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# The Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) Guide: A New Approach to Cardiac Risk Assessment

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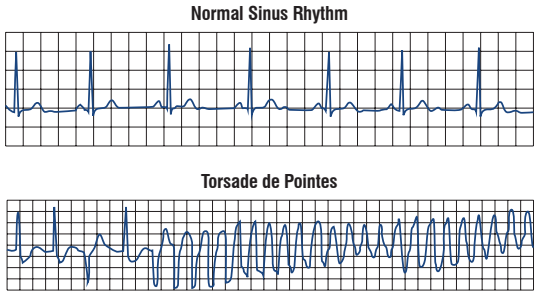
## Historic Overview – Delayed Repolarization Hazard

The association of certain cardiovascular drugs such as quinidine with risk of sudden cardiac death (quinidine syncope) was recognized nearly 100 years ago, but the mechanism was obscure until the 1960s, when quinidine syncope was found to be associated with prolongation of the electrocardiographic QT interval and a cardiac arrhythmia that displayed a distinctive electrocardiographic signature known as Torsade de Pointes (TdP, "twisting of the points"), a polymorphic ventricular arrhythmia which is potentially fatal (Figure 1). In the same era, congenital long QT syndromes (LQTS), rare familial diseases with similar cardiac phenotypes (i.e., QTc intervals in excess of 500 ms, episodes of TdP, and in some cases sudden cardiac death) were described.

**Figure 1. Human electrocardiograms illustrating normal and torsadogenic activity**



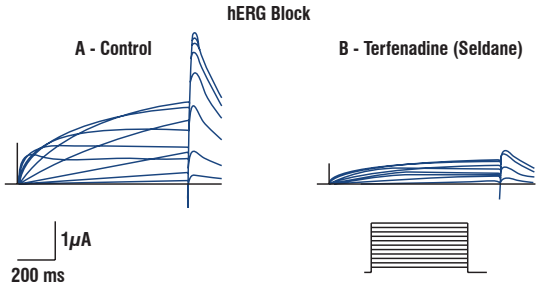

Courtesy of Dr. A. J. Moss.

With the deciphering of the genome in the 1990s, one form of LQTS (LQTS2) was found to be linked to hERG (human ether-a-go-go-related gene), encoding a cardiac potassium channel responsible for the fast component of the delayed rectifier current ($I_{Kr}$) which mediates cardiac action potential repolarization. Loss-of-function mutations in hERG were found to result in suppression of $I_{Kr}$, mimicking the underlying mechanism of cardiac drugs such as quinidine, which can cause QT prolongation and TdP. Also in the 1990s, several non-cardiac drugs, including terfenadine (Seldane) and cisapride (Propulsid), were associated with rare instances of QT prolongation and TdP.

The case of Seldane (terfenadine) is emblematic of the problem with drug-induced delayed repolarization and led investigators to identify the link between TdP and non-cardiac drugs. Seldane, an $H_1$ histamine receptor antagonist, entered the market in 1985 as the first non-

sedating antihistamine for symptomatic treatment of allergic rhinitis (sneezing, itchy or runny nose, watery eyes). In preclinical drug development, Seldane gave no cardiac risk signals (no effects on cardiac action potential or electrocardiographic QT interval). But in clinical trials it showed ~6 msec increase in the duration of the QT interval (i.e., a 2% increase) in the presence of metabolic inhibition (Honig et al., 1993). Moreover, postmarket reporting identified TdP occurring at a rate of ~0.3 cases/1 million prescriptions (Pink Sheet, 1990), too rare to have been detected in clinical trials. An ionic mechanism was proposed by Dr. Arthur Brown's laboratory in the mid-1990s (Roy et al., 1996) when they showed Seldane to be a potent hERG blocker *in vitro* (Figure 2).

**Figure 2. Effect of terfenadine on hERG potassium channel current ($I_{Kr}$) expressed in *Xenopus* oocytes**




Roy, M.-L. Dumaine, R & Brown, A.M, *Circulation* 1996;817-823

The drug was removed from the market in 1998 and eventually replaced by its active metabolite, Allegra (fexofenadine), which carries no hERG or TdP risk.

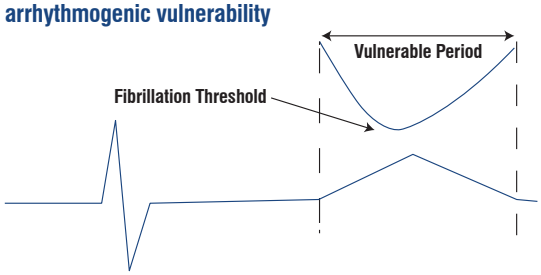
Other marketed noncardiac drugs that were found to be torsadogenic, and subsequently withdrawn, are listed in Table1. It is noteworthy that this list of withdrawn drugs covers a broad spectrum of pharmacological classes, but all share the characteristically potent hERG block relative to the effective therapeutic concentration (i.e., a low safety margin). Not all members within a class will be torsadogenic, viz., terfenadine and fexofenadine.

**Table 1. Drugs withdrawn for TdP**

| Drug | Class | Date Withdrawn | Safety Margin (hERG $IC_{50}$/$ETPC_{free}$) |
|---|---|---|---|
| Terodiline | Urinary Incontinence | 1991 | 31.0 |
| Sparfloxacin | Antibiotic | 1996 | 1.5 |
| Terfenadine | Antihistamine | 1998 | 1.3 |
| Astemizole | Antihistamine | 1999 | 0.8 |
| Grepafloxacin | Antibiotic | 1999 | 13.0 |
| Cisapride | Prokinetic | 2000 | 5.3 |
| Droperidol | Tranquilizer | 2001 | 1.1 |
| Levomethadyl | Opiate Dependence | 2003 | 4.8 |

The mechanism that makes hERG inhibition hazardous can be understood through the concept of the vulnerable period of the cardiac excitatory cycle. It has long been known that applying a brief electrical shock at a critical time in the repolarization phase of the action potential can elicit ectopic beats and, in extreme cases, ventricular fibrillation. The critical period has been shown to correspond to the peak of the electrocardiographic T-wave, where the fibrillation threshold is lowest (Figure 3).

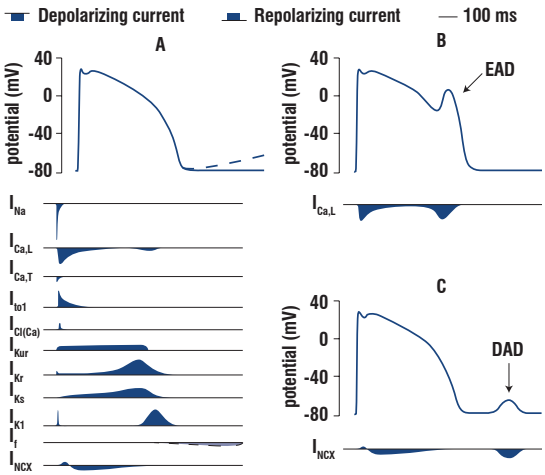
**Figure 3. Diagram of electrocardiogram correlated with arrhythmogenic vulnerability**




The underlying ionic mechanism that accounts for the vulnerable period is shown below. Repolarization (Figure 4A) is driven by the net charge movement mediated by outward currents (i.e., hERG [$I_{Kr}$] and KvLQT1/mink [$I_{Ks}$]), and inward currents (e.g., Cav1.2 [$I_{Ca,L}$]). Blockade of potassium current prolongs the phase of repolarization in a voltage range that allows reactivation of $I_{Ca,L}$, and possibly development of late Nav1.5 ($I_{Na}$) that further increases the critical period. Inward $I_{Ca,L}$ currents are thought to be responsible for generating early afterdepolarizations (EADs, Figure 4B) that trigger

ectopic beats (premature ventricular contractions) and re-entrant arrhythmia. Prolongation of repolarization also may generate delayed afterdepolarizations (DADs, Figure 4C), via the electrogenic $Na^+$ -$Ca^{2+}$ exchange transporter, that can trigger arrhythmia. Thus, drug-induced effects on any one of these ionic currents can have the potential to upset the balance necessary for normal rhythmicity. Because of its unusual structural features, hERG is the most promiscuous target among cardiac ion channels, and selective hERG blockers are responsible for nearly all drug-induced delayed repolarization and its arrhythmogenic consequence. However, compensatory block of inward current by nonselective drugs with multiple ion channel effects (MICE) may mitigate the proarrhythmic effects of hERG inhibition via delayed repolarization (Lacerda et al., 2010). Moreover, block of other potassium channels or augmentation of late inward $Ca^{2+}$ or $Na^+$ currents may create torsadogenic risk even in the absence of hERG inhibition (Lacerda et al., 2008).

**Figure 4. Ionic mechanism of ventricular action potential**




## Development of Regulatory Guidelines

The severity of the cardiac risk of noncardiac drugs is illustrated by the fact that QT prolongation and TdP is currently the single most common reason for withdrawal or restriction of marketed drugs. However, the rarity of observing TdP during clinical development makes this an unusable endpoint and leads to regulatory focus on hERG inhibition and QTc testing as surrogates. QTc is a particularly weak surrogate. Although the measurement can be made with a high degree of sensitivity and precision, it lacks specificity for TdP prediction (i.e., TdP is always preceded by long QT, but long QT often is unaccompanied by TdP). hERG inhibition as a preclinical indicator has great sensitivity, but also suffers from lack of specificity. Nonetheless, the current regulatory approach has promoted hERG inhibition to a position as a go/no-go decision point in early drug discovery, and a thorough QTc study powered to detect a 10 ms increase (with an upper bound 95% confidence interval) is required in clinical development of most drugs.

Recommendations for cardiac risk evaluation were harmonized among regulatory agencies and industry through the adoption in 2005 of ICH S7B (http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S7B/Step4/S7B_Guideline.pdf) entitled "The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT-interval prolongation) by Human Pharmaceuticals" and ICH E14 (http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Guideline.pdf) entitled "The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs." The S7B guideline recommended the *in vitro* hERG patch clamp assay and *in vivo* QTc evaluation in nonrodent animals. However, these preclinical studies were not considered sufficient to obviate torsadogenic risk. Therefore, a focused electrocardiographic study, typically in healthy volunteers (except in oncology), and the "thorough QT" study (TQTS) embodied in the E14 document is recommended for all new drugs that have systemic exposure. The goal of the TQTS is to demonstrate that a supratherapeutic dose and therapeutic dose result in less than 10 ms increase in QTc at the one-sided upper 95% confidence limit. This threshold for risk evaluation, which amounts to an increase of just a few percent, is set intentionally low to increase the sensitivity of the test.

These three tests (*in vitro* hERG, *in vivo* animal QTc, and TQTS) became a minimum standard for most drug development programs. The S7B and E14 guidelines were particularly significant because they embodied the current scientific understanding of torsadogenic risk. Their effectiveness is apparent from the fact that, since their adoption, no drugs have been withdrawn for torsadogenic risk and the number of reported drug-induced TdP cases for noncardiac drugs has decreased. However, it has been estimated that up to 60% of new molecular entities developed as potential drugs have tested positive for hERG block and were subsequently terminated from development (Raschi et al., 2008), despite their potential therapeutic benefit versus torsadogenic risk. Moreover, approved drugs that have precautionary labeling because of marginal QT-prolongation will face restricted applicability and competitive disadvantage.

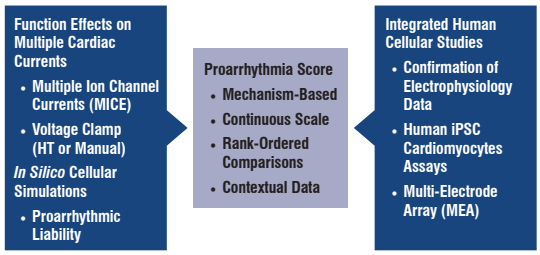
### Comprehensive *In Vitro* Proarrhythmia Assay (CiPA)

Advances in our understanding of the relationship between cardiac ion channel dysfunction and arrhythmogenesis, and newly available technologies for *in vitro* testing, has prompted a re-evaluation of the strategy for identifying cardiac risk. The new strategy, entitled "Comprehensive *In Vitro* Proarrhythmia Assay" (CiPA), shifts the emphasis away from QT-prolongation and focuses on predicting proarrhythmic torsadogenic hazard risk through an expansion of the *in vitro* component of nonclinical safety evaluation, which could be performed in the discovery stage of drug development to guide selection of candidate compounds with the most favorable profiles (Sager et al., 2014). This new approach is intended to provide a more accurate representation of the torsadogenic potential compared to the current focus on delayed repolarization.

It is anticipated that CiPA will reduce or eliminate the necessity for conducting a thorough QT study during clinical development. However, ECG evaluation during Phase I would continue to be an important component of cardiac risk evaluation and discordances between CiPA and Phase I ECG results would need to be reconciled.

CiPA is envisioned to consist of four components: (1) an evaluation of the effects of test compounds on cardiac ion channel assays; (2) *in silico* modeling of the cardiac action potential based on the ion channel results to integrate the data with cardiac function; (3) experimental measurement of drug effects in human ventricular myocytes to confirm the modeling result; and (4) scoring the results (Figure 5). The results would provide the basis for assigning a proarrhythmia score on a graduated scale, rather than a go/no-go flag (Figure 5).

**Figure 5. Diagrammatic representation of CiPA (derived from Sager et al., 2014)**




## Implementation of CiPA at Charles River Discovery

### Multiple Ion Channel Evaluation (MICE)

The seven cardiac ion channels listed in Table 2 represent the primary determinants of the ventricular action potential (Figure 4) that are currently being validated in the CiPA initiative as proarrhythmic determinants. These cardiac ion channels are the main safety targets for hazard identification. Each ion channel has been expressed heterologously in either CHO or HEK293 cells at Charles River and standardized assays are performed by gigaseal patch clamp either in an automated platform QPatch HT® (Sophion Bioscience A/S, Denmark) or in conventional manual patch clamp. Other cardiac targets can also be included in the evaluation to expand analysis of potential mechanisms. These include: Kv1.5 ($I_{Kur}$), Kir3.1/3.4 ($I_{K,ACh}$), Kir6.2/SUR2A ($I_{K,ATP}$), Cav3.2 ($I_{Ca,T}$), and NCX1 ($I_{Na,Ca}$).

**Table 2. Cardiac Channel Panel**

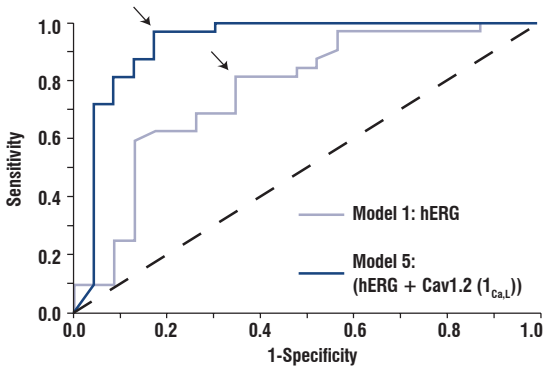
| Ion Channel | Cell Line Catalog Number | Assay Platform |
|---|---|---|
| Cav 1.2 ($I_{Ca,L}$) | CT6004 | QPatch HT or Manual Patch Clamp |
| Nav1.5 ($I_{Na}$) | CT6007 | QPatch HT or Manual Patch Clamp |
| Late Nav1.5 ($I_{Na}$) | CT6007 | Manual Patch Clamp |
| hERG ($I_{Kr}$) | CT6002 | QPatch HT or Manual Patch Clamp |
| Kv7.1/KCNE1 ($I_{Ks}$) | CT6101 | QPatch HT or Manual Patch Clamp |
| Kir2.1 ($I_{K1}$) | CT6127 | QPatch HT or Manual Patch Clamp |
| Kv4.3/KChip2.2 ($I_{to}$) | CT6171 | QPatch HT or Manual Patch Clamp |

Charles River Discovery has published evidence that the evaluation of Multiple Ion Channel Effects (MICE) results in more accurate discrimination of torsadogenic and nontorsadogenic drugs (Kramer et al., 2013). We tested the hypothesis by measuring the blocking potencies of 32 torsadogenic (+TdP) and 23 nontorsadogenic (-TdP) drugs on hERG, and the depolarizing Cav1.2 and Nav1.5 channel currents. These data were then used to construct logistic regression (LR) models to predict TdP risk. Consistent with our hypothesis, we found that the LR model constructed with hERG potencies alone was not as predictive of TdP risk as models that were constructed using hERG plus MICE variables (Cav1.2, Nav1.5). These results are referenced in CiPA documents as essential support for using MICE assays rather than the hERG assay alone.

Charles River now offers automated patch clamp of MICE assays as a tool for cardiac safety evaluation in drug discovery and nonclinical drug development. Our automated system provides gigaseals and has been validated against manual patch clamp, the reference standard. Manual patch clamp is slow, labor intensive and expensive. Automation is critical to controlling cost and time.

Results from cardiac panel profiling can then be benchmarked against a database of 90 reference compounds with known clinical outcomes to identify potential arrhythmogenic hazard. Alternatively, $IC_{50}$ values can be input into a logistic regression model (Kramer et al., 2013; Figure 6).

**Figure 6. Comparison of logistic regression models applied to 55 reference compounds. ROC analysis of Model 1 (hERG alone) and 5 (hERG and Cav1.2 ($I_{Ca,L}$)). The arrows indicate the cutoff point associated with the J-index. The dashed line indicates the performance of a model that does not discriminate. The table shows 2 x 2 contingencies for both models. (Kramer et al., 2013)**



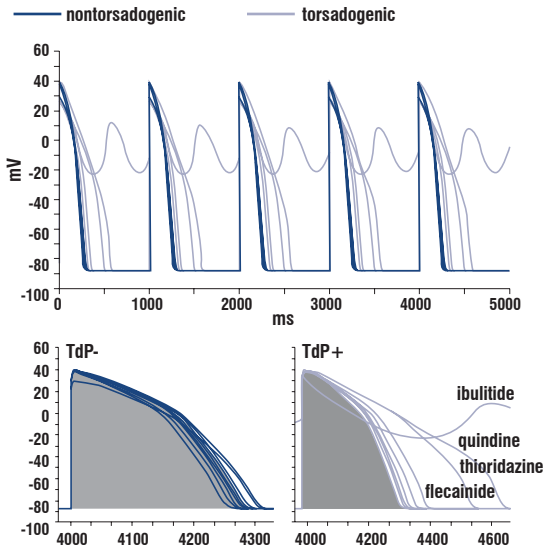

| | Model 1 (hERG) | | Model 5 (hERG + $I_{Ca,L}$) | |
|---|---|---|---|---|
| Predicted outcome | -TdP | +TdP | -TdP | +TdP |
| Correctly classified | 25 | 15 | 31 | 19 |
| Incorrectly classified | 6 | 8 | 1 | 3 |

## *In Silico* Modeling of Cardiac Action Potentials

Interpretation of cardiac ion channel patch clamp data can be further extended by entering the results into mathematical models of the electrical response of human cardiomyocytes. The goal is to simulate drug-induced modulation of the ventricular action potential, searching for possible arrhythmic events or changes in the action potential duration that are predictive of torsadogenic risk. To this end, we input ion channel concentration-response

data from patch clamp of recombinant cell lines into the O'Hara–Rudy action potential model (O'Hara et al., 2011) to predict drug effects on QT and rhythm. Figure 7 shows the last 5 of 1,000 simulated action potentials elicited at 1 Hz for each of 22 nontorsadogenic (blue traces) and 32 torsadogenic (red traces) drugs. The conductances were decreased based on the published block potencies for hERG, Cav1.2 and Nav1.5 obtained using automatic patch clamp platforms and the effective therapeutic plasma concentration (ETPC) (Kramer et al., 2013). Note that only one drug (ibutilide) shows arrhythmic activity at the clinical concentration. However, torsadogenic and nontorsadogenic drugs are well segregated when changes in the shape of the action potential are considered. This is shown in the bottom panel of Figure 7, where the shaded area is the space covered by the control action potential. Note that, in general, torsadogenic drugs prolong the action potential whereas nontorsadogenic drugs shorten it or are not different from control. Logistic regression analysis indicates that this different response is statistically significant.

**Figure 7. Action potential model (O'Hara et al., 2011) prediction of 55 reference compounds. Upper panel: the last 5 of 1,000 simulated action potentials elicited at 1 Hz for each of 22 nontorsadogenic and 32 torsadogenic drugs are shown. Lower panels: Segregated action potentials for torsadogenic and nontorsadogenic compounds. Shaded area denotes the space covered by the control action potential.**




## Stem Cell-Derived Human Cardiomyocyte Assays

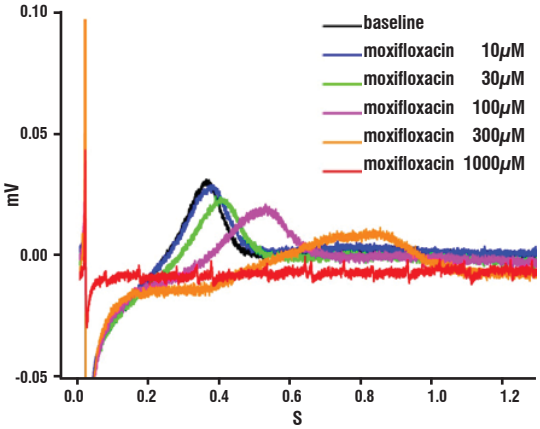
Induced pluripotent stem cells (iPSC), reprogrammed from adult human cells, can be differentiated into cardiomyocytes and grown in culture dishes to form a spontaneously beating layer of myocytes that displays electrical properties similar to an intact human heart. Human iPSC-derived cardiomyocytes represent the second major component of CiPA. They have several advantages including:

- Derivation from adult rather than embryonic cells
- Differentiation into major cardiac subtypes – predominantly ventricular
- Retention of cardiac phenotype during cell culture
- Supply in quantity sufficient for multi-well recording platforms

Charles River uses iPSC-derived human myocytes grown in monolayer tissue culture as a multicellular test system that recapitulates the physiologic properties of the human heart. Instrument technologies have advanced over the last several years such that label-free measurement of these "heart-in-a-dish" monolayers is possible in medium-throughput (96-well plate) format.

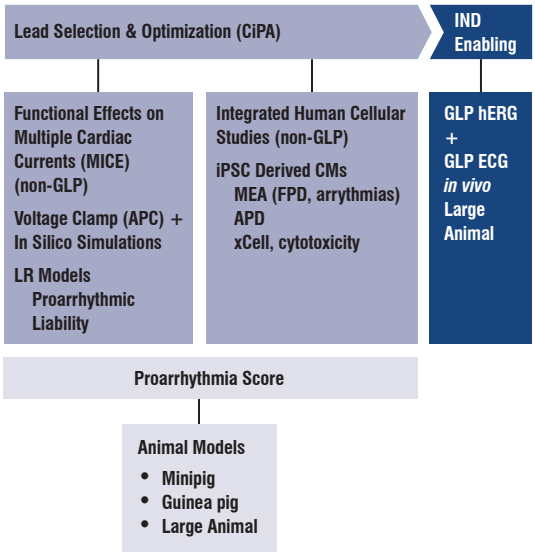
Our multiple-electrode array (MEA) assay evaluates two-dimensional excitation propagation by recording spontaneous ECG-like field potentials (Figure 8). Drug effects on field potential parameters, including the field potential duration (FPD) which is a surrogate for the QT interval, are recorded. Importantly, reference torsadogenic drugs not only prolong the FPD, they induce arrhythmias that are readily detected on the MEA platform. Conversely, safe nonarrhythmic drugs do not induce arrhythmias. The field potential provides a readout of the integrated functioning of intact myocytes and allows identification of multiple cardiac hazards including arrhythmia, premature beats, beat rate slowing, conduction velocity decrease, beat prolongation and other electrical irregularities. These data indicate that the MEA assay can be used for early *in vitro* detection of proarrhythmia risk before clinical use, which is the central goal of the CiPA initiative. Additional benefits of the MEA assay are that the myocytes can be monitored during drug testing over long periods of time and at high concentrations that would not be tolerated in animals or humans.

**Figure 8. MEA recordings from human myocyte syncytia. Field potentials recorded at baseline (before addition), and after exposure to indicated concentrations of moxifloxacin. For each trace, recordings were made for 2 minutes, sodium spikes were detected and aligned, then recordings were averaged. The FPD, measured from the sodium spike to the peak of the repolarization "T-wave," was prolonged by 30, 100 and 300 μM moxifloxacin; 1,000 μM moxifloxacin induced arrhythmic beating.**




## Suggested Implementation of CiPA Strategy

**Figure 9. Suggested Implementation of CiPA Strategy**




As shown in Figure 9, we view the CiPA approach as an integral part of lead optimization where a small number of compounds have been identified as potential candidates. In this instance, the gigaohm seal automated patch clamp is optimal for accurate assessment of potency in the MICE assay and proarrhythmic risk assessment. Nonetheless, large volume screening of compounds against the most critical cardiac channels (i.e., hERG, Nav1.5, and Cav1.2), conducted in high-throughput megaohm seal patch clamp format before lead optimization, can be useful for identifying the potential hazard of hERG-selective compounds. The figure also shows that CiPA does not displace preclinical ECG studies in conscious, unanesthetized animals. The overall goal is for CiPA and animal studies to minimize the necessity of a clinical TQT study as mandated by the E14 guidance to detect clinical QT prolongation as a surrogate marker for proarrhythmia.

In summary, CiPA has the potential to change the face of nonclinical cardiac safety assessment by replacing early "go/no-go" testing for hERG risk, with an updated methodology based on careful review and critique of current strategies, coupled with a mechanistic approach to understanding the basis of drug-induced arrhythmia. Moreover, replacement of the TQT study with careful clinical assessment of electrophysiologic effects in Phase 1 ECG safety studies, after compounds have been thoroughly vetted in the nonclinical testing, is anticipated to reduce unwarranted attrition of compounds in development, and ultimately benefit patients through development of new and better drugs.

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