Review

Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development


¹Safety Assessment UK, AstraZeneca R&D Alderley Park, Macclesfield, Cheshire SK10 4TG, UK  
²Cardiovascular Pharmacology, AstraZeneca R&D Mölndal, 431 83 Mölndal, Sweden  
³Animal Welfare Group, AstraZeneca R&D Alderley Park, Macclesfield, Cheshire SK10 4TG, UK  
⁴Drug Safety, AstraZeneca R&D Charnwood, Loughborough, Leicestershire LE11 5RH, UK  
⁵Covance Laboratories Limited, Otley Road, Harrogate, North Yorks HG3 1PY, UK  
⁶Merck Research Laboratories, P.O. Box 4, West Point, PA 19486-00047, USA  
⁷GlaxoSmithKline Safety Assessment, The Frythe, Welwyn, Herts AL6 9AR, UK  
⁸Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK  
⁹Department of Cardiological Sciences, St George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK

Received 1 July 2002; accepted 21 November 2002

Abstract

Objective: To attempt to determine the relative value of preclinical cardiac electrophysiology data (in vitro and in vivo) for predicting risk of torsade de pointes (TdP) in clinical use. Methods: Published data on hERG (or Iₖₑ) activity, cardiac action potential duration (at 90% repolarisation; APD₉₀), and QT prolongation in dogs were compared against QT effects and reports of TdP in humans for 100 drugs. These data were set against the free plasma concentrations attained during clinical use (effective therapeutic plasma concentrations; ETPC_unbound). The drugs were divided into five categories: (1) Class Ia and III antiarrhythmics; (2) Withdrawn from market due to TdP; (3) Measurable incidence/numerous reports of TdP in humans; (4) Isolated reports of TdP in humans; (5) No reports of TdP in humans. Results: Data from hERG (or Iₖₑ), IC₅₀, APD₉₀, QTc in animals and QTc in humans were generally close to or superimposed on the ETPC_unbound values. This relationship was uncoupled in the other categories, with more complex relationships between the data. In Category 1 (except amiodarone), the ratios between hERG/Iₖₑ, IC₅₀ and ETPC_unbound (max) ranged from 0.1- to 31-fold. Similar ranges were obtained for drugs in Category 2 (0.31- to 13-fold) and Category 3 (0.03- to 35-fold). A large spread was found for Category 4 drugs (0.13- to 35 700-fold); this category embraced an assortment of mechanisms ranging from drugs which may well be affecting Iₖₑ currents in clinical use (e.g. sparfloxacin) to others such as nifedipine (35 700-fold) where channel block is not involved. Finally, for the majority of Category 5 drugs there was a >30-fold separation between hERG/Iₖₑ activity and ETPC_unbound values, with the notable exception of verapamil (1.7-fold), which is free from QT prolongation in man; this is probably explained by its multiple interactions with cardiac ion channels. Conclusions: The dataset confirms the widely-held belief that most drugs associated with TdP in humans are also associated with hERG K⁺ channel block at concentrations close to or superimposed upon the free plasma concentrations found in clinical use. A 30-fold margin between Cmax and hERG IC₅₀ may suffice for drugs currently undergoing clinical evaluation, but for future drug discovery programmes, pharmaceutical companies should consider increasing this margin, particularly for drugs aimed at non-debilitating diseases. However, interactions with multiple cardiac ion channels can either mitigate or exacerbate the prolongation of APD and QT that would ensue from block of Iₖₑ currents alone, and delay of repolarisation per se is not necessarily torsadogenic. Clearly, an integrated assessment of in vitro and in vivo data is required in order to predict the torsadogenic risk of a new candidate drug in humans.

*Corresponding author. Tel.: +44-1625-514-810; fax: +44-1625-513-779.
1. Introduction

Drug-induced QT interval prolongation and the appearance of torsade de pointes (TdP) has become recognised as a potential risk during treatment with a broad range of drugs including repolarisation-delaying antiarrhythmics [1], various antihistamines [2,3], antipsychotics [4], antimicrobial agents [5], and miscellaneous others [5–10]. For the vast majority of these agents it has been demonstrated that the slowing of repolarisation is a consequence of inhibition of the rapidly activating delayed rectifier potassium current ($I_{Kr}$) in cardiac tissues, which is conveyed by the human ether-a-go-go-related gene-encoded voltage-dependent potassium channel (hERG K$^+$ channel; IUPHAR classification: K$^+_11.1$ [11]).

In 1997, the Committee for Proprietary Medicinal Products (CPMP) published a ‘Points to Consider’ document that made recommendations for non-clinical and clinical approaches to assess the risk of QT interval prolongation and TdP for non-cardiovascular drugs [12]. Since the appearance of this document, there has been much debate within the pharmaceutical industry as to the relative merits of various in vitro and in vivo techniques in detecting such activity preclinically [13–15]. A survey of practice within the industry conducted approximately 2 years after publication of the CPMP document indicated that whereas the industry was taking this issue very seriously, there was wide variability in approach [16].

One reason for the lack of confidence in the predictive value of the preclinical tests is that each drug associated with TdP in man appears to tell a different story in terms of its electrophysiological profile. Clearly there is a consensus about the importance of interactions with the hERG K$^+$ channel but even so there is no agreement, either within the pharmaceutical industry or drug regulatory authorities, about what constitutes a safe margin. Risk assessment is further complicated when a drug acts on other cardiac ion channels in addition to the hERG K$^+$ channel, as is often the case.

We therefore undertook a literature survey of non-clinical and clinical data on an initial list of 100 drugs, in order to assess the predictivity of the various types of data. The drugs were first categorised according to their torsadogenic propensity, then information on effective free therapeutic plasma concentration (ETPC\textsubscript{unbound}) with inhibition of hERG/$I_{Kr}$ current, effects on cardiac action potential duration in vitro, prolongation of QT interval in dogs, and QT prolongation in man were collated and tabulated. It is hoped that these data, presented together for the first time, will assist in the ongoing debate around the preclinical detection of torsadogenic propensity, provide evidence for setting provisional safety margins, and encourage further research.

2. Methods

The approach was as follows:

1 A list of 100 drugs was compiled, covering a range of therapeutic classes. This list included the majority of drugs that have been associated with TdP or QT prolongation in man, together with other drugs that have no such association, but which have come under a certain degree of scrutiny because of therapeutic class, drug interactions, etc., in addition to drugs designed to delay cardiac repolarisation (i.e. Class Ia and III antiarrhythmics).

2 These drugs were assigned to five categories of torsadogenic propensity:

   - **Category 1**: Repolarisation-prolonging (Class Ia and Class III) antiarrhythmics (which have $I_{Kr}$ block as an integral pharmacodynamic mechanism, and QT prolongation as an intended, desirable effect).
   - **Category 2**: Drugs that have been withdrawn or suspended from the market in at least one major regulatory territory due to an unacceptable risk of TdP for the condition being treated.
   - **Category 3**: Drugs that have a measurable incidence of TdP in humans, or for which numerous case reports exist in the published literature.
   - **Category 4**: Drugs for which there have been isolated reports of TdP in humans.
   - **Category 5**: Drugs for which there have been no published reports of TdP in humans. This category also contains some drugs (e.g. ketoconazole) which are associated with drug interactions leading to TdP, but which have not been associated with cases of TdP when used alone.

N.B. Erythromycin appears twice in the above list, as there are two formulations with different torsadogenic propensities: intravenous (Category 3) and oral (Category 4). Mibefradil was withdrawn from the market after less than a year due to drug interactions leading to TdP; however, there was no clear evidence that this drug caused...
TdP by itself [17], so it has not been assigned to Category 2. Instead, it has been assigned to Category 4, as there is one report of TdP in the literature [18].

3. The following data were collated:
   (a) Drug name, drug category (as above), molecular weight;
   (b) Published values and ranges for % plasma protein binding and ETPC$_{\text{total}}$; these were obtained from online databases for drugs in current use (or from standard medical reference texts for older drugs) where possible (e.g. Refs. [19,20]), in order to limit the number of references cited. ETPC$_{\text{unbound}}$ (nM) were then calculated for each drug. Where published values for ETPC$_{\text{unbound}}$ (nM) were available and were greater than those calculated, this higher value was used.
   (c) Published IC$_{50}$ values for I$_{Kr}$ in mammalian cardiac tissue and from hERG-transfected cell lines. HERG data from Xenopus oocytes were not used unless there were no other hERG or I$_{Kr}$ data available. Data from hERG or I$_{Kr}$ assays (hereafter referred to as ‘hERG/I$_{Kr}$’) and all other electrophysiological data (see below) were derived from original references obtained via MEDLINE (between 1966 and May 2002).
   (d) Published potency values for eliciting a 10–20% increase in the duration of the cardiac action potential at 90% repolarisation (APD$_{90}$) in in vitro preparations. A 10% increase in APD$_{90}$ is typically the minimum increase that reaches statistical significance in such studies, and is also the degree of change considered physiologically significant by the majority of pharmaceutical companies in a recent survey [16]. Frequently it was not possible to determine the concentration producing a 10–20% increase in APD$_{90}$ interval, so the concentration producing the percentage increase in APD$_{90}$ closest to this range was used.
   (e) Published values for doses associated with 10–20% QTc prolongation in beagle dogs, or other species if dog data were unavailable. A 10% increase in QTc is typically the minimum increase that reaches statistical significance in conscious dog studies, and is also the degree of change considered physiologically significant by the majority of pharmaceutical companies in the same survey [16]. Frequently it was not possible to determine the dose producing a 10–20% increase in QTc interval, so the dose producing the percentage increase in QTc closest to this range was used. There are also examples where the percentage change is either not quoted in the original source reference or is not possible to calculate because of the absence of baseline data.
   (f) Published values for plasma concentrations associated with 10–20% QTc prolongation in beagle dogs, or other species if dog data were unavailable. Such data were found to be rarely available outside Category 1.
   (g) Published values for doses associated with 10–20% QTc prolongation in humans. This degree of change was chosen in order to be consistent with data obtained from dogs. Plasma concentrations are presented where available, but such data are extremely rare outside Category 1.

4. For 52 drugs where both ETPC$_{\text{unbound}}$ and hERG (or I$_{Kr}$) IC$_{50}$ data were available, the ETPC$_{\text{unbound}}$ range was plotted schematically alongside the electrophysiology data. For terfenadine, given that the QT prolonging effects are due to the parent compound whereas the antihistamine activity is due to the metabolite, data for ETPC$_{\text{unbound}}$ were obtained from studies that had measured terfenadine in the presence of a cytochrome P$_{450}$ inhibitor.
   5. The ratios of the lowest published hERG/I$_{Kr}$ IC$_{50}$ value divided by the upper value for the ETPC$_{\text{unbound}}$ range were calculated and plotted on a logarithmic scale for all 52 drugs.
   6. Of these 52 drugs, reasonably complete concentration–effect data were available for three drugs (one from each of categories 1, 2 and 5) for hERG/I$_{Kr}$, APD$_{90}$, dog QT interval, and human QT interval, and these were plotted as overlay plots alongside the ETPC$_{\text{unbound}}$ range.

Full datasets with source references are accessible via the electronic version of this article (http://www.elsevier.com/homepage/sab/cardio/doc/Redfern_full.pdf), or are available from the authors on request.

3. Results

The initial list of 100 drugs covered a wide variety of therapeutic indications and dosing regimens. However, numerous drugs in this list had incomplete information, either for ETPC$_{\text{unbound}}$ data or electrophysiological data. Ultimately, from the original list of 100 drugs, 52 had data on both ETPC$_{\text{unbound}}$ and hERG/I$_{Kr}$.

Fig. 1 is a schematic plot of ETPC$_{\text{unbound}}$ data alongside the in vitro and in vivo electrophysiology data, for the 52 drugs where data were available for both ETPC$_{\text{unbound}}$ and hERG/I$_{Kr}$. For ETPC$_{\text{unbound}}$ data, where ranges are available these are plotted; otherwise, a single concentration is presented. For the hERG/I$_{Kr}$ data, the band is plotted from the minimum published IC$_{50}$ value upwards. Data on effects on APD$_{90}$ (lowest published values for concentrations causing a 10–20% increase in APD$_{90}$ in in vitro preparations) were available for the majority of these drugs, including all drugs in Categories 1 and 2. Data on effects on QTc in animals (lowest published values for
concentrations causing a 10–20% increase in QTc in vivo) and on QTc in humans (lowest published values for concentrations causing a 10–20% increase in QTc in humans) are also plotted where available.

3.1. hERG/I_{Kr}

There was a trend with respect to the hERG/I_{Kr}, IC_{50} values, in that the potency tended to be weaker when
moving from Categories 2 to 5. However, the range of potencies within Category 1 was about as large as that covering the other four categories. Far more striking was the separation between ETPC\textsubscript{unbound} (max) and the lowest quoted hERG/I\textsubscript{Kr} IC\textsubscript{50} value. With the exception of amiodarone, most Category 1 drugs (‘Class Ia and Class III antiarrhythmics’) had hERG/I\textsubscript{Kr} activity close to, or superimposed on, their ETPC\textsubscript{unbound} values. The same was true for Categories 2 (‘withdrawn/suspended due to TdP’) and 3 (‘measurable incidence/numerous reports of TdP’). There was a mixed picture for drugs assigned to Category 4 (‘isolated reports of TdP in humans’). Finally, for Category 5 drugs (‘no reports of TdP in humans’), there was a clear separation between hERG/I\textsubscript{Kr} activity and ETPC\textsubscript{unbound} values. There was one notable exception to this: verapamil, which is free from QT prolongation despite hERG/I\textsubscript{Kr} activity close to its ETPC\textsubscript{unbound} range.

### 3.2. APD\textsubscript{90}

Fig. 1 also indicates that for drugs in Category 1, concentrations causing a 10–20% increase in APD\textsubscript{90} were reasonably close to the concentrations showing hERG/I\textsubscript{Kr} activity and the ETPC\textsubscript{unbound} range, as might be expected, with the exception of amiodarone. Most of the drugs in Category 2 also increased APD\textsubscript{90} at concentrations close to the lowest published values for hERG/I\textsubscript{Kr} IC\textsubscript{50}. Data on effects on APD\textsubscript{90} were only available for four drugs in Category 3 (thioridazine, bepridil, flecainide and erythromycin) for which hERG/I\textsubscript{Kr} data were also available. In each case APD\textsubscript{90} was increased, but at concentrations higher than those required to inhibit hERG/I\textsubscript{Kr}; for bepridil and flecainide, APD\textsubscript{90} was shortened at higher concentrations. There was no clear pattern to effects on APD\textsubscript{90} in Category 4, with some drugs increasing this parameter and others decreasing it or having biphasic effects. Of the six drugs in Category 5 for which APD\textsubscript{90} data (in addition to hERG/I\textsubscript{Kr} data) were available, chlorpheniramine increased APD\textsubscript{90}, verapamil had a biphasic effect, mefloquine and diltiazem decreased it, and ebastine and tamoxifen had no effect.

### 3.3. QTc in vivo

Similarly, for Category 1 drugs, concentrations causing a 10–20% increase in QTc in vivo were generally close to the ETPC\textsubscript{unbound} range, the hERG/I\textsubscript{Kr} IC\textsubscript{50} and the concentrations causing a 10–20% increase in APD\textsubscript{90} (Fig. 1). Here again, the notable exception was amiodarone, where the QTc change was observed within the ETPC\textsubscript{unbound} range, whereas the in vitro electrophysiology changes required much higher concentrations (as mentioned above). In Category 2, a 10–20% increase in QTc in vivo was achieved at lower concentrations than the hERG/I\textsubscript{Kr} IC\textsubscript{50} for cisapride, sertindole and terfenadine, whereas for astemizole this effect was achieved within the published range of values for hERG/I\textsubscript{Kr} IC\textsubscript{50} (Fig. 1).

### 3.4. QTc in humans

Again, for drugs in Category 1, the plasma concentrations resulting in a 10–20% increase in QTc in humans were close to those causing electrophysiological effects in the preclinical tests (Fig. 1). Unfortunately, such data were rarely available outside this category, so no clear pattern can be discerned.

### 3.5. Margins between hERG/I\textsubscript{Kr} IC\textsubscript{50} and ETPC\textsubscript{unbound}

The margins between free therapeutic plasma concentration and activity at hERG/I\textsubscript{Kr} for these drugs are shown in Fig. 2. These are the ratios of the lowest quoted hERG/I\textsubscript{Kr} IC\textsubscript{50} value divided by the upper value for the ETPC\textsubscript{unbound} range, plotted on a logarithmic scale. Within Category 1 (Class Ia and III antiarrhythmics), all the drugs listed had margins of <30-fold, with the exception of tedsamil (31-fold) and amiodarone (1400-fold), which both have a low torsadogenic propensity. All drugs withdrawn/suspended from sale due to TdP (Category 2) had margins well below 30-fold (range 0.01- to 13-fold). Currently-marketed drugs associated with a measurable incidence/numerous reports of TdP (Category 3) also had margins of less than 30-fold, with the exception of pimozide which was just outside this value (35-fold). For drugs in Category 4 (‘isolated reports of TdP’), there was a wide range of margins, from 0.13-fold (sparfloxacin) to 35 700-fold (nifedipine). Finally, for those drugs where there are no reports of TdP in clinical use (Category 5), the margins ranged from 23-fold to 3311-fold, with the exception of verapamil (1.7-fold) and ketoconazole (11-fold). The apparent ‘outliers’ within each category are commented on in the Discussion.

Relatively few drugs in Category 5 have been investigated for effects on APD\textsubscript{90} and QTc, so equivalent plots of APD\textsubscript{90} and QTc margins would be of limited value, especially as there are complications of species differences, biphasic concentration–response curves, and imprecise or incomplete data (e.g. concentrations giving 10–20% increases not always available). Instead, representatives of categories 1, 2 and 5 have been chosen for overlay plots, to illustrate what would be possible with complete datasets. Fig. 3 is a set of overlay plots for three drugs where concentration–effect data are available for most of the electrophysiological variables. These more detailed overlay plots confirmed and extended the above findings. For dofetilide (an example of a Category 1 drug) the concentration–effect plots for hERG/I\textsubscript{Kr}, APD\textsubscript{90} and human QTc were superimposable. In the case of terfenadine (Category 2), the effects on APD\textsubscript{90} and QT were either
### 4. Discussion

#### 4.1. Mechanisms involved in torsade de pointes

TdP is a potentially life-threatening ventricular tachyarrhythmia characterised by QRS complexes continuously...

---

**Fig. 2.** Log margins of lowest published values for hERG IC$_{50}$ divided by the upper end of the ETPC$_{bound}$ range. Plots to the left of the origin indicate hERG activity within the therapeutic plasma concentration range. The vertical dotted line indicates a ratio of 30. For terfenadine, the margin is based on a value of 4 nM for ETPC$_{bound}$ (max) as a concentration attained in the presence of a P$_i$ inhibitor. For source data refer to Methods.

<table>
<thead>
<tr>
<th>Log margin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

**Minimal or absent.** For these two drugs, the clinical free plasma concentration range encroached on the foot of the hERG concentration–response plots, whereas for tamoxifen (Category 5) there was clear separation. Also, this drug had no effect on APD$_{50}$ and QTc, and has not been associated with TdP.
Category 1: dofetilide

Category 2: terfenadine

Category 5: tamoxifen

Fig. 3. Overlay plots of concentration–effect data for \( I_{\text{hERG}} \), APD90, QT in dog, and QT in human for representatives of categories 1, 2 and 5. In vivo data are plotted as unbound plasma concentration. Rectangle indicates effective therapeutic plasma concentration (unbound) in humans; for terfenadine, this indicates the range obtained in the presence of a \( \text{P}_{\text{glyc}} \) inhibitor.
changing in morphology around an imaginary isoelectric line. The trigger for drug-induced TdP arrhythmias appears to be ventricular extra beats which can be induced by early depolarisations originating in cells with relatively long repolarisation phases (typically Purkinje fibres and/or midmyocardial cells). Whether focal activity or re-entrant pathways are responsible for the perpetuation of the arrhythmia is still unclear [8,21,55,56,66] and both mechanisms may co-exist [22]. TdP episodes can either be self-terminating or degenerate into ventricular fibrillation.

Compelling evidence exists for \( I_{Ks} \) inhibition as a major risk factor for TdP, as will be discussed. Agents that act primarily by blocking the slowly activating delayed rectifier potassium channel (\( I_{Ks} \)), or by retarding the inactivation (or the recovery from the inactivation) of the sodium and the L-type calcium channels impede repolarisation, and thus could be expected to provoke TdP. Mefloquine is an example of an \( I_{Ks} \) blocker (it is slightly more potent at inhibiting this current than at \( I_{Kr} \) [23], but also inhibits \( I_{Ca,L} \) [24]) within our dataset, yet it has not been associated with TdP. More widespread clinical experience with drugs that are relatively selective for \( I_{Ks} \) would be required before excluding a contribution of \( I_{Ks} \) block as a risk factor for TdP in humans. Other risk factors for drug-induced TdP include female gender, bradycardia, hypokalemia, drug–drug interactions, rapid drug administration (intravenous), structural heart disease, prolonged baseline QT interval and genetic variants (polymorphisms and mutations) [25]. It has been suggested that these risk factors act by reducing the net repolarising current, thereby limiting the ‘repolarisation reserve’ [26]. TdP can also occur as a result of localised cardiac ischaemia, and such arrhythmias are seen, albeit rarely, after myocardial infarction [27].

### 4.2. Limitations of the dataset

It is worthwhile discussing the limitations of our dataset before attempting to draw any conclusions from it. Firstly, it is restricted to drugs that have reached the market (or at least, late stage clinical development), some of which have been tested for effects on QT in dogs, in a cardiac action potential preparation in vitro, and (for half of them) at hERG/\( I_{Kr} \). Up until now, quite understandably, in vitro electrophysiologists have focused their investigations on those drugs that have either been associated with QT prolongation and TdP in man, or have come under suspicion due to their therapeutic class. It is quite likely that a ‘hERG screening of the pharmacopoea’ would unearth some potent hERG \( K^- \) channel blockers that have never been associated with QT prolongation or TdP, to add to one such compound in our dataset: verapamil (see below). Secondly, the relative torsadogenic propensity in clinical use is difficult to judge within each of our categories, as this would also have to take into account the size and nature of the patient population, and the typical duration of treatment. In addition, some of the apparent incidences of TdP in Category 4 (‘isolated reports of TdP’) may have been misdiagnosed. Thirdly, estimates of free plasma concentrations are dependent on the accuracy of published data, particularly plasma protein binding. Finally, it should be noted that the purpose of compiling our dataset was entirely with future drug development in mind, and the collated information should not be applied retrospectively to re-assess torsadogenic risk for any of the drugs which comprise it. Furthermore, any new nonclinical electrophysiology findings on marketed drugs should not change risk assessment—if a drug has been used safely for several years without causing TdP, subsequent demonstration of activity in a hERG assay (for example) does not suddenly render it unsafe.

### 4.3. Torsadogenic propensity of drugs that block the hERG \( K^- \) channel

The number of drugs prolonging QT in man (excluding antiarrhythmics) is well over 100 [10], many of which have a long history of safe clinical use. An obvious question then arises: why are some drugs that are associated with QT prolongation apparently devoid of torsadogenic effects, whereas others are considered such a risk that they have been withdrawn or suspended from clinical use? Obviously, a decision to withdraw/suspend from sale does not necessarily relate solely to the incidence of TdP, as other factors such as the severity of the disease target and the availability of alternative drugs are taken into account before such a decision is reached. From the available evidence it does not appear to be related to dosing regimen or duration of therapy.

One possible explanation is the margin between the concentrations required for clinical efficacy and the concentrations producing inhibition of hERG/\( I_{Kr} \) currents, prolongation of APD\(_{90}\) or prolongation of QT interval. The hERG/\( I_{Kr} \) data are simpler to work with, as there is a quantitative value (the IC\(_{50}\)) which is rarely available for effects on the other parameters (APD\(_{90}\) and QT interval). Also, whereas for Category 1 drugs the various effects (\( I_{Kr} \) inhibition, increase in APD\(_{90}\) and QT interval) occur at around the same concentration for each drug, within the other categories, drugs very often have different effects on APD\(_{90}\) in different tissues, biphasic concentration–effect relationships, or both. In addition, the action potential data are subject to the complications of species differences, frequency-dependence, possible residual effects of anaesthetics used and, with some drugs, binding to non-biological surfaces within the perfusion system, whereas QT data are susceptible to problems caused by inappropriate correction for changes in heart rate and (for anaesthetised preparations) effects of commonly-used anaesthetics on QT interval [16]. Clearly we should focus on margins rather than absolute potency at the hERG \( K^- \) channel: although all but one (grepafloxacin) of the drugs in Category 2 (‘withdrawn/suspended from the market due to TdP’)
were more potent hERG/I_{Kr} blockers than any of the drugs in Category 5 (‘no reports of TdP’), the important differences between our 5 categories are to be found in the margin data (Fig. 2).

From our dataset, generally speaking, drugs with little or no margin between ETPC_{unbound} and hERG/I_{Kr}, IC_{50} are associated with TdP in man, whereas those with a large margin are not. Notable exceptions to this are discussed below. Setting aside these ‘outliers’, the range of margins within each of Categories 1–3 were similar (approximately 0.01- to 30-fold), whereas those in Category 5 were greater than 30-fold, with the exception of phenytoin (23-fold) and cibenzoline (24-fold) which fell just short of this margin. Category 4 (‘isolated reports of TdP’) was an interesting ‘mixed bag’, containing one drug with hERG/I_{Kr} activity within its therapeutic plasma concentration range (sparfloxacin), whereas the remainder at least had some therapeutic margin, as expected. It is possible that some other property (or lack of one) of this antibiotic mitigates against a high incidence of TdP.

4.4. ‘Anomalous’ drugs

Although we should view margins as a continuum, a 30-fold margin between ETPC_{unbound} and hERG/I_{Kr}, IC_{50} appears to be a line of demarcation between the majority of drugs associated with TdP and those which are not. However, there are some notable exceptions to this: amiodarone (1400-fold), fluoxetine (106-fold), ciprofloxacin (183-fold), diphenhydramine (880-fold), and nifedipine (35 700-fold), which have each been associated with TdP. Conversely, the margin for verapamil is less than twofold, yet this drug does not prolong QT, let alone induce TdP, and ketoconazole also has a small (11-fold) margin but apparently is not proarrhythmic. What are the possible explanations for the ‘false negatives’ and the two ‘false positives’? Amiodarone is an atypical Class III antiarrhythmic, and some commentators have questioned whether it should really be considered to belong to this class [14], as it also exhibits Class I, II and IV activity, together with vasodilator and anti-ischaemic effects [28], and requires chronic treatment for development of its antiarrhythmic effects [28]. It is worth comparing and contrasting the pharmacology of bepridil and verapamil, as they are both classed as ‘calcium channel antagonists’, yet both have a variety of other actions. In the case of bepridil, this is a complex drug with actions on other ionic currents including I_{Kr} [29,30], and it also has negative chronotropic effects [31] which would contribute towards promoting the conditions that enable TdP to occur. Verapamil on the other hand does not have bradycardic actions, does not affect I_{Kr} current [30] and does not prolong QT [32]. Ketoconazole also has a small margin (11-fold), yet has not been associated with TdP when administered as the sole medication. Its well-known interactions with terfenadine leading to TdP have largely been ascribed to its inhibition of CYP3A4 [2]. However, ketoconazole does prolong the QT interval by itself in guinea-pigs [33–35], and so an additive effect in blocking the hERG K^{+} channel may contribute to the proarrhythmic potential of this co-therapy. There has been only one report of TdP with fluoxetine since its launch in the mid-1980s, in an elderly female patient [36]. The authors hypothesised that this episode could have been due to enhancement of 5-HT-induced vasoconstriction, which has been observed to occur in coronary arteries with damaged endothelium. The incidence of TdP with ciprofloxacin is extremely low (0.3 cases per 10 million prescriptions), this value based on two reports as part of a historical cohort study over a 5.5-year period [37]. These were not accompanied by any ECG recordings, details of the patients, or information on any co-therapy, so it remains unproven as to whether the episodes were due directly to the drug. In terms of explaining these events, ciprofloxacin could cause release of histamine [38], which in turn could cause coronary vasoconstriction [39], and it is conceivable that this could have pathophysiological consequences in susceptible individuals under very rare circumstances. It is not too fanciful to propose this as an explanation of an event occurring in two recipients of a total of ~66 million prescriptions. Diphenhydramine has been widely available since the mid-1940s, both as a prescription drug and in various over-the-counter medicines, yet we could only find two apparent cases of TdP in the literature. As there were no details given as to co-therapy, underlying disease, or the arrhythmia itself, these two cases cannot confidently be ascribed to an effect of this drug. As with ciprofloxacin, these reports were part of a historical cohort study and were not accompanied by any details of the patients [40]. These reports are surprising in view of the absence of TdP with this drug even after overdose [41]. Perhaps the largest anomaly of all is the huge margin for nifedipine. There have been two reports of TdP in over 20 years of clinical use of this drug. Both of these were in patients with cardiovascular disease, and both episodes were attributed to localised myocardial ischaemia caused by coronary ‘steal’ due to this vasodilator [42–45]. It is obvious from the ratio of ETPC_{unbound} to hERG IC_{50} for nifedipine that these two cases were not due to effects on I_{Kr} currents. It is also worth mentioning erythromycin: most of the reported cases of TdP have been observed after rapid intravenous injection of the drug, where plasma concentrations can reach the micromolar range [46].

4.5. Possible explanations for the size of margin

Examination of hERG concentration–effect curves in transfected cell lines (see Fig. 3 for examples) reveals that for all drugs there is at most approximately one log order of magnitude between a ‘threshold effect’ and the IC_{50}. Therefore, in theory one would expect that a 10-fold margin between ETPC_{unbound (max)} and the hERG IC_{50}
would suffice, whereas in practice the ‘safe’ margin appears to be 30-fold (Fig. 2). There are various possible explanations for this. Firstly, occasionally in individuals the plasma concentrations may exceed the values for ETPC_{unbound} (max) used in our dataset, and the concentrations may encroach on the functioning of the hERG K^+ channel. This could occur as a result of accidental overdose, or because of individual variations in plasma protein binding [47] or in drug metabolism. Secondly, many of these agents are lipophilic, and accumulation may occur in myocytes either intracellularly or within the cytoplasmic membrane, so that the local concentration in the vicinity of the hERG K^+ channel may exceed the free plasma concentration [48]. Thirdly, the potency at transfected hERG channels may be lower than that occurring in vivo, due to the absence of ancillary subunits and other biochemical factors. In addition, there may be individual variations in the functionality of the hERG K^+ channel (e.g. channel density), or fluctuations in function due to changes in electrolyte concentrations.

A debate has also begun about whether to use IC_{50} values or even IC_{10} values for hERG instead of the IC_{50}. The rationale for retaining the use of IC_{50} values is obvious, as this is the most accurate measurement that can be made from a sigmoidal log concentration–effect curve, and in the case of hERG assays, as indicated above, the shape and slope of the curves are very similar across nearly all drugs tested. It is therefore better to retain the most accurate and familiar measure of drug potency at the hERG K^+ channel (i.e. the IC_{50}) and adjust the safety margins upwards accordingly, rather than to set a smaller margin based on an intercept derived from an unreliable part of the sigmoidal log concentration–effect curve, barely above the background noise. However, this statement should not be taken to mean that a 10–20% inhibition of I_{Kr} current is of negligible physiological significance: from our dataset it is reasonable to conclude that a 10–20% inhibition of I_{Kr} current in a hERG assay would translate into detectable increases in APD_{90} and QT interval, at least for those compounds where the inhibition of I_{Kr} is not offset by interactions at other cardiac ion channels.

### 4.6. Mixed ion channel activity

From our dataset, it would appear that drugs which are relatively selective for the hERG K^+ channel compared to other cardiac ion channels (especially Class Ia and III antiarrhythmics, with the exception of amiodarone) have concentration–effect curves for inhibition of hERG/\(I_{Kr}\), APD_{90} and QT interval (in dog and human) that are virtually superimposable (Figs. 1 and 3). Those with mixed ion channel activity (e.g. terfenadine) do not: the concentrations required for prolongation of APD_{90} and QT interval are dissociated from those blocking hERG/\(I_{Kr}\). Presumably their effects on other ion channels (primarily the sodium and L-type calcium channels) offset the effects of inhibition of \(I_{Kr}\) on APD_{90} and QT interval.

In order to assess the impact of mixed channel activity on torsadogenic propensity, we should consider the torsadogenic potential of selective hERG K^+ channel blockers. The following drugs from the dataset are relatively selective hERG K^+ channel blockers: almokalant, dofetilide, cisapride, astemizole, and sertindole. Each of these drugs has a potency at hERG in the low nM range (i.e. 50 nM or less), and has either been associated with TdP in clinical trials (dofetilide, almokalant), or has been withdrawn from sale due to an unacceptably high incidence of TdP (cisapride, astemizole, sertindole). Therefore, it would seem that relatively selective hERG K^+ channel blockers thus far have been associated with TdP in humans, when plasma concentrations enter the range for inhibition of the channel. So if hERG K^+ channel block is a key risk factor, it is conceivable that the plasma concentration required for arrhythmogenesis with some mixed-activity compounds could be quite precise—at a certain concentration, the combined ion channel effects may interact to produce the conditions for arrhythmogenesis. In this hypothetical scenario, the risk of TdP would be reduced at concentrations above and below this critical point. Evidence exists for this hypothesis in the case of quinidine, for example [49].

One point to make here is that we should not take comfort in a non-antiarrhythmic candidate drug with mixed channel activity that is active at the hERG K^+ channel at concentrations close to its therapeutic range, but produces relatively little QT prolongation. Given that the holy grail of Class III antiarrhythmic research programmes is to achieve agents with the ‘right’ balance of ion channel activity [50], the likelihood of a non-antiarrhythmic research programme achieving this by good fortune is surely quite low. Put another way, we are more likely to end up with a ‘terfenadine’ than a ‘verapamil’. We should ask ourselves whether we want potent activity at a range of cardiac ion channels in (say) a drug to treat arthritis.

### 4.7. Other risk factors

Although it may be the case that the relatively selective hERG K^+ channel blockers in our list are torsadogenic, drugs with the same potency at the channel may not necessarily carry the same torsadogenic risk. The nature of the interaction with the channel may be important: for example, it has been postulated that there may be multiple binding sites on the channel [51] even for a single drug (bepridil) [29], as well as for different classes of drugs, including the possibility of intracellular binding sites [52]. Also, different drugs bind to the channel in its different states (closed, open, inactivated) [51,53]. There are likely to be further developments in this area. Regardless of how a particular drug binds to the hERG K^+ channel, the prolongation of APD_{90} that ensues may not be the defining
factor in terms of arrhythmogenesis: the ensuing shape and stability of the action potential may be critical. Prolongation of APD$_{90}$ together with shortening of APD$_{90}$ (i.e. ‘triangulation’) appears to be associated with known arrhythmogenic compounds in a rabbit Langendorff-heart preparation, and leads to the appearance of EADs, whereas prolongation of APD$_{90}$ per se is antiarrhythmic [54]. Other risk factors for arrhythmogenesis are temporal instability of the action potential duration within a train, and reverse-frequency dependence [54]. Finally, at the multicellular level there is heterogeneity in sensitivity of cell types across the ventricular wall to prolongation of APD by any given drug, and the sensitivity range varies between drugs [55,56].

There are several examples in our dataset of drugs that would be expected to completely inhibit $I_{Kr}$ currents within their therapeutic range (e.g. quinidine, ibutilide terodiline, thioridazine). Yet even when there is complete inhibition of $I_{Kr}$ current within the myocardium, episodes of TdP do not occur continuously: such electrophysiological conditions merely increase the risk of such episodes occurring. So inhibition of $I_{Kr}$ currents leads to two distinct, crucial abnormalities, both reflected by QT prolongation: delayed repolarisation and instability of action potentials in Purkinje fibres and M-cells, leading to the focal genesis of EADs, and dramatic transmural heterogeneity of ventricular repolarisation, enabling propagation of the premature depolarising wavefront and the development of TdP [8,21,55,56,66]. Drugs that merely prolong action potential without causing instability, or do not result in QT dispersion, would not necessarily be torsadogenic.

5. Summary and recommendations

5.1. Importance of $I_{Kr}$ inhibition as a feature of torsadogenic agents

Our dataset has confirmed that $I_{Kr}$ inhibition is a common feature of drugs that induce TdP in man (with the exception of drugs that may induce or exacerbate myocardial ischaemia, resulting in TdP as an extremely rare event). The available evidence indicates that block of hERG K$^+$ channels (either selectively or in conjunction with effects on other ion channels) is associated with TdP if it occurs at concentrations close to those achieved in clinical use. Block of hERG K$^+$ channels is a risk factor in drug-induced TdP, and probably the predominant risk factor, but it requires a combination of factors, possibly coming together in a rare combination at one point in time (i.e. over a few cardiac cycles), to trigger the event.

5.2. Preclinical strategies for assessing torsadogenic risk

Whilst our understanding of the relationship between inhibition of $I_{Kr}$ and the risk of TdP is progressing rapidly, a pragmatic approach for the pharmaceutical industry would be to limit structural/physicochemical similarities to hERG K$^+$ channel blockers, then be guided by the chemical series with the least hERG activity. Structural requirements for binding to the hERG K$^+$ channel have been characterised, at least for antihistamines [57–59] and class III antiarrhythmics [60], and the binding sites on the hERG protein have been modelled [61,62]; nonetheless, a significant effort is required to better understand these structure–activity relationships. However, series with hERG activity need not necessarily be rejected: it depends on the severity of the therapeutic target (see below). Data on hERG activity is useful in the early stages of drug discovery, but as the research project progresses, not only is it prudent to establish the effects on the cardiac action potential, this is recommended by regulatory authorities prior to human exposure [12]. The final risk assessment prior to Phase I clinical trials has to be based on both the in vitro and in vivo data, with the latter derived from both safety pharmacology and toxicology studies. Recommendations on optimal study design (both in vitro and in vivo) have been offered previously [16], several commentators have proposed strategies for preclinical [7,48,63–65] and clinical assessment [7,48,66] of torsadogenic risk, and the current views of regulatory authorities have also been discussed, at least informally [67–69]. The capability of the pharmaceutical industry to detect QT prolongation in preclinical tests has improved substantially over recent years, but a significant challenge still remains to identify proarrhythmic potential reliably.

The following recommendations merely relate to the information that should be acquired before a decision is taken to enter clinical evaluation; exactly when these tests are done is a matter of choice for the individual companies, and perhaps for individual projects within those companies. So, our proposal would simply be as follows:

(i) determine an IC$_{50}$ at hERG in a transfected mammalian cell line;
(ii) test the effects of the compound on mammalian cardiac action potential in any well-characterised in vitro preparation;
(iii) test the compound for effects on QT interval in vivo, either by telemetry or using anaesthesia, applying either a suitable correction for heart rate changes or a regression approach [16], or alternatively, cardiac pacing.

Much debate has taken place since the CPMP document [12] concerning the selection of an in vitro preparation for detecting drug-induced changes in cardiac action potential, and species differences have been studied systematically in the case of Purkinje fibres [70]. However, we feel that if accompanied by evaluation of hERG activity, the choice of preparation is less important than when this was the mainstay of the in vitro evaluation. The reason for this is
that if a drug slows repolarisation in a concentration-dependent manner as predicted from its activity at hERG, then it can reasonably be assumed that it does not possess significant activity at other cardiac ion channels (e.g. cisapride). If it does not (e.g. terfenadine), then a further investigation of effects at other cardiac ion channels is required. Computer models may assist with interpretation of effects on action potential characteristics [71]. So, in brief, any well-characterised in vitro cardiac preparation may be used, according to the availability of tissue and the expertise within the laboratory, so long as the outcome is interpreted alongside the hERG data.

5.3. Safety margins

Our dataset suggests that a margin of 30-fold between hERG IC_{50} and C_{max} would be adequate to ensure an acceptable degree of safety from arrhythmogenesis, with a low risk of obtaining false positives. The same margin has been proposed previously based on opinion [65], and has also been suggested on the basis of a smaller dataset of drugs [72]. We would recommend that this margin is acceptable for all drugs currently undergoing clinical development. However, for the future, one should aim for higher margins where possible, as then the concerns over drug interactions, variable pharmacokinetics, and all the other risk factors would recede. This would also address issues of inter-laboratory variability in hERG IC_{50} data: we have used the lowest published hERG IC_{50} values to arrive at provisional safety margins, but it is likely that any single laboratory would derive a value for any particular drug somewhere within the published range rather than at the lower limit.

Obviously margins should reflect disease severity and medical need. For example, one could envisage that a 10-fold margin might be acceptable for drugs used in diseases which are lethal if untreated (e.g. cancer, AIDS, some other infections, etc.), a 30-fold margin may be acceptable for drug treatments for serious debilitating diseases (e.g. stroke, Parkinson’s disease, schizophrenia, epilepsy, asthma, arthritis, etc.), but a margin of 100-fold or even higher might be required in the case of less serious diseases (e.g. Raynaud’s, seasonal rhinitis, eczema, etc.). These higher margins would also be appropriate for drugs prescribed to psychiatric patients at risk from suicidal overdose. Furthermore, one has to consider changes to therapeutic target, patient population, and route of administration.

The emphasis here should be on ‘provisional margins as a starting point’—an integrated evaluation of in vitro and in vivo electrophysiology data is essential for risk assessment, and will ultimately be superseded by clinical data. Drugs with small margins for hERG activity are not necessarily unsafe, as evidenced by several that have been in safe use for years, generally due to the mitigating effects of actions at other ion channels. However, for the purposes of future drug development, candidate drugs with a margin smaller than 30-fold (for hERG IC_{50}, 10% increase in APD_{90}, or 10% increase in QT interval—whichever is the smallest margin) over the C_{max} would probably require evaluation in a proarrhythmic model, and the clinical programme may have to be augmented [12]. At the other end of the margin scale, despite our best efforts, some new drugs may induce TdP in susceptible individuals as extremely rare events by indirect mechanisms unrelated to actions at ion channels.

5.4. ‘Class effects’

It is clear from our dataset that potency in hERG assays, prolongation of APD_{90} and QT, and torsadogenic risk, have little to do with therapeutic class. The hERG K^+ channel shows promiscuous binding characteristics across a wide range of chemical structures [53]. Obviously, if similar molecular structures have been synthesized using an opportunistic approach in order to improve on a marketed drug, then if one of these drugs has hERG activity, this may well be shared by all its competitors to a greater or lesser extent, but this is not a ‘class effect’ in the strictest sense of the term. Except in the case of Class Ia and III antiarrhythmics, for adverse effects so remote from their target organs, and with a molecular mechanism quite distinct from their primary molecular target, the use of the term ‘class effect’ is unhelpful here. It is probably also unnecessary to invoke this term when considering torsadogenic risk, so long as preclinical data from the strategy outlined above (hERG IC_{50}, effects on cardiac action potential, QT interval) are collated for submission to regulatory authorities. Astemizole and cetirizine are both non-sedating antihistamines, yet whereas astemizole is an extremely potent hERG blocker with a margin around threefold, and was withdrawn after approximately 16 years on the market due to an unacceptable risk of TdP, cetirizine is a very weak hERG blocker, with a margin approaching 2000-fold, and has been used clinically for the same period of time without a single report of TdP.

5.5. Concluding remarks

Although ‘torsade de pointes’ was first characterized by Dessertenne in 1966 [73], the electrocardiographic features had been reported more than 40 years earlier [74], and sudden deaths due to antipsychotic drugs were documented soon after their introduction in the 1950s [75]. It is therefore not a new phenomenon, and the CPMP’s 1997 ‘Points to Consider’ document [12] was arguably long overdue. Hopefully our survey of the published literature has helped to clarify the task of preclinical risk assessment with respect to proarrhythmic potential of new drugs. The more drugs with a long track record of clinical use for which there are full datasets on in vitro and in vivo cardiac electrophysiology, the clearer our understanding will be-
come. The challenge to the pharmaceutical industry is to minimise hERG activity so as to eliminate the major risk factor, but not at the expense of discarding therapeutically useful drugs that are urgently required to treat a myriad of diseases for which there is presently a paucity of quality treatment.

References
