Proarrhythmia liability assessment and the comprehensive in vitro Proarrhythmia Assay (CiPA): An industry survey on current practice

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A R T I C L E  I N F O

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A B S T R A C T

Introduction: The Safety Pharmacology Society (SPS) has conducted a survey of its membership to identify industry practices related to testing considered in the Comprehensive In vitro Proarrhythmia Assay (CiPA).

Methods: Survey topics included nonclinical approaches to address proarrhythmia issues, conduct of in silico studies, in vitro ion channel testing methods used, drugs used as positive controls during the conduct of cardiac ion channel studies, types of arrhythmias observed in non-clinical studies and use of the anticipated CiPA ion channel assay.

Results: In silico studies were used to evaluate effects on ventricular action potentials by only 15% of responders. In vitro assays were used mostly to assess QT prolongation (95%), cardiac Ca2+ and Na+ channel blockade (82%) and QT shortening or QRS prolongation (53%). For de-risking of candidate drugs for proarrhythmia, those assays most relevant to CiPA including cell lines stably expressing ion channels used to determine potency of drug block were most frequently used (89%) and human stem cell-derived or induced pluripotent stem cell cardiomyocytes (46%). Those in vivo assays related to general proarrhythmia derisking include ECG recording using implanted telemetry technology (88%), jacketed external telemetry (62%) and anesthetized animal models (53%). While the CiPA initiative was supported by 92% of responders, there may be some disconnect between current practice and future expectations, as explained.

Discussion: Proarrhythmia liability assessment in drug development presently includes study types consistent with CiPA. It is anticipated that CiPA will develop into a workable solution to the concern that proarrhythmia liability testing remains suboptimal.

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1. Introduction

A need to seek understanding of mechanisms leading to drug-induced arrhythmias, with a view to better predicting drug-induced proarrhythmia with fewer false positives, has resulted in the development of the comprehensive in vitro proarrhythmia (CiPA) assay (Huang et al., submitted for publication). Several biomarkers of ventricular repolarization-associated proarrhythmia risk have been considered in drug safety testing over the last few decades including QT prolongation (Authier, Pugsley, Troncy, & Curtis, 2010; Stramba-Badiale et al., 1997), QT dispersion (Day, McComb, & Campbell, 1990), early afterdepolarizations (Cranefield & Aronson, 1988) and Ttrial (triangulation, reverse use-dependence, instability and dispersion of repolarization) (Hondegem, Carlsson, & Duker, 2001; Lawrence, Bridgel kernel-Taylor, Pollard, Hammond, & Valent, 2006). Advocating the use of multiple in vitro ion channels in drug safety evaluation has emerged as an important strategy to assess the proarrhythmia liability of drug candidates during early stages of development (Kramer et al., 2013). While cell
lines with stable heterologously expressed ion channels have been used as testing tools for decades, an increasing interest in the validation and application of human stem cell derived cardiomyocytes for proarrhythmia risk assessment is apparent (Guo et al., 2013; Himmel, 2013; Kirby, Qi, Phatak, Smith, & Malaney, 2016; Navarrete et al., 2013; Qu & Vargas, 2015; Qu, Gao, Fang, & Vargas, 2013). Nonetheless, several factors have been shown to modulate ion channel voltage clamp study results (e.g., IC50 values) including the experimental temperature, the physicochemical nature of the drug studied, the voltage protocol used and whether the drug is actively perfused or static in the experimental chamber (Luo & Guthrie, 2005). Thus, numerous modifications have been proposed to optimize manual and automated patch clamp studies (Brüggemann, Stoelzel, George, Behrends, & Fertig, 2006; Comley, 2014; Hamill, Marty, Neher, Sakmann, & Sigworth, 1981; Lepple-Wienhues, Ferlinz, Seeger, & Schäfer, 2003; Obergussberger et al., 2015; Polonchuk, 2012; Sigworth & Klemic, 2005; Wakefield, Pollard, Redfern, Hammond, & Valentin, 2002). As an unfortunate consequence, a lack of consistency in testing protocols and study designs is present across laboratories within the pharmaceutical industry and CROs. Yet, nonclinical models for assessing proarrhythmia liability remain the cornerstone of regulatory drug safety testing and establishing best practices and careful evaluation of the translational value of the data obtained remains a priority (Hammond et al., 2001; Redfern et al., 2003). Since their inception, in silico models have also gained considerable complexity regarding their ability to generate cardiac action potentials since they integrate species differences with respect to ion channel profiles with in vitro data (Mirams et al., 2011; O’Hara & Rudy, 2012; Okada et al., 2015; Rodriguez et al., 2015). In the clinical arena, novel approaches to replace standard thorough QT (TQT) studies have been developed and are being implemented for use in marketing authorization submissions (Cavero, Holzgreve, & Clements, 2016; Shah, Maison-Blanche, Robert, Denis, & Duvauchelle, 2016). More recently, dissection of the T wave into early and late phases of repolarization (Johannesen et al., 2014; Vicente et al., 2015) has enabled a mechanistic evaluation of the proarrhythmia liability of drugs using clinical ECG data. Thus, proarrhythmia liability assessment is advancing at an accelerated pace from application of in silico modeling to clinical safety testing. As the regulatory paradigm for proarrhythmia liability assessment is evolving (Vicente, Stockbridge, & Strauss, 2016), reviewing current testing practices as they relate to drug development was undertaken by the SPS and the results are summarized herein.

2. Methods

This survey was distributed by the SPS to 887 safety pharmacologists, toxicologists and pharmacologists working globally in the pharmaceutical industry, at CROs, regulatory agencies, academia or the technology provider industry. Survey topics included (a) nonclinical approaches to address proarrhythmia issues, (b) conduct of in silico studies, (c) in vitro ion channel testing methods used, (d) drugs used as positive controls during the conduct of cardiac ion channel studies, (e) types of arrhythmias observed in non-clinical studies and (f) use of the anticipated CIPA ion channel assay. Survey questions are reported in the results section together with the percentages of the answers.

3. Results

All results are presented as the percentage of the total number of responses received per question. Results are presented with all affiliations combined. In addition, we show the percentage of the 887 scientists who were asked that responded to each question (i.e., the response rate).

3.1. Study survey demographics

Scientists (n = 887) from various fields of expertise (Fig. 1A) and geographic locations (Fig. 1B) were invited to participate in the survey and 85 (or 10%) responded. Of the 85 responders, a preponderance was from Europe (46%), followed by North America (38%) and Asia (17%). Responders originated from diverse business organizations (Fig. 1C) and company sizes (Fig. 1D) with 57% employed at companies with ≥1000 employees. Thus, one cannot exclude the potential that multiple responders from the same organization may have contributed to this survey (an issue that was not quantified in the responses) despite a request that only one response be provided from the same organization. Most responders were employed by pharmaceutical companies, followed by CROs and biopharmaceutical companies (Fig. 1C). The survey identified many interesting facts including the primary therapeutic areas that were pursued by the various companies (Fig. 1E). Oncology (74%) and neurology (68%) were the therapeutic indications frequently targeted for drug development followed by cardiovascular (62%), inflammation (58%), metabolic diseases (51%), infectious disease (39%) and lastly, rare/orphan diseases (36%). When asked, 60% of responders were involved with the design, conduct, interpretation, or review of in silico assays (Fig. 1F) and the response rate increased to 87% for those involved in the conduct of in vitro ion channel assays (Fig. 1G). A majority (61%) of responders reported that their company developed small molecules (new chemical entities; NCE) while 27% of responders worked for institutions developing large molecules/biologics.

3.2. Approaches that address proarrhythmia & CIPA related drug testing: survey results

The most frequent (75%) nonclinical approach used to address proarrhythmia issues by responders was to conduct a battery of in vitro (i.e., binding and ion channel electrophysiology studies, etc.) and in vivo CV safety pharmacology assays to screen drug candidates and establish a risk profile during the drug discovery phase of development (Table 1). Conduct of “fit-for-purpose” nonclinical safety pharmacology tests based on an integration of observations derived from chemistry structure-activity relationships (SAR), toxicology study findings and other scientific considerations (e.g., drug indication, drug class, pharmacology, pharmacokinetics/pharmacodynamics etc.) was reported to be used by 31% of responders. When asked which assays were used in the last 5 years to assess cardiac/cardiovascular effects, the responses were analyzed in terms of those in vitro assays with potential application to CIPA method implementation and included cell lines stably expressing ion channels for determination of drug block potency (89%), ion channel binding assays (57%), human stem cell-derived or induced pluripotent stem cell cardiomyocytes (iPSC-CM) (46%) and hERG channel trafficking (42%) and isolated tissue bath preparations (41%). Those cardiac/cardiovascular assays/models currently used to evaluate cardiac safety include ECG recordings using implanted telemetry systems (88%) followed by jacketed external telemetry (JET) (62%), anesthetized animal models (53%), cardiac action potential recordings using e.g., Purkinje fibers (51%), isolated Langendorff hearts (38%) and isolated cardiac wedge preparations (18%), with both proarrhythmia animal models and Zebrafish assays used by ≤12% of responders (Table 2).

3.2.1. Conduct of in silico assays

One third of responders frequently used in silico studies to evaluate drug effects on hERG (‘hERG’ assays) while 35% of them had never used in silico studies for this purpose (Table 3). Only 15% of responders reported frequently using in silico studies to evaluate drug effects on the cardiac action potential and 39% of responders had never used in silico studies at their company. Non-cardiac drug safety evaluations that were reportedly using in silico models included genetic toxicity evaluations and the study of effects on other cardiac ion channels. When in silico studies were used, assays were reported to be conducted as part of the early discovery safety study assessment by a large proportion of responders (43%); however, 44% report that in silico modeling is not used at their respective companies (Table 4, panel A).
Table 1

Nonclinical approaches used to address proarrhythmia issues.

| Answer options | Response percent | Response count |
|-----------------|------------------|----------------|
| Conduct a battery of in vitro (i.e., binding studies, ion channel studies, etc.), and in vivo CV safety pharmacology assays to screen drug candidates and establish risk during drug discovery | 75% | 58 |
| Conduct “fit-for-purpose” nonclinical safety pharmacology tests based on an integration of observations derived from chemistry (SAR), Toxicology findings and other scientific considerations (e.g., drug indication, drug class, pharmacology, PK/PD etc.) | 31% | 24 |
| Perform safety testing based on GLP findings from safety pharmacology and toxicology studies only | 20% | 15 |
| Do not know | 4% | 3 |

* Responders selected all that applied.

Table 2

Cardiovascular assays used by the pharmaceutical industry.

| Answer options | Frequently used | Rarely used | Response count |
|-----------------|-----------------|-------------|----------------|
| Cell lines stably expressing ion channels for determination of drug block potency | 89% | 11% | 66 |
| ECG recording using implanted telemetry technology | 82% | 12% | 67 |
| Jacketed External Telemetry (JET) | 62% | 38% | 63 |
| Ion channel binding assays | 57% | 43% | 68 |
| Anesthetized animal models | 53% | 47% | 58 |
| Cardiac action potential recordings (e.g., Purkinje fiber, papillary muscle, others...) | 51% | 49% | 61 |
| Human stem cell-derived or induced pluripotent stem cell cardiomyocytes (iPS-CM) | 46% | 54% | 68 |
| hERG (I[Ks]) channel trafficking | 42% | 58% | 72 |
| Isolated tissue bath preparations | 41% | 59% | 63 |
| Isolated Langendorff heart | 38% | 62% | 63 |
| Isolated cardiac wedge preparation | 18% | 82% | 51 |
| Proarrhythmia animal models | 12% | 88% | 49 |
| Zebrafish assays | 4% | 96% | 47 |

* Responders selected all that applied.

3.2.2. In vitro ion channel electrophysiology assays

When asked whether drug-induced arrhythmias were encountered in the conduct of non-clinical studies only 22–44% of responders provided some indication of their observation (Table 2). Of the types of drug-induced arrhythmias queried, PVCs were most frequently reported for IKr blockade (Fig. 5). However, of the 59% reporting only 10% frequently report abandoning a pharmacophore due to inability to eliminate hERG channel blockade. Interestingly, 22% state that although hERG channel block may not be eliminated chemically they have not discontinued development of the pharmacophore. A majority (82%) also reported that in vitro ion channel testing procedures include the use of a positive control in each study (Table 5) with the assays performed at either room temperature (71%) or at physiological temperature (58%). Washout data was obtained by a minority of responders (43%). Automated (75%) or manual (71%) patch clamp systems were used by a similar proportion of responders with 48% conducting in vitro assays to Good Laboratory Practice (GLP).

As expected, Ios/Kv11.1 (91%), Ica,L/Cav1.2 (80%), Ifs/Nav1.5 (57%) and Ito/Kv7.5 (53%) were the most frequently interrogated cardiac ion channels when used in stably expressed cell lines (Table 6). Although not as frequent, Ifs/Kv1.5 (29%), Ito/Kv4.3 (22%), Ifs/Kv1/2.1–2.3 (22%) and Ifs/HCN4 (20%) were also evaluated. Drugs used as positive controls that were most frequently tested in the validation/qualification of each cardiac ion channel assay included E-4031 (65%), verapamil (62%), nifedipine (59%), dofetilide (59%), cisapride (56%), flecainide (53%) and terfenadine (52%) (Table 7). Note that because of survey responders being from both the pharmaceutical industry and CRO’s, some compounds referenced in this table may have been reported by both if such studies were contracted out and conducted by CRO study director scientists. Similarly, positive control drugs that were used frequently for cardiac ion channel studies in order to interpret and compare the IC50 values obtained for NCE’s also included a range of K+, Ca2+ and Na+ channel blockers such as E-4031 (75%), verapamil (72%), terfenadine (69%), dofetilide (67%), nifedipine (64%), flecainide (62%), cisapride (58%), quinidine (57%) and sotalol (55%) (Table 8). One third of responders (34%) estimated the variability (i.e., the range from lowest to highest value obtained) in IC50 values to be <25% while 22% indicated that outcome variability was not formally evaluated and 24% did not know the level of variability of their assays (Table 9). A majority of responders (66%) used a supramaximal concentration of their positive control drug when conducting an in vitro ion channel study in order to determine whether the assay responded appropriately whereas half (49%) used a concentration of the positive control in the range of its IC50 value to assess sensitivity (Table 10). Most (79%) always used the same positive control drug in their ion channel assay whereas a minority selected positive controls to include a comparator/similar drug to the compound being tested (22%) while only 13% used a positive control drug based on the chemistry of the compound being tested. Only 7% used a positive control based on the therapeutic indication of the compound to test (7%) (Table 11).

3.2.3. Arrhythmias

When asked whether drug-induced arrhythmias were encountered in the conduct of non-clinical studies only 22–44% of responders provided some indication of their observation (Table 2). Of the types of drug-induced arrhythmias queried, PVCs were most frequently reported for the standard CV safety model using dogs (80%); however, PVCs were also observed in NHP (59%), rabbit (29%) and guinea pigs (17%) (Table 2). Venricular tachycardia was most frequently observed in dogs (79%) followed by NHP (58%) and rabbits (39%) (Table 2). Ventricular tachycardia was most frequently observed in dogs (79%) followed by NHP (58%) and rabbits (39%) (Table 2). AVB was another common arrhythmia reported (77%) in drug studies involving the use of dogs. Responders also observed drug-induced ventricular fibrillation most frequently in dogs (55%) and NHP (55%). Note that the dog and NHP are the most frequently used non-clinical safety pharmacology...
species thus there is a likely increased propensity for observations of various arrhythmia's developing when NCE's are tested in these species.

3.2.4. Human stem cells derived or iPSC cardiomyocytes

Human stem cell derived or iPSC-CM were considered by most responders as a valuable addition to the spectrum of proarrhythmia screening assays (75%); however, only 26% considered such assays as an economically valuable addition to the safety study arsenal of nonclinical studies (Table 13). While 21% of responders considered human stem cell derived or iPSC-CM representative of adult cardiomyocytes and provide reliable data as a nonclinical safety assay, an equal number (19%) considered that these cells expressed relevant endogenous cardiac ion channels in culture to provide reliable data. Remarkably, only 17% of responders consider that human stem cell derived or iPSC-CM could replace cell lines that stably express human cardiac ion channels.

Table 3
Conduct of in silico studies.

| Answer options                                                                 | Never used | Rarely used | Frequently used | Response count |
|--------------------------------------------------------------------------------|------------|-------------|-----------------|----------------|
| In silico studies are used to evaluate drug effect on hERG                      | 35%        | 32%         | 33%             | 79             |
| In silico studies are used to evaluate drug effect on the Cardiac Action Potential | 42%        | 42%         | 15%             | 78             |
| In silico studies are used to evaluate other effects (Please specify)          | 39%        | 38%         | 33%             | 71             |
| - Genetic toxicity (6)                                                         |            |             |                 |                |
| - Effects on other CV ion channels (2)                                         |            |             |                 |                |
| - Brain penetration (2)                                                        |            |             |                 |                |
| - Others (9)                                                                  |            |             |                 |                |

* Responders selected all that applied.

Table 4
Time of the conduct of in silico (ion channel and AP) and in vitro cardioc electrophysiology assays.

Panel A
What best describes the timing of in silico (ion channel or AP) modeling assays conducted by your company?*

| Answer options                                                                 | Response percent | Response count |
|--------------------------------------------------------------------------------|-----------------|----------------|
| In silico modeling assays are not used by my company                          | 44%             | 33             |
| In silico assays are conducted early in drug discovery                        | 43%             | 32             |
| In silico assays are conducted before any nonclinical data is obtained     | 17%             | 13             |
| In silico assays are conducted during drug development (i.e., after compound nomination) | 13%             | 10             |
| In silico assays are conducted during nonclinical GLP studies               | 12%             | 9              |
| In silico assays are conducted after Phase 1 clinical trials | 1%             | 1              |

Panel B
What best describes the timing of in vitro ion channel electrophysiology assays conducted by your company?*

| Answer options                                                                 | Response percent | Response count |
|--------------------------------------------------------------------------------|-----------------|----------------|
| In vitro electrophysiology assays are not used by my company                  | 7%              | 5              |
| In vitro assays are conducted early in drug discovery/before nonclinical data is obtained | 80%             | 61             |
| In vitro assays are conducted during drug development (i.e., after compound nomination) | 40%             | 30             |
| In vitro assays are conducted during nonclinical GLP studies                 | 30%             | 23             |
| In vitro assays are conducted after Phase 1 clinical trials | 3%              | 2              |

* Responders selected all that applied.

Fig. 2. Applications of in vitro assays in the assessment of cardiovascular liabilities.

Fig. 3. Number of test article concentrations used to determine drug potency (IC50) for channel block (select one).

Fig. 4. The percent of responders that calculate confidence intervals for IC50 values when conducting in vitro ion channel assays.
Has your company ever abandoned a pharmacophore due to an inability to chemically eliminate the potential for hERG channel blockade? (Select one)

- Yes, but rarely (10%)
- Yes, frequently (22%)
- No (49%)
- I do not know (19%)

Fig. 5. The impact of hERG channel blockade on pharmacophore development.

Table 5
The in vitro ion channel testing procedures used.

| Answer options                                      | Response percent | Response count |
|-----------------------------------------------------|------------------|----------------|
| Positive control included for each study            | 82%              | 63             |
| Washout data obtained                               | 43%              | 33             |
| GLP compliant                                       | 48%              | 37             |
| Manual patch clamp                                  | 71%              | 55             |
| Automated patch clamp                               | 75%              | 58             |
| Test conducted at room temperature                  | 71%              | 55             |
| Test conducted at physiological temperature         | 58%              | 45             |

* Responders selected all that applied.

Table 6
Cardiac ion channels tested when using stably expressed cell lines.

| Answer options                                      | Rarely used | Frequently used | Response count |
|-----------------------------------------------------|-------------|----------------|----------------|
| lk,Nav1.5 (sodium channels)                          | 23%         | 77%            | 65             |
| lk,cav1.2 (calcium channels)                         | 20%         | 80%            | 64             |
| lk,Rv4.3 (fast transient outward potassium channel) | 78%         | 22%            | 50             |
| lk,Kv1.5 (ultra-rapid potassium channel)             | 71%         | 29%            | 51             |
| lk,Kv1.4 (slow transient outward potassium channel) | 84%         | 16%            | 44             |
| lk,Kv11.1 (hERG)                                    | 9%          | 91%            | 67             |
| lk,Kv11.1 (KvLQT)                                   | 47%         | 53%            | 55             |
| lk,R2.1–2.3                                       | 78%         | 22%            | 45             |
| Cav2.1 (P/Q calcium channel)                         | 93%         | 8%             | 40             |
| Cav3.2 (T-type calcium channel)                      | 87%         | 13%            | 45             |
| HCN2 (K/Na hyperpolarization-activated pacemaker current) | 95%     | 5%             | 41             |
| IHCN4 (prominent cardiac pacemaker ‘funny’ current)  | 80%         | 20%            | 45             |
| lach,GuK/IKR3.3/3.4 (G-protein coupled inward rectifying K channel) | 88% | 13% | 40 |
| lach,GuK/IKR2.2/SUR2A (ATP-sensitive inward rectifying K channel) | 95% | 5% | 39 |

* Responders selected all that applied.
ICH S7B (US FDA, 2005). As shown in Table 4B, in vitro assays, when used, were conducted early in drug discovery (i.e., frontloaded) during drug development. In vitro assays were predominantly used to assess potential effects of drugs on cardiac cellular electrophysiology processes (Table 2).

A majority (59%) reported that their company had abandoned at least one pharmacophore (or potential ‘toxicophore’) based on data obtained from assays characterizing drug-mediated blockade of hERG. This is an interesting finding that supports the rationale for the CiPA paradigm, originally proposed as the next logical step in drug proarrhythmia testing (Fermini et al., 2016; Gintant et al., 2015). As such, efforts are underway to provide a clear roadmap in the evaluation of the cardiac ion channel assays involved and provide oversight with regard to validation of a standardized testing strategy (Colatsky et al., 2016; Crumb, Vicente, Johannesen, & Strauss, 2016; Sager, Gintant, Turner, Pettit, & Stockbridge, 2014).

In vitro ion channel assays are sensitive to experimental study conditions and data interpretation benefits from the inclusion of positive control drug(s). Most (81%) reported including a positive control agent in each study. A majority (71%) reported conducting in vitro ion channel assays at room temperature, which could reflect the current inability of some automated patch clamp systems to control experimental temperature. Potential differences in the predictive value of assays conducted at physiological or room temperatures are not fully characterized (Zhou et al., 1998) and the optimal testing conditions remain an open agenda item as CiPA efforts progress.

Testing for drug effects on a panel of cardiac ion channels is required for a mechanistic prediction of the net effect of a drug on the ventricular action potential. The various cardiac ion channels were assessed in the following order of importance by responders: Kv11.1 > Ca2+ > Na+, 1.5 > K, 7.1 > K, 1.5 > K, 4, 3 > K, 2.1–2.3. These results suggest that the strategy to evaluate the proarrhythmic risk of an NCE can differ significantly amongst safety pharmacologists. The well characterized contribution of individual ionic currents to the genesis of the ventricular action potential has yet to produce a validated, integrated in silico proarrhythmia liability assay.

Understanding the need to properly evaluate the proarrhythmic risk of a NCE is imperative to safety pharmacologists. It should be recognized that the incidence of nearly all arrhythmia types (apart from heritable channelopathy related arrhythmias) increases with age (Chow, Marine, & Fleg, 2012). Arrhythmias are the most common cause of sudden cardiac death (SCD) in patients with hypertrophic or dilated cardiomyopathy (O’Mahony, Elliott, & McKenna, 2013; Wu & Das, 1999) and ischaemic heart disease (Huikuri, Castellanos, & Myerburg, 2001). Consequent to this, it needs to be recognized that the use of prescription drugs also increases with age (Qato, Wilder, Schumm, Gillet, & Stockbridge, 2014).

### Table 7

| Drugs used as positive controls for validation/qualification of cardiac ion channel assays. | Answer options | Response percent | Response count |
|---|---|---|---|
| Amiodarone | 25% | 20 |
| Amtriptiline | 17% | 14 |
| Atenolol | 28% | 23 |
| Bepridil | 22% | 18 |
| Cisapride | 56% | 45 |
| Clarithromycin | 9% | 7 |
| Dilatazem | 27% | 22 |
| Dofetilide | 59% | 48 |
| E-4031 | 65% | 53 |
| Flecainide | 53% | 43 |
| Ibutidine | 11% | 9 |
| Imipramine | 16% | 13 |
| Loratidine | 12% | 10 |
| Quinidine | 46% | 37 |
| Mexiletine | 27% | 22 |
| Mexofloxacin | 35% | 28 |
| Nifedipine | 50% | 48 |
| Pentamidine | 25% | 20 |
| Pentobarbital | 5% | 4 |
| Pimozide | 17% | 14 |
| Procainamide | 10% | 8 |
| Sotalol | 43% | 35 |
| Terfenadine | 52% | 42 |
| Verapamil | 62% | 50 |
| I do not know | 12% | 10 |

* Responders selected all that applied.

### Table 8

| Drugs used as positive controls for the interpretation and comparison to the test drug during the conduct of cardiac ion channel studies. | Answer options | Rarely used | Frequently used | Response count |
|---|---|---|---|---|
| Amiodarone | 70% | 30% | 33 |
| Bepridil | 81% | 19% | 31 |
| Cisapride | 44% | 58% | 43 |
| Clarithromycin | 91% | 9% | 23 |
| Dilatazem | 63% | 37% | 30 |
| Dofetilide | 33% | 67% | 43 |
| E-4031 | 21% | 79% | 53 |
| Flecainide | 38% | 62% | 42 |
| Ibutidine | 88% | 12% | 25 |
| Imipramine | 83% | 17% | 29 |
| Loratidine | 85% | 15% | 26 |
| Quinidine | 43% | 57% | 35 |
| Mexiletine | 73% | 27% | 30 |
| Mexofloxacin | 56% | 44% | 32 |
| Nifedipine | 36% | 64% | 45 |
| Pentamidine | 72% | 28% | 32 |
| Pentobarbital | 84% | 16% | 25 |
| Pimozide | 79% | 21% | 28 |
| Procainamide | 78% | 22% | 27 |
| Sotalol | 45% | 55% | 38 |
| Terfenadine | 31% | 69% | 39 |
| Verapamil | 28% | 72% | 43 |
| I do not know | – | – | – |

* Responders selected all that applied.

### Table 9

| hERG IC50 value variability for positive control drugs used in the assay. | Answer options | Response percent | Response count |
|---|---|---|---|
| I do not know | 24% | 18 |
| It was not formally evaluated | 22% | 17 |
| 25% | 13% | 10 |
| 75–100% | 4% | 3 |
| 100–200% | 1% | 1 |
| >200% | 1% | 1 |

* Responders selected all that applied.
of arrhythmia species (torsades de pointes) can be lethal. were it not for the fact that proarrhythmia (especially production of toxic effects) between 1 and 4% in humans (Kennedy et al., 1985) and because it is identiﬁed (Authier et al., 2010). This would all be inconsequential because risk is low, not because their nature (Curtis et al., 2013). More serious ventricular arrhythmias including ventricular tachycardia and ventricular fibrillation were reported in all species, especially in dogs, likely also due to their frequent use in cardiac safety studies (Lindgren et al., 2008). When compared to other species, the proportion of responders that observed AVB in dogs was much higher than that in other species. While most responders considered that human stem cell derived or iPSC cardiomyocytes were a valuable addition to the overall proarrhythmia screening assay, potential limitations to their use appeared to include reliability of the cells to represent adult cardiomyocyte phenotypes, cardiac ion channel expression stability, and ﬁnancial considerations. It appears that a certain apprehension within the SP community remains in the implementation of this assay beyond validation. A large proportion of responders (40%) acknowledged that the actual predictive value of nonclinical safety assays for the clinical TQT response has not been formally evaluated. However, publications have addressed the predictive value of QTc measurements in beagle dogs, if not for the TQT outcome, then for QT effects in humans. Ollerstam et al. (2006) explored the PK-PD relationships for QTc prolongation by doxetilide in dogs and humans. This approach has been extended to a larger number of compounds (Parkinson et al., 2013). Ewart et al. (2014) showed very good concordance between dog and human QTc effects for 150 proprietary compounds from 12 pharmaceutical companies. Furthermore, the Health and Environmental Sciences Institute (HESI) Pro-Arrhythmia Working Group recently published a paper (Vargas et al., 2015) that identiﬁed both human and non-rodent animal studies that assessed QT signal concordance between species, based on 40 marketed drugs. The study primarily found that QT interval data derived from relevant non-rodent models had a 90% chance of predicting QT ﬁndings in humans. However, the perception revealed by this survey highlights the need for CIPA to assemble all the available published data on concordance between nonclinical (in silico, in vitro and in vivo) assays/models in order to critically evaluate current non-clinical assays, evaluate drugs with broad ion channel blocking characteristics and derive data-driven recommendations for use.

Table 11
Positive control drugs selected for use in cardiac ion channel studies.

| Answer options                                                                 | Response percent | Response count |
|--------------------------------------------------------------------------------|------------------|----------------|
| We always include the same positive control drugs                              | 79%              | 60             |
| We select positive control drugs based on chemistry of the compound to test    | 13%              | 10             |
| We select positive controls based on the therapeutic indication of the compound to test | 7%               | 5              |
| We select positive controls to include comparator/similar drugs to the compound to test | 22%              | 17             |

* Responders selected all that applied.

Alexander, 2016) which positions drug-induced arrhythmia as a serious safety concern. However, the susceptibility of the young animal population to spontaneous arrhythmia (Gauvin, Tilley, Smith, & Baird, 2009) notably differs from that observed in an older patient population, albeit limited studies are conducted to characterize spontaneous arrhythmias in standard laboratory animals of normal health at any age. Rather, such studies, primarily academic in nature, involve development of arrhythmias by simulating pathology, e.g., by coronary artery ligation (Cheung, Pugsley, & Walker, 1993; Hagerty, Wainwright, & Kane, 1996). Unfortunately, the sensitivity of pre-clinical safety models to drug-induced arrhythmia development has been erroneously perceived to be lower than the sensitivity in the human population placing additional pressure on development of alternate arrhythmia risk assessment methods. This is easily illustrated by the fact that the non-sedating antihistamine, terfenadine, which is regarded as a positive control for torsades de pointes proarrhythmia assessment, in fact elicited torsades de pointes in only a small proportion of patients prescribed the drug (Pugsley et al., 2008). Therefore the imperative to evolve a more predictive approach to proarrhythmia liability testing appears correct; however, the justiﬁcation that animal models used in the assessment for drug-induced proarrhythmia activity lack sensitivity is ﬂawed. A lack of sensitivity in models is appropriate given the low risk of proarrhythmia in humans for all drugs, even drugs with established liability. The safety pharmacologist should recognize that the most important part of the problem is one of preclinical proarrhythmia bioassay sensitivity and speciﬁcity; particularly the ability to detect liability with a feasibly small number of experimental preparations. We make this point because the CiPA initiative, commendable though it is, may be attempting to solve the problem of detecting low risk liability by collecting multiple readouts all of which lack speciﬁcity – because risk is low, not because the magical formula of multiple target effect provide reliable data.

Table 12
Types of arrhythmia observed in nonclinical studies.

| Have you encountered drug induced arrhythmia in nonclinical studies?*     | Rat | Guinea pig | Rabbit | Dog | Pig | Non-human primates |
|---------------------------------------------------------------------------|-----|------------|--------|-----|-----|-------------------|
| I don’t know                                                              | 74% | 68%        | 71%    | 68% | 74% | 58%               |
| Premature ventricular contractions (PVCs)                                 | 15% | 17%        | 29%    | 80% | 15% | 59%               |
| Ventricular tachycardia                                                    | 24% | 27%        | 39%    | 79% | 12% | 58%               |
| Ventricular fibrillation                                                   | 23% | 27%        | 36%    | 53% | 14% | 53%               |
| Atrioventricular (AV) block                                               | 14% | 32%        | 20%    | 77% | 7%  | 41%               |

* Responders selected all that applied.

conceivable that they may respond differently to drugs, it is usually prudent to deﬁne their nature (Curtis et al., 2013). More serious ventricular arrhythmias including ventricular tachycardia and ventricular fibrillation were reported in all species, especially in dogs, likely also due to their frequent use in cardiac safety studies (Lindgren et al., 2008). When compared to other species, the proportion of responders that observed AVB in dogs was much higher than that in other species. While most responders considered that human stem cell derived or iPSC cardiomyocytes were a valuable addition to the overall proarrhythmia screening assay, potential limitations to their use appeared to include reliability of the cells to represent adult cardiomyocyte phenotypes, cardiac ion channel expression stability, and ﬁnancial considerations. It appears that a certain apprehension within the SP community remains in the implementation of this assay beyond validation. A large proportion of responders (40%) acknowledged that the actual predictive value of nonclinical safety assays for the clinical TQT response has not been formally evaluated. However, publications have addressed the predictive value of QTc measurements in beagle dogs, if not for the TQT outcome, then for QT effects in humans. Ollerstam et al. (2006) explored the PK-PD relationships for QTc prolongation by doxetilide in dogs and humans. This approach has been extended to a larger number of compounds (Parkinson et al., 2013). Ewart et al. (2014) showed very good concordance between dog and human QTc effects for 150 proprietary compounds from 12 pharmaceutical companies. Furthermore, the Health and Environmental Sciences Institute (HESI) Pro-Arrhythmia Working Group recently published a paper (Vargas et al., 2015) that identiﬁed both human and non-rodent animal studies that assessed QT signal concordance between species, based on 40 marketed drugs. The study primarily found that QT interval data derived from relevant non-rodent models had a 90% chance of predicting QT ﬁndings in humans. However, the perception revealed by this survey highlights the need for CiPA to assemble all the available published data on concordance between nonclinical (in silico, in vitro and in vivo) assays/models in order to critically evaluate current non-clinical assays, evaluate drugs with broad ion channel blocking characteristics and derive data-driven recommendations for use.

Table 13
Use of human stem cell derived or iPSC cardiomyocytes.

| In your view, human stem cell derived or iPSC cardiomyocytes are:* | Response percent | Response count |
|--------------------------------------------------------------------|------------------|----------------|
| Representative of adult cardiomyocytes and provide reliable data as a nonclinical safety assay | 21%             | 15             |
| A valuable addition to the proarrhythmia screening assays          | 75%             | 54             |
| Can replace cell lines with stable expression of human ion channels | 17%             | 12             |
| Have a stable expression of cardiac ion channels in culture to provide reliable data | 19%             | 14             |
| Are economically valuable to be added to proarrhythmia assays      | 26%             | 19             |
| I don’t know                                                       | 13%             | 9              |

* Responders selected all that applied.

Alexander, 2016) which positions drug-induced arrhythmia as a serious safety concern. However, the susceptibility of the young animal population to spontaneous arrhythmia (Gauvin, Tilley, Smith, & Baird, 2009) notably differs from that observed in an older patient population, albeit limited studies are conducted to characterize spontaneous arrhythmias in standard laboratory animals of normal health at any age. Rather, such studies, primarily academic in nature, involve development of arrhythmias by simulating pathology, e.g., by coronary artery ligation (Cheung, Pugsley, & Walker, 1993; Hagerty, Wainwright, & Kane, 1996). Unfortunately, the sensitivity of pre-clinical safety models to drug-induced arrhythmia development has been erroneously perceived to be lower than the sensitivity in the human population placing additional pressure on development of alternate arrhythmia risk assessment methods. This is easily illustrated by the fact that the non-sedating antihistamine, terfenadine, which is regarded as a positive control for torsades de pointes proarrhythmia assessment, in fact elicited torsades de pointes in only a small proportion of patients prescribed the drug (Pugsley et al., 2008). Therefore the imperative to evolve a more predictive approach to proarrhythmia liability testing appears correct; however, the justification that animal models used in the assessment for drug-induced proarrhythmia activity lack sensitivity is flawed. A lack of sensitivity in models is appropriate given the low risk of proarrhythmia in humans for all drugs, even drugs with established liability. The safety pharmacologist should recognize that the most important part of the problem is one of preclinical proarrhythmia bioassay sensitivity and specificity; particularly the ability to detect liability with a feasibly small number of experimental preparations. We make this point because the CiPA initiative, commendable though it is, may be attempting to solve the problem of detecting low risk liability by collecting multiple readouts all of which lack specificity – because risk is low, not because the magical formula of multiple target effect profile has not yet been identified (Authier et al., 2010). This would all be inconsequential were it not for the fact that proarrhythmia (especially production of torsades de pointes) can be lethal.

The current survey identiﬁed PVCs as the most frequently reported arrhythmia type, especially in large animal species used in the conduct of standard SP studies. However, the survey did not explore the subtype of arrhythmia speciﬁcally deﬁned as a PVC. However, because these arrhythmias are commonly observed with a prevalence estimated between 1 and 4% in humans (Kennedy et al., 1985) and because it is
The anticipated utilization of the CiPA assay differed between responders as some considered it would be better used as a screening tool (56%), while others considered its implementation at the lead stage of drug development (42%) or as part of the FIH package (34%). As the initiative progresses, it is hoped that the CiPA assay is able to impact drug development efforts and better establish a framework to guide proarrhythmia risk assessment. To this effect it is worth mentioning that during the ICH Assembly meeting held in Jacksonville, FL on December 9–10, 2015, the decision was taken to reopen the ICH S7B and E14 Discussion Group with the objective to review emerging data that may influence the content of both ICH S7B and E14 in the future (Anonymous, 2016).

Survey limitations included the potential participation of several employees from the same organization. The response rate (i.e. 10%) was lower than other industry surveys undertaken by the SPS but was comparable to the previous survey on the same topic. The lower response rate may be explained by the specialized nature of CiPA related assays.

5. Conclusion

Proarrhythmia liability assessment in drug development presently includes study types consistent with CiPA. It is anticipated that CiPA will develop into a workable solution to the concern that proarrhythmia liability testing remains suboptimal. The main anticipated value, however, is to avoid falsely identifying drugs as unsafe that in fact are safe in humans (Gintant, Sager, & Stockbridge, 2016; Wallis, 2010), although examples of such drugs in current use are limited to verapamil, ranolazine and alfuzosin. This is rather different from developing a more sensitive integrated approach for detecting proarrhythmia liability. It is important the two are not confused. The present survey indicates that current practice has yet to assimilate this disconnect.

Conflicts of interest

None of the authors have any conflicts of interest, other than their employment in commercial pharmaceutical companies, academic institutions, or contract research organizations. No information is presented in this paper that advocates for or promotes commercial products from any of our organizations.

Disclaimer

This publication reflects the views of the authors and does not represent views or policies of any organization, including the FDA. The views of the authors should not be construed to represent FDA’s views or policies.

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Table 14

| Question | Response percent | Response count |
|----------|-----------------|----------------|
| It was not formally evaluated | 40% | 30 |
| Is 60–75% | 9% | 7 |
| Is 75–90% | 21% | 16 |
| Is >90% | 15% | 11 |
| I do not know | 21% | 16 |

* Responders selected all that applied.

Table 15

| Question | Response percent | Response count |
|----------|-----------------|----------------|
| As a screening tool | 56% | 44 |
| At the lead development stage | 42% | 33 |
| As part of the FIH package | 34% | 27 |
| Strictly as indicated by the regulatory agencies (check box exercise) | 17% | 13 |
| I do not know | 13% | 10 |

* Responders selected all that applied.

Table 16

| Question | Response percent | Response count |
|----------|-----------------|----------------|
| How do you anticipate using the CiPA ion channel assays? | | |
| As a screening tool | 56% | 44 |
| At the lead development stage | 42% | 33 |
| As part of the FIH package | 34% | 27 |
| Strictly as indicated by the regulatory agencies (check box exercise) | 17% | 13 |
| I do not know | 13% | 10 |

* Responders selected all that applied.
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