction is applied to form a tight seal between the membrane of the cell and the surface of the glass pipette. More suction is applied to disrupt the membrane and an electrical circuit is established

Table 1: Reference hERG inhibitors used for the evaluation of the automated patch clamp platform CytoPatch

COMPOUND	DESCRIPTION	IC_{50} CYTOPATCH	IC_{50} MANUAL PATCH CLAMP	RATIO
E4031	Class III antiarrhythmic drug	70.3nM	8.3nM	8.5
Cisapride	Gastroprokinetic agent	32.8nM	13.6nM	2.4
Astemizole	Antihistamine drug	3.50nM	2.1nM	1.7
Caffeine	Central nervous system stimulant	7.42mM	10.5mM	0.71
Dofetilide	Class III antiarrhythmic drug	19.2nM	25.5nM	0.75
Terfenadine	Antihistamine	36.7nM	47.5nM	0.77
Quinidine	Class I antiarrhythmic drug	0.97µM	2µM	0.49
Verapamil	L-type calcium channel blocker	0.56µM	0.37µM	1.5

potential to inhibit the IKr current that is conducted through the hERG ion channel.

To address the pharmaceutical industry's shift to safety testing earlier in drug development, WIL Research has invested in automated patch clamping services. WIL Research acquired the automated patch clamp platform CytoPatch (**Figure 1**) due to the high quality of the platform and data it generates[10]. This platform is unique in that it includes a chip made out of quartz glass containing a patch clamp pipette. This ensures tight binding between the pipette and cells, resulting in stable measurement and high-quality data. The quartz chip contains two microfluidic channels (**Figure 2**). The first channel is present in the glass pipette and is filled with an intracellular solution. The second channel, called the cytocentring channel, captures cells by applying negative pressure. The quartz chips are embedded by plastic packaging. This packaging contains a third channel that applies extracellular buffer or a test compound, such as a drug candidate. The lower part of **Figure 2** depicts the principle of a patch clamp measurement with a CytoPatch chip.

With exception of the process of cell catching, the principle of the CytoPatch resembles the manual patch clamping technique. Before cell catching, positive pressure is applied to the pipette to keep it clean. The cells are then captured by the cytocentring channel. Subsequently, negative pressure is applied to the pipette to form a tight seal. Finally, the negative pressure in the pipette is further increased to rupture the membrane. Electrical access to the cells is obtained and recordings can start. During a measurement there is continuous flow with extracellular solution, which stabilises the seal.

Evaluation of the automated patch clamp platform cytopatch

To demonstrate proper operation of the CytoPatch system, WIL Research and Cytocentrics Bioscience GmbH have performed an Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ). The CytoPatch software includes an audit trail, user access administration and is 21 CFR part 11 and GLP compliant. The IQ, OQ and PQ passed all acceptance criteria.

CytoPatch was evaluated by testing eight reference hERG inhibitors (**Table 1**). The IC_{50} values were calculated and compared with manual patch clamp data obtained in-house or from Cytocentrics (**Table 1**). The obtained IC_{50} values were similar for both methods. The only difference between

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IC_{50} values was with E4031, which exhibited an 8.5-fold difference. This difference could be explained by the use of different batches of E4031. The correlation between the IC_{50} values determined with the CytoPatch and those determined using manual patch clamp is depicted in **Figure 3**. A high correlation of 0.97 between the two methods was observed representing the high data quality obtained with the CytoPatch instrument.

How automated patch clamps differ

Automated methods have a higher throughput than manual patch clamp methods, demand a smaller amount of test article (a few milligrams) and only require trained technicians rather than electrophysiologists to operate the devices. Based on these characteristics, the automated methods can be easily integrated in lead optimisation programmes for drug development. **Table 2** shows a comparison of various automated patch clamp platforms and manual patch clamping.

The CytoPatch platform delivers, in comparison to the other automated patch clamp platforms, a lower throughput (approximately 20 data points per day versus up to about 100 to 1,000 data points per day for the other platforms when looking at hERG screening). But because the CytoPatch platform is designed with a modular concept, up to 20 devices can be connected to form one multi-channel CytoPatch instrument. Using this concept, the throughput can be increased to approximately 400 data points per day. Thus, when throughput alone is the most important criterion for selecting a patch clamp platform, the PatchXpress (Molecular Devices) or Qpatch (Sophion) platforms have the best characteristics.

Other characteristics such as the presence of a glass pipette, the ability to form gigaseals, fast and continuous perfusion, and 21 CFR part 11 and GLP compliancy are key criteria for measuring the quality of automated patch clamp platforms, as summarised in **Table 2**. All automated patch clamp devices in **Table 2** have the characteristic that gigaseals are formed. In contrast to other platforms the CytoPatch does not demand fluoride buffers to enhance seal formation, which results in a more physiological model. Of the automated systems, the CytoPatch is the only system that is 21 CFR part 11 and GLP compliant, that makes use of a real glass pipette and contains a fast and continuous perfusion system that allows for voltage gated and ligand gated ion channels tests. Therefore the CytoPatch ensures the highest data quality and similarity with the manual patch clamp method.

Future outlook: translating *in vitro* safety studies into clinical QT-prolongation

The vast majority of the clinical cases of drug-induced QT-prolongation have been observed to be

	PATCHXPRESS (MOLECULAR DEVICES), QPATCH (SOPHION), PATCHLINER (NANION)	CYTOPATCH	MANUAL PATCH CLAMPING
Throughput	High	Medium	Low
Test article demand	Low	Low	High
Operator	Trained technicians	Trained technicians	Electrophysiologist
Seal	Gigaseal	Gigaseal	Gigaseal
Glass pipette	No	Yes	Yes
Continuous and fast perfusion	No	Yes	Yes
21 CFR part 11 and GLP compliant	No	Yes	Yes

Table 2: Comparison of the characteristics of various automated patch clamp platforms and manual patch clamping with respect to hERG screening

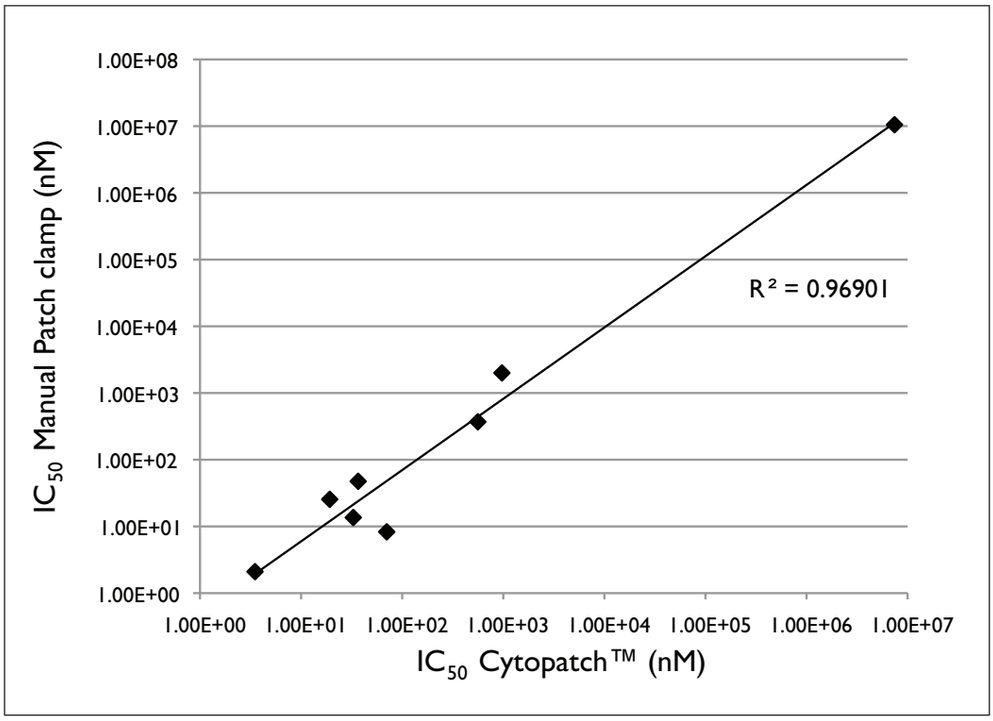

Figure 3
Correlation of the hERG IC_{50} values obtained using the CytoPatch device with those obtained using manual patch clamp

due to blockade of the hERG ion channel. However, as more and more data becomes available about drug-induced QT-prolongation, it is apparent that there is not always a correlation between hERG inhibition and QT-prolongation. There are numerous examples of false positives and false negatives in the *in vitro* hERG assay[13-21] (**Table 3**). Therefore, it can be concluded that additional (*in vitro*) assays are needed to better predict the cardiac safety profiles of compounds.

The main reason for erroneous conclusions of *in vitro* hERG assays is that several compounds affect ion channels other than hERG or affect multiple ion channels. Since the ventricular action potential is coordinated by interplay of ion channels – of which $Na_v1.5$, $Ca_v1.2$, $K_v4.2/4.3$, $K_v7.1$, hERG and Kir2.1 are the main ones – drug interaction with one of these other channels can affect the QT-time.

To get a better cardiac safety profile of a drug candidate, screening of a compound against a panel of cell lines with overexpression of the main ion channels involved in the ventricular action potential has been proposed[22]. Performing such a profiling by using manual patch clamping would be too laborious for screening purposes; however, the availability of automated patch clamp platforms has made it possible to perform such a screening[5].

A new development is the application of induced pluripotent stem (iPS) cell-derived human cardiomyocytes. These cells express several relevant cardiac ion channels at comparable levels to primary human cardiomyocytes and can be used for a total ion channel measurement. Future application of these iPS cell-derived human cardiomyocytes may give a better prediction for cardiac toxicity. Performing a total ion channel measurement is feasible with the automated patch clamp methods discussed here.

Summary

Drug-induced QT prolongation is recognised as a major hurdle in the successful development of drug candidates. The main cause of drug-induced QT prolongation is due to hERG channel blockade. A

False positives hERG assay	Verapamil Clomiphene Clemastine Fluoxetine Citalopram Moxifloxacin Ranolazine
False negatives hERG assay	Alfuzosin Chloroquine

Table 3: Examples of drugs for which there was no correlation between inhibition in the *in vitro* hERG assay and QT-prolongation observed *in vivo*

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direct assay of hERG channel inhibition is therefore routinely part of the safety pharmacology package conducted to support initiation of clinical trials. Several medium or high-throughput automated patch clamp platforms have become available that make it possible to move the hERG assay earlier in the preclinical phase to early lead optimisation. This strategy can help to improve the success rate of drug candidates.

To support this new testing strategy, WIL Research now offers the CytoPatch automated patch clamp platform. Evaluation of this platform has shown that there is a high correlation with manual patch clamping. Of the automated patch clamp platforms tested, the CytoPatch platform most closely mimics manual patch clamping.

Since the correlation between hERG inhibition and drug-induced long QT syndrome is not perfect, the testing of drug effects on other ion channels is recommended to be included in future *in vitro* testing strategies. DDW

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