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

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ABSTRACT

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 Ca^{2+} 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.

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 TRIaD (triangulation, reverse use-dependence, instability and dispersion of repolarization) (Hondeghem, Carlsson, & Duker, 2001; Lawrence, Bridgeland-Taylor, Pollard, Hammond, & Valentin, 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

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and application of human stem cell derived cardiomyocytes for proarrhythmia risk assessment is apparent (Guo et al., 2013; Himmel, 2013; Kirby, Qi, Phatak, Smith, & Malany, 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.*, IC_{50} 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, Stoelzle, George, Behrends, & Fertig, 2006; Comley, 2014; Hamill, Marty, Neher, Sakmann, & Sigworth, 1981; Lepple-Wienhues, Ferlinz, Seeger, & Schäfer, 2003; Obergrussberger 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, Holzgrefe, & 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.

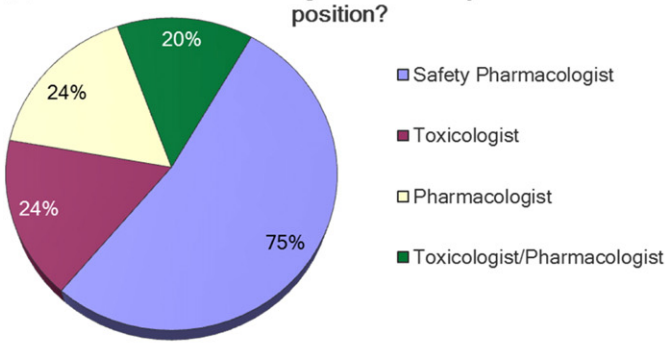
3.2. Approaches that address proarrhythmia & CiPA related drug testing: survey results

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).

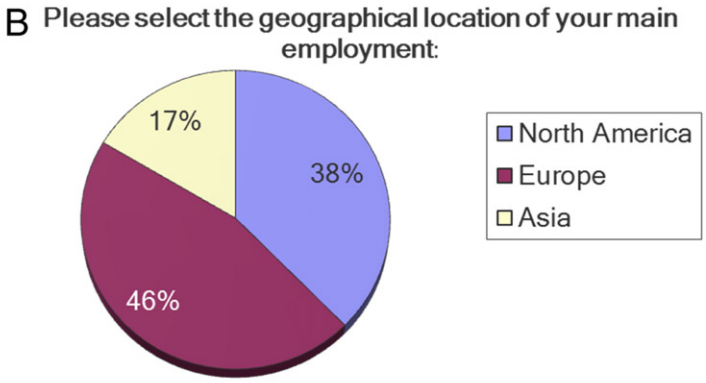
3.2.1. Conduct of in silico assays

One third of responders frequently used *in silico* studies to evaluate drug effects on I_{Kr} ('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).

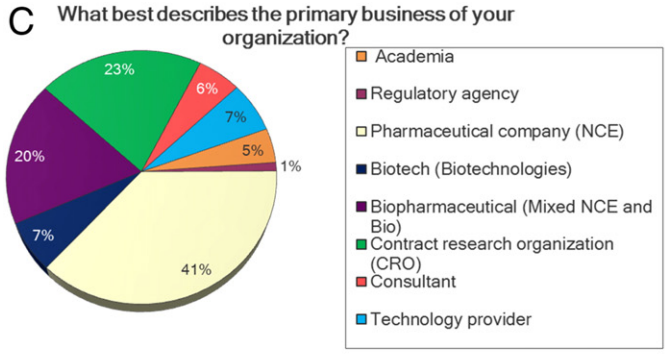
A


Which of the following best describe your current position?
20%
24%
24%
75%
Safety Pharmacologist
Toxicologist
Pharmacologist
Toxicologist/Pharmacologist

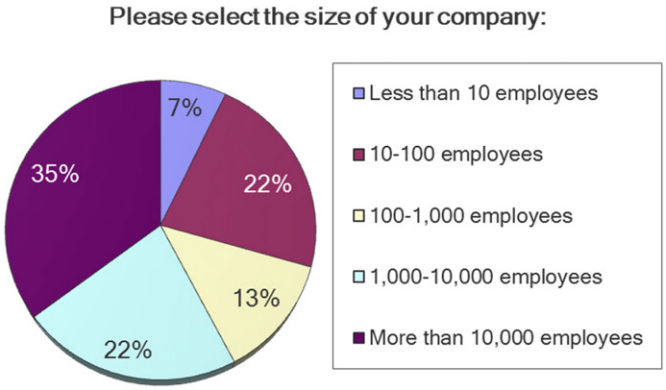
B


Please select the geographical location of your main employment:
17%
38%
46%
North America
Europe
Asia

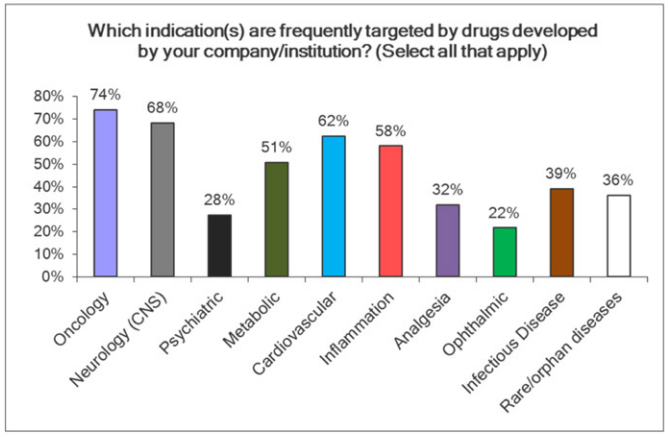
C


What best describes the primary business of your organization?
23%
6%
7%
5%
1%
20%
7%
41%
Academia
Regulatory agency
Pharmaceutical company (NCE)
Biotech (Biotechnologies)
Biopharmaceutical (Mixed NCE and Bio)
Contract research organization (CRO)
Consultant
Technology provider

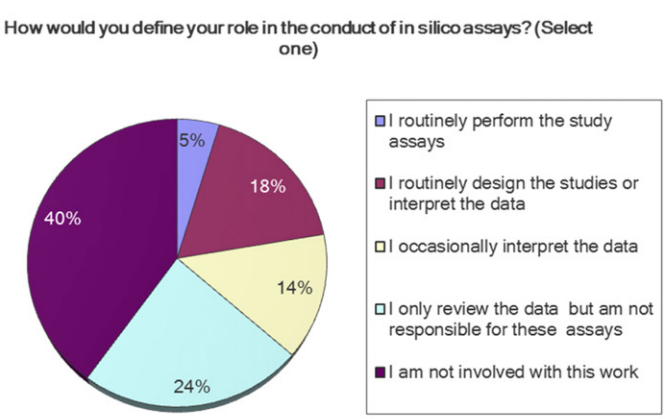
D


Please select the size of your company:
7%
22%
13%
22%
35%
Less than 10 employees
10-100 employees
100-1,000 employees
1,000-10,000 employees
More than 10,000 employees

E


Which indication(s) are frequently targeted by drugs developed by your company/institution? (Select all that apply)
80%
70%
60%
50%
40%
30%
20%
10%
0%
74%
68%
28%
51%
62%
58%
32%
22%
39%
36%
Oncology
Neurology (CNS)
Psychiatric
Metabolic
Cardiovascular
Inflammation
Analgesia
Ophthalmic
Infectious Disease
Rare/orphan diseases

F


How would you define your role in the conduct of in silico assays? (Select one)
5%
18%
40%
14%
24%
I routinely perform the study assays
I routinely design the studies or interpret the data
I occasionally interpret the data
I only review the data but am not responsible for these assays
I am not involved with this work

G

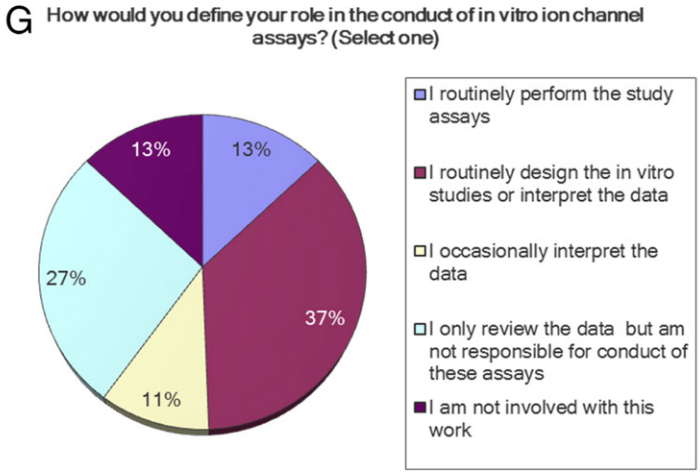

How would you define your role in the conduct of in vitro ion channel assays? (Select one)
13%
13%
27%
37%
11%
I routinely perform the study assays
I routinely design the in vitro studies or interpret the data
I occasionally interpret the data
I only review the data but am not responsible for conduct of these assays
I am not involved with this work

Table 1
Nonclinical approaches used to address proarrhythmia issues.

What best describes the nonclinical approach used to address proarrhythmia issues in your company?[a]		
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
I do not know	4%	3

[a] Responders selected all that applied.

Table 2
Cardiovascular assays used by the pharmaceutical industry.

Which of the following cardiac/cardiovascular assays has your company used in the last 5 years?[a]			
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	88%	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 (iPSC-CM)	46%	54%	68
hERG (I_{Kr}) 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

[a] Responders selected all that applied.

3.2.2. In vitro ion channel electrophysiology assays

When asked to describe the timing of conduct of *in vitro* ion channel electrophysiology assays, most responders (80%) reported using *in vitro* assays during conduct of early safety frontloading (or discovery) phase of investigation before complete nonclinical data was obtained (Table 4, panel B). *In vitro* assays were most frequently used to assess QT prolongation (95%). Cardiac Ca^{2+} (82%) and Na^{+} channel blockade (82%), QT shortening (53%) and QRS prolongation (53%) were also reported as common applications of *in vitro assays* (Fig. 2). Only 33% of responders reported using *in vitro* assays to assess positive or negative cardiac inotropic effects, the remainder likely did not conduct such assessments. When establishing the IC_{50} values for ion channel blockade most responders (37%) reported using four (4) test article concentrations (Fig. 3). A minority (36/85 or 42%) reported calculating confidence intervals for the IC_{50} values determined using *in vitro* ion channel assays (Fig. 4). Many responders (59%) reported that their company had abandoned a pharmacophore due to an inability to eliminate the potential for I_{Kr} blockade (Fig. 5). However, of the 59% reporting only 10% frequently report abandoning a pharmacophore due to an 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, I_{Kr}/Kv11.1 (91%), $I_{Ca,L}$/Cav1.2 (80%), I_{Na}/Nav1.5 (77%) and I_{Ks}/Kv7.1 (53%) were the most frequently interrogated cardiac ion channels when used in stably expressed cell lines (Table 6). Although not as frequent, I_{Kur}/Kv1.5 (29%), $I_{to,f}$/Kv4.3 (22%), I_{K1}/Kir2.1–2.3 (22%) and I_f/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 IC_{50} values obtained for NCE's also included a range of K^{+}, Ca^{2+} and Na^{+} channel blockers such as E-4031 (79%), 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 IC_{50} 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 IC_{50} 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 12). 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 12). Ventricular tachycardia was most frequently observed in dogs (79%) followed by NHP (58%) and rabbits (39%). 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

Fig. 1. Proarrhythmia liability assessment and CiPA Survey Demographics. Panels A–G describe the characteristics of those responding to the survey in terms of expertise (A), geographical location (B), organization affiliation (C), size of the organization (D), indication most frequently targeted by drugs developed from 2011 to 2016 (69 respondents) (E) and role in the conduct of *in silico* assays (F) and *in vitro* assays (G).

Table 3
Conduct of *in silico* studies.

Does your company conduct *in silico* studies (used in the last 5 years or routinely used)?[a]				
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 _____ (fill in the blank with checkboxes) in my assessment of drug safety	39%	38%	23%	71
In silico studies are used to evaluate other effects (Please specify)				19
- Genetic toxicity (6) - Effects on other CV ion channels (2) - Brain penetration (2) - Others (9)				

[a] Responders selected all that applied.

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

Panel A

What best describes the timing of *in silico* (ion channel or AP) modeling assays conducted by your company?[a]		
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?[a]		
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/selection)	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

[a] Responders selected all that applied.

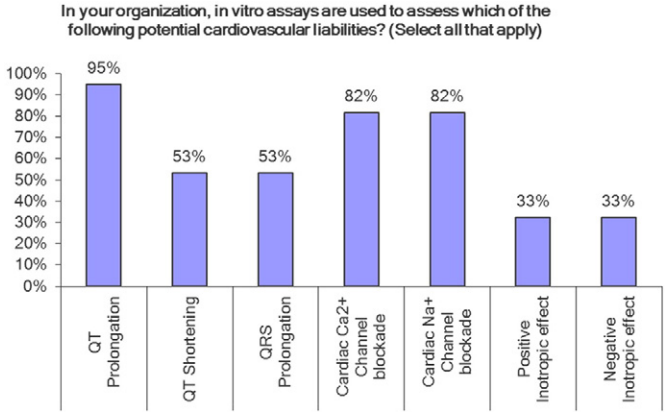


Fig. 2. Applications of *in vitro* assays in the assessment of cardiovascular liabilities.

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.

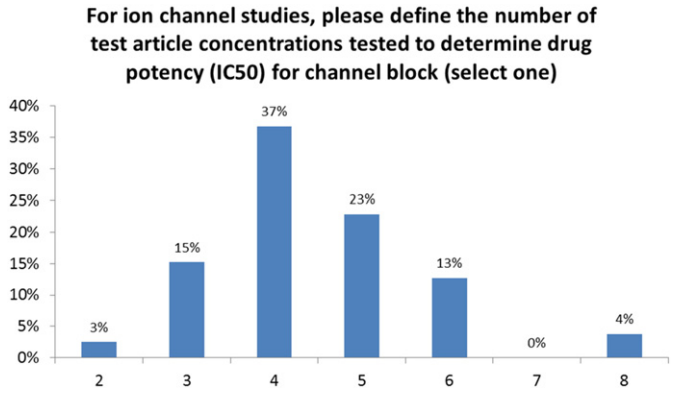


Fig. 3. Number of test article concentrations used to determine drug potency (IC_{50}).

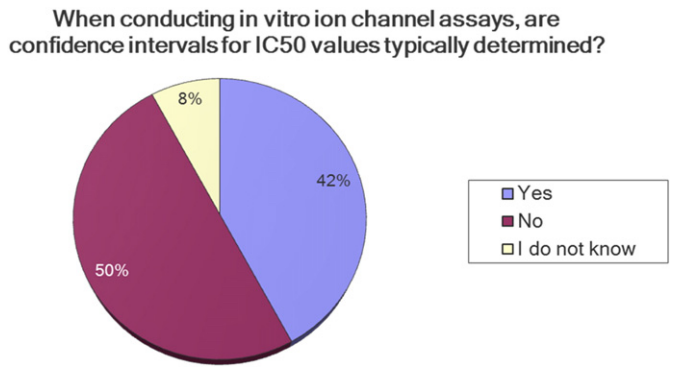


Fig. 4. The percent of responders that calculate confidence intervals for IC_{50} values when conducting *in vitro* ion channel assays.

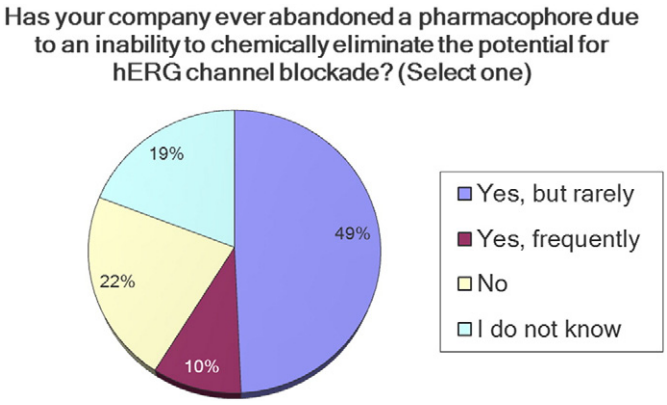


Fig. 5. The impact of hERG channel blockade on pharmacophore development.

Table 5
The *in vitro* ion channel testing procedures used.

Please define the *in vitro* ion channel testing procedures used in your organization.[a]		
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

[a] Responders selected all that applied.

When responders were asked to estimate the predictivity for clinical responses (*e.g.*, study findings from conduct of the Thorough QT (TQT) study) of the nonclinical safety assays used in the integrated risk assessment, only 21% selected 75–90% while only 15% selected >90% (Table 14). 21% of respondents do not know the estimated predictivity for clinical responses of the non-clinical safety assays used while 40% did not formally evaluate predictivity.

3.2.5. CiPA

Currently most responders view the ion channel portion of CiPA as a screening tool (56%) that is likely to be used primarily at the lead development stage (42%) of the drug development spectrum. Only 34% would consider including it as a component of the first-in-human (FIH) regulatory submission package (Table 15) while 17% consider the CiPA ion channel assay as a requirement strictly indicated by the regulatory agencies (*i.e.*, it currently would be only conducted as a check box exercise). Note, however, that 13% of responders currently do not know how they would anticipate using the CiPA ion channel assays in drug development.

4. Discussion

The present survey aimed to identify current strategies that are used by those in the pharmaceutical industry, in particular safety pharmacologists, for non-clinical safety testing as they relate to proarrhythmia liability assessment. In addition, a focus was placed on assays that are currently under evaluation within the CiPA initiative. The geographic region that contributed the largest number of responses was Europe. This proportion contrasts with prior SPS surveys (Authier, Vargas, Curtis, Holbrook, & Pugsley, 2013; Authier et al., 2016; Friedrichs, Patmore, & Bass, 2005; Lindgren et al., 2008) for which North America was the predominant region. The percentage of responders involved in the development of small molecules reached 61%, which likely reflects a selection bias whereby scientists developing NCE's are more likely to be involved in proarrhythmia liability testing. It should be noted that CiPA is currently considered for small molecule proarrhythmia liability assessment.

A pre-established battery of assays to screen drug candidates was used by most (75%) responders. This is perhaps somewhat surprising given that the common perception within SP testing is that approaches are taken in which studies are tailored with regard to indication, risk/benefit considerations and other factors such as whether the molecule is a biological or NCE (Pugsley, Authier, & Curtis, 2008). However, as has been pointed out previously, in the case of NCEs, potency in these assays has little to do with therapeutic class (Redfern et al., 2003). Amongst available assays, cell lines with stably expressed ion channels and telemetry acquired ECG were used by most (89% and 88% of responders, respectively). Other assays had varying degrees of utilization which likely reflects historical practices within each organization, the perceived proarrhythmic risk of the drug candidate (*i.e.*, science driven selection of proarrhythmia assays) based upon therapeutic area and the validation data available to support the added value of a given test as part of the overall risk assessment. As expected, given that CiPA is an emerging drug safety screening paradigm that is not yet fully validated, application of *in silico* assessment was not widely implemented. At present, *in silico* assays were most frequently reported (43%) to be used early in drug discovery. When *in vitro* assays were used, screening for I_{Kr} block remained the most frequent application followed by assessment of the effects on cardiac action potentials (Pollard et al., 2010; Valentin, 2010; Valentin, Pollard, Lainée, & Hammond, 2010). This likely reflects the 'hERG-centric' approach to proarrhythmia testing which has prevailed within SP for well over a decade, consistent with issuance of

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

If you are using cell lines with stably expressed ion channels, which of the following have you tested in the last 5 years and which ion channels are routinely tested?[a]			
Answer options	Rarely used	Frequently used	Response count
I_{Na}/Nav1.5 (sodium channels)	23%	77%	65
$i_{ca,l}$/cav1.2 (calcium channels)	20%	80%	64
$I_{to,f}$/Kv4.3 (fast transient outward potassium channel)	78%	22%	50
I_{Kur}/Kv1.5 (ultra-rapid potassium channel)	71%	29%	51
$I_{to,s}$/Kv1.4 (slow transient outward potassium channel)	84%	16%	44
I_{Kr}/Kv11.1 (hERG)	9%	91%	67
I_{Ks}/Kv7.1 (KvLQT1)	47%	53%	55
I_{K1}/Kir2.1–2.3	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
I_f/HCN4 (prominent cardiac pacemaker 'funny' current)	80%	20%	45
I_{KAch}/(GIRK)Kir3.1/3.4 (G-protein coupled inward rectifying K channel)	88%	13%	40
I_{KATP}/Kir6.2/SUR2A (ATP-sensitive inward rectifying K channel)	95%	5%	39

[a] Responders selected all that applied.

Table 7
Drugs used as positive controls for validation/qualification of cardiac ion channel assays.

Please indicate the control drugs used for ion channel assay validation/qualification in your organization in the last 5 years.[a]		
Answer options	Response percent	Response count
Amiodarone	25%	20
Amitriptyline	17%	14
Astemizole	28%	23
Bepridil	22%	18
Cisapride	56%	45
Clarithromycin	9%	7
Diltiazem	27%	22
Dofetilide	59%	48
E-4031	65%	53
Flecainide	53%	43
Ibutilide	11%	9
Imipramine	16%	13
Loratidine	12%	10
Quinidine	46%	37
Mexilitine	27%	22
Moxifloxacin	35%	28
Nifedipine	59%	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

[a] 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 I_{Kr}. 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).

Table 8
Drugs used as positive controls for the interpretation and comparison to the test drug during the conduct of cardiac ion channel studies.

Please indicate the drugs that were used as positive controls for ion channel studies (interpretation and comparison with the test drug) in your organization in the last 5 years.[a]			
Answer options	Rarely used	Frequently used	Response count
Astemizole	70%	30%	33
Bepridil	81%	19%	31
Cisapride	44%	58%	43
Clarithromycin	91%	9%	23
Diltiazem	63%	37%	30
Dofetilide	33%	67%	43
E-4031	21%	79%	53
Flecainide	38%	62%	42
Ibutilide	88%	12%	25
Imipramine	83%	17%	29
Loratidine	85%	15%	26
Quinidine	43%	57%	35
Mexilitine	73%	27%	30
Moxifloxacin	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	–	–	12

[a] Responders selected all that applied.

Table 9
hERG IC_{50} value variability for positive control drugs used in the assay.

Please estimate the variability (*i.e.*, the range from lowest to highest value) in IC_{50} values that you have determined for hERG inhibition using the same positive control drug over the past year.[a]		
Answer options	Response percent	Response count
I do not know	24%	18
It was not formally evaluated	22%	17
<25%	34%	26
25–75%	13%	10
75–100%	4%	3
100–200%	1%	1
>200%	1%	1

[a] Responders selected all that applied.

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: $K_v11.1 > Ca_v1.2 > Na_v1.5 > K_v7.1 > K_v1.5 > K_v4.3 > K_{ir}2.1–2.3$. 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). Concurrent to this, it needs to be recognized that the use of prescription drugs also increases with age (Qato, Wilder, Schumm, Gillet, &

Table 10
Use of a positive control drug during the conduct of *in vitro* ion channel assays.

When using a positive control drug in an *in vitro* ion channel assay, how is the positive control utilized?[a]		
Answer options	Response percent	Response count
To determine if the assay responds appropriately by using a supramaximal concentration of the positive control	66%	47
To determine assay sensitivity by using a concentration of the positive control that is similar to its IC_{50} value	49%	35
A positive control drug is not used in ion channel assays	3%	2

[a] Responders selected all that applied.

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

Please indicate how positive control drugs are selected for ion channel studies[a]		
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

[a] Responders selected all that applied.

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

In your view, human stem cell derived or iPSC cardiomyocytes are:[a]		
Answer options	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 do not know	13%	9

[a] 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 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 dofetilide 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 12
Types of arrhythmia observed in nonclinical studies.

Have you encountered drug induced arrhythmia in nonclinical studies?[a]							
	Rat	Guinea pig	Rabbit	Dog	Pig	Non-human primates	Response count
I don't know	74%	68%	71%	68%	74%	58%	31
Premature ventricular contractions (PVCs)	15%	17%	29%	80%	15%	59%	41
Ventricular tachycardia	24%	27%	39%	79%	12%	58%	33
Ventricular fibrillation	23%	27%	36%	55%	14%	55%	22
Atrioventricular (AV) block	14%	32%	20%	77%	7%	41%	44

[a] Responders selected all that applied.

Table 14
Predictivity of nonclinical safety assays for the clinical (TQT) response.

Please estimate the predictivity for clinical responses (*e.g.*, TQT) of the nonclinical safety assays used in your organization when considering integrated QT risk assessment[a]		
Answer options	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

[a] Responders selected all that applied.

Table 15
Anticipated use of the CiPA ion channel assay.

How do you anticipate using the CiPA ion channel assays?[a]		
Answer options	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

[a] Responders selected all that applied.

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 recent 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.

Conflict 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.

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