

# Simulation of multiple ion channel block provides improved early prediction of compounds' clinical torsadogenic risk

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## Aims

The level of inhibition of the human Ether-à-go-go-related gene (hERG) channel is one of the earliest preclinical markers used to predict the risk of a compound causing Torsade-de-Pointes (TdP) arrhythmias. While avoiding the use of drugs with maximum therapeutic concentrations within 30-fold of their hERG inhibitory concentration 50% (IC<sub>50</sub>) values has been suggested, there are drugs that are exceptions to this rule: hERG inhibitors that do not cause TdP, and drugs that can cause TdP but are not strong hERG inhibitors. In this study, we investigate whether a simulated evaluation of multi-channel effects could be used to improve this early prediction of TdP risk.

## Methods and results

We collected multiple ion channel data (hERG, Na, L-type Ca) on 31 drugs associated with varied risks of TdP. To integrate the information on multi-channel block, we have performed simulations with a variety of mathematical models of cardiac cells (for rabbit, dog, and human ventricular myocyte models). Drug action is modelled using IC<sub>50</sub> values, and therapeutic drug concentrations to calculate the proportion of blocked channels and the channel conductances are modified accordingly. Various pacing protocols are simulated, and classification analysis is performed to evaluate the predictive power of the models for TdP risk. We find that simulation of action potential duration prolongation, at therapeutic concentrations, provides improved prediction of the TdP risk associated with a compound, above that provided by existing markers.

## Conclusion

The suggested calculations improve the reliability of early cardiac safety assessments, beyond those based solely on a hERG block effect.

## Keywords

Computer modelling • Drug development • Pharmacology • Risk prediction • Torsade-de-pointes

## 1. Introduction

Many drug compounds have a tendency to associate with, and to block, cardiac ion channels; this can lead to abnormal propagation of electrical action potentials (APs) through the heart tissue. This action is 'designed in' to anti-arrhythmic drugs, but is often an unwanted side effect of non-cardiac drugs. In the complex geometry of the heart, this change in electrical propagation can cause

degeneration of the normal heart rhythm into life-threatening Torsade-de-Pointes (TdP) arrhythmia.

TdP can arise months into treatment,<sup>1</sup> and only in a very small subset of the patients receiving a drug<sup>2</sup> (individuals are predisposed to TdP dependent on a large number of factors, such as: the presence of congenital long QT syndromes, heart failure, bradycardia, electrolyte imbalance, gender, hepatic or renal impairment, impaired metabolism, and co-administration of certain drugs<sup>3</sup>). It is therefore difficult

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to predict preclinically, or assess during clinical trials on healthy individuals, which drugs will have the potential to cause TdP and which are safe. As such, a number of drugs have been withdrawn from the market due to an unacceptable TdP risk (for example, astemizole, cisapride, terfenadine, and thioridazine were withdrawn between 1997 and 2002).<sup>4–7</sup>

The rapid delayed rectifying potassium channel [ $I_{Kr}$ , with a human isoform known as Ether-à-go-go-related gene (hERG)] is both important in controlling repolarization of the ventricular myocyte AP, and particularly susceptible to block by many different compounds.  $I_{Kr}$  blockade leads to a lengthening of the AP duration (APD) and has long been associated with an increased TdP risk.<sup>8</sup>

Apart from the predisposing factors, we might suppose that the level of TdP risk conferred by a particular drug will depend upon the affinity of the drug compound for different ion channels, and its concentration. The affinity of a compound for a channel is quantified using its inhibitory concentration 50% ( $IC_{50}$ ) value: the concentration of the drug that will cause the current flowing through an ion channel to be reduced by 50%. The concentration of a compound to which cardiac ion channels are exposed is assumed to be the effective free therapeutic plasma concentration (EFTPC); that is, the concentration of unbound/free compound in the blood plasma when the drug is given at its therapeutic dose.

The earliest indicator used to assess torsadogenic risk in the pharmaceutical compound development process is usually based on the hERG  $IC_{50}$  value; compounds that block hERG too readily are discarded. Channel interactions are detected in multi-target toxicity screens, and hERG  $IC_{50}$  values are generally established from automated patch-clamp experiments on expression system cell lines.

However, there are many exceptions to this association between hERG block and TdP: both hERG inhibitors that do not cause TdP (e.g. verapamil and propafenone), and less commonly, drugs that cause TdP that are weak hERG inhibitors (e.g. tedisamil).<sup>9</sup> These cases can arise when a drug blocks other ion channels: particularly susceptible are  $I_{Na}$  (fast sodium channel) and  $I_{CaL}$  (the L-type calcium channel).<sup>10,11</sup> Blockade of these channels will lead to a shortening of APD, countering some of the effects of a hERG block. It has been suggested previously that multi-channel effects must be considered when evaluating the TdP risk.<sup>11,12</sup>

Safety tests undertaken later in the drug development process attempt to address multi-channel effects, by taking into account the action of a compound on the tissue as a whole. These tests are usually based on the ECG: QTc interval prolongation from *in vitro* animal models (such as wedge preparations and Langendorff-perfused heart<sup>13</sup>), *in vivo* animal models, clinical trials, and eventually human thorough QT trials, specified by the ICH E14 document.<sup>14</sup> There are concerns that thorough QT trials may be overly restrictive, as some drugs registered pre-ICH E14 prolong QT but are not associated with high rates of TdP.<sup>15</sup> Concerns over cardiovascular side effects now account for an estimated 30% of potential compound discontinuations.<sup>16</sup> It would be ideal to screen out troublesome compounds at the earliest possible opportunity—saving money, time, and lives.

In 2003, in an effort to provide such a screening process, Redfern et al.<sup>17</sup> evaluated the clinical TdP risk of many drugs, proposed that a measure of  $[hERG IC_{50}]/[EFTPC_{max}]$  be used as a TdP risk indicator, and that a value of 30 or over is a provisional safety margin. This was suggested as an improvement over simply  $[hERG IC_{50}]$ ; in the following we shall quantify the improvement in predictive power that this

achieved. De Bruin et al.<sup>18</sup> confirmed that the marker proposed by Redfern et al. was an indicator of risk, by showing a statistically significant correlation between  $[hERG IC_{50}]/[EFTPC_{max}]$  and the number of abnormal cardiac events occurring per patient.

We aim to quantify how much information the earliest stage of safety screening can give on clinical TdP risk. By performing experiments, and by mining the literature, we gather  $IC_{50}$  values for two other channels in addition to hERG, namely  $I_{Na}$  and  $I_{CaL}$ , for a total of 31 drugs. We use mathematical cardiac electrophysiology models of ventricular cells to incorporate the information on the degree of drug block for each channel, and then predict the changes in cell behaviour that would result under different protocols. We then correlate these model predictions with the clinical risk of TdP as indicated by the Redfern et al.<sup>17</sup> risk classification, quantifying their predictive power and identifying risk indicators.

## 2. Methods

In this section, we detail the following steps: classification of drugs into TdP risk categories; measurement of  $IC_{50}$  values; simulation of possible risk indicators; and prediction of risk categories according to the indicators.

### 2.1 Clinical risk classification

We have taken the TdP risk classification system proposed by Redfern et al.<sup>17</sup> defining the following categories in terms of clinical human TdP risk:

1. Class Ia and III anti-arrhythmics; generally associated with a large, but acceptable, risk of TdP.
2. Drugs that have been withdrawn from the market (by at least one major regulatory authority) due to unacceptable TdP risk.
3. Drugs with a measurable incidence of TdP, or for which numerous case reports exist.
4. Drugs for which there have been isolated case reports of TdP.
5. Drugs for which there have been no published reports of TdP.

We refer to these categories as the 'risk categories'. Lawrence et al.<sup>13</sup> updated the list, adding further drugs and reclassifying others. Further modifications have been made: thioridazine has been moved from risk category 3 to 2, as it was withdrawn from general use in 2005, due to association with excessive QT prolongation and cases of TdP.<sup>19</sup> Quetiapine has been reclassified from category 5 to 4, as it has recently been associated with isolated TdP case reports.<sup>20</sup> Risk categories for all the drugs in this study can be seen in Table 1.

### 2.2 $IC_{50}$ and EFTPC values

Redfern et al.<sup>17</sup> performed a thorough literature search for hERG  $IC_{50}$  values and EFTPC data for over 90 drugs; these form the basis of our data set. To investigate multi-channel effects, we performed experiments on 15 compounds to measure  $I_{Na}$  and  $I_{CaL}$   $IC_{50}$  values directly. Concentration–response curves for  $I_{Kr}$  were measured in HEK-293 cells stably transfected with hERG cDNA (for amiodarone and prenylamine;  $IC_{50}$  values in the literature were highly variable and absent, respectively); for  $I_{Na}$  in HEK-293 cells stably transfected with hNaV1.5 cDNA; and for  $I_{CaL}$  in isolated ventricular myocytes from guinea pig, killed by cervical dislocation following stunning; using a conventional whole-cell patch-clamp technique.<sup>21</sup> Full details of our experimental protocols can be found in Supplementary material online, S1. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). All animals were treated in accordance with UK Home Office regulations [Animals (Scientific Procedures) Act 1986: London: Her Majesty's

**Table 1** Risk categories, IC<sub>50</sub> values, EFTPCs and references for all of the drugs in this study. References for this table are given in full in Supplementary material online, S6.

Generic drug name	TdP risk	Na IC <sub>50</sub> (nM)	CaL IC <sub>50</sub> (nM)	hERG IC <sub>50</sub> (nM)	EFTPC <sub>max</sub> (nM)
Ajmaline	1	8200 <sup>34</sup>	71 000 <sup>35</sup>	1040 <sup>36</sup>	300–1500 <sup>37</sup>
Amiodarone	1	4800 <sup>38</sup>	270 <sup>39</sup>	30 <sup>a</sup>	0.1–0.5 <sup>17</sup>
Amitriptyline	4	20 000 <sup>40</sup>	11 600 <sup>41</sup>	3280 <sup>42</sup>	11–41 <sup>17</sup>
Bepiridil	3	3700 <sup>a</sup>	211 <sup>a</sup>	33 <sup>43</sup>	10–33 <sup>17</sup>
Chlorpromazine	3	4300 <sup>44</sup>	n/a <sup>44</sup>	1470 <sup>45</sup>	3–38 <sup>17</sup>
Cibenzoline	5	7800 <sup>47</sup>	30 000 <sup>48</sup>	22 600 <sup>49</sup>	502–976 <sup>17</sup>
Cisapride	2	14 700 <sup>a</sup>	n/a <sup>a</sup>	6.5 <sup>50</sup>	2.6–4.9 <sup>17</sup>
Desipramine	4	1520 <sup>a</sup>	1709 <sup>a</sup>	1390 <sup>51</sup>	27–108 <sup>17</sup>
Diltiazem	5	9000 <sup>52</sup>	450 <sup>53, 54</sup>	17 300 <sup>55</sup>	53–122 <sup>17</sup>
Diphenhydramine	4	41 000 <sup>a</sup>	228 000 <sup>a</sup>	5200 <sup>49</sup>	22–34 <sup>17</sup>
Dofetilide	1	300 000 <sup>56, 57</sup>	60 000 <sup>57</sup>	5 <sup>18</sup>	0.4–2.0 <sup>17</sup>
Fluvoxamine	4	39 400 <sup>a</sup>	4900 <sup>a</sup>	3100 <sup>17</sup>	15–377 <sup>58</sup>
Haloperidol	3	7000 <sup>59</sup>	1700 <sup>59</sup>	27 <sup>60</sup>	1.2–3.6 <sup>17</sup>
Imipramine	4	3600 <sup>44</sup>	8300 <sup>45</sup>	3400 <sup>17</sup>	35–106 <sup>17</sup>
Mexiletine	4	43 000 <sup>61</sup>	100 000 <sup>62</sup>	50 000 <sup>63</sup>	1445–4129 <sup>17</sup>
Mibefradil	4	980 <sup>64</sup>	156 <sup>65</sup>	1800 <sup>49</sup>	6–12 <sup>17</sup>
Nifedipine	4	37 000 <sup>44</sup>	60 <sup>66</sup>	275 000 <sup>67</sup>	3.1–7.7 <sup>17</sup>
Nitrendipine	5	36 000 <sup>44</sup>	0.35 <sup>68</sup>	10 000 <sup>17</sup>	0.11–3.02 <sup>17</sup>
Phenytoin	5	49 000 <sup>a</sup>	103 000 <sup>a</sup>	100 000 <sup>17</sup>	4000–4500 <sup>17</sup>
Pimozide	3	54 <sup>69</sup>	162 <sup>70</sup>	20 <sup>17</sup>	0.1–1.0 <sup>17</sup>
Prenylamine	2	2520 <sup>a</sup>	1240 <sup>a</sup>	65 <sup>a</sup>	9–17 <sup>71</sup>
Propafenone	4	1190 <sup>a</sup>	1800 <sup>a</sup>	440 <sup>72</sup>	26–241 <sup>17</sup>
Propranolol	5	2100 <sup>a</sup>	18 000 <sup>a</sup>	2828 <sup>73</sup>	12–26 <sup>74</sup>
Quetiapine	4	16 900 <sup>a</sup>	10 400 <sup>a</sup>	5800 <sup>75</sup>	10–33 <sup>17</sup>
Quinidine	1	16 600 <sup>a</sup>	15 600 <sup>a</sup>	300 <sup>76</sup>	924–3237 <sup>17</sup>
Risperidone	5	102 000 <sup>a</sup>	73 000 <sup>a</sup>	150 <sup>17</sup>	0.61–1.81 <sup>17</sup>
Sertindole	3	2300 <sup>77</sup>	8900 <sup>77</sup>	14 <sup>17</sup>	0.02–1.59 <sup>17</sup>
Tedisamil	1	20 000 <sup>78</sup>	n/a <sup>79</sup>	2500 <sup>17</sup>	75–85 <sup>17</sup>
Terfenadine	2	971 <sup>a</sup>	375 <sup>a</sup>	8.9 <sup>73</sup>	0.1–9.0 <sup>17</sup>
Thioridazine	2	1830 <sup>a</sup>	1300 <sup>a</sup>	33 <sup>17</sup>	208–979 <sup>17</sup>
Verapamil	5	41 500 <sup>a</sup>	100 <sup>a</sup>	143 <sup>55</sup>	25–81 <sup>17</sup>

<sup>a</sup>Measured as part of this work as described in Section 2.2 and Supplementary material online, S1. Reference 63 states a value of '≫10 000' and so 50 000 was taken.

Stationery Office 1986], and the work was approved by GlaxoSmithKline internal ethical review.

To expand our drug data set to include at least four drugs from each category, we utilized the Aureus Pharma database (<http://www.aureus-pharma.com/Pages/Products/Aurquest.php>) and a manual literature search to find I<sub>Na</sub> and I<sub>CaL</sub> IC<sub>50</sub> values for 16 further compounds. Where more than one IC<sub>50</sub> value was available in the literature, we have followed Redfern *et al.* in utilizing the lower value in our analysis. We were therefore able to establish I<sub>Kr</sub>, I<sub>Na</sub>, and I<sub>CaL</sub> IC<sub>50</sub> values and EFTPC data for 31 compounds; a full list is presented in Table 1.

The three IC<sub>50</sub> values and maximum EFTPC values for these drugs are plotted against the risk categories in Figure 1. The lack of association displayed in Figure 1 suggests that these 'raw' IC<sub>50</sub> values will have little predictive power for the risk category, a concept we quantify in section 2.4.

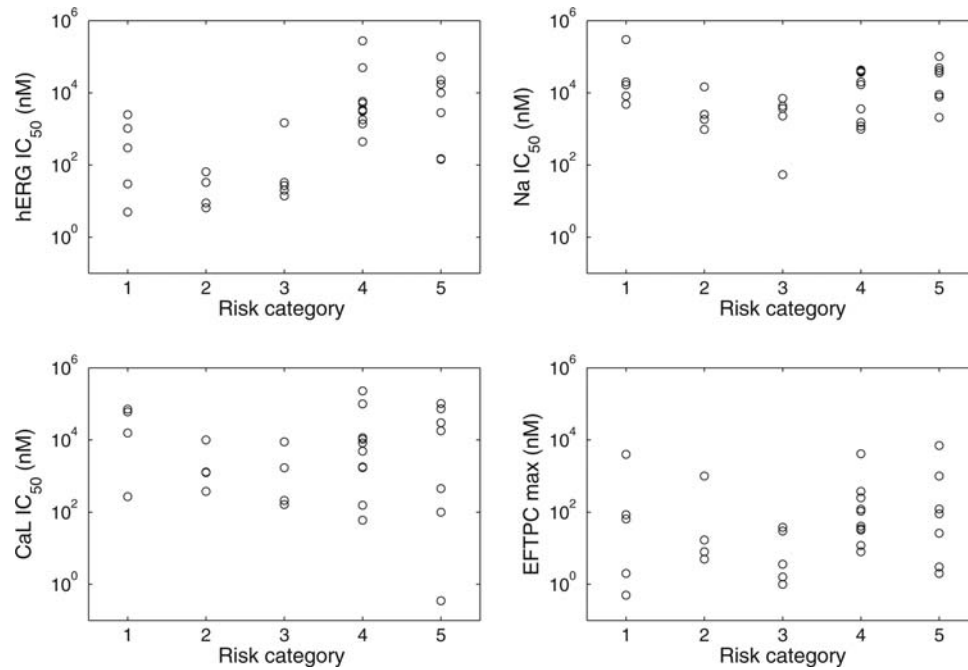
## 2.3 Simulations

In addition to using the 'raw' IC<sub>50</sub> and EFTPC values to associate a drug with a risk category, we hypothesize that some function of these values may provide a stronger association. We turn to mathematical cardiac

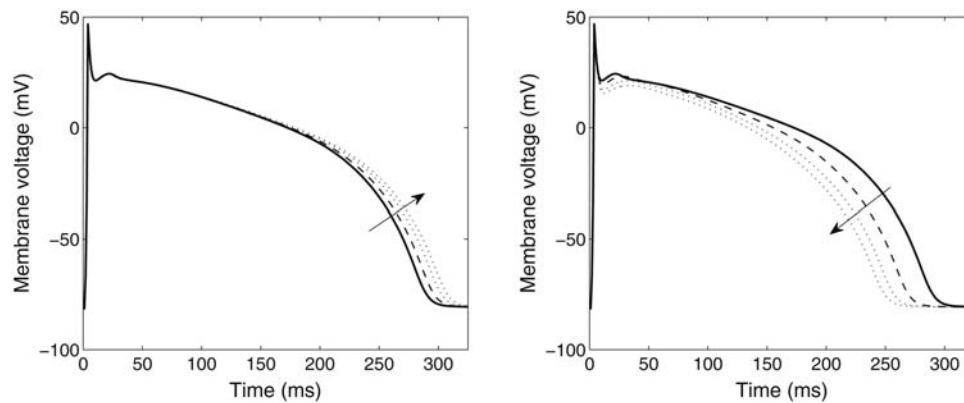
electrophysiology models of ventricular myocytes; these models integrate information about individual channel currents to describe their collective behaviour, and AP formation. We use these models to predict changes to whole-cell behaviour under drug action, using the IC<sub>50</sub> values and concentration data as model inputs, dictating the degree of drug-induced channel block. The aim is to find model outputs that correlate with the risk categories more strongly than the markers shown in Figure 1, and so provide *in silico* TdP risk indicators.

Mathematical cardiac electrophysiology models are systems of (typically) tens of highly non-linear ordinary differential equations (ODEs), governing the evolution of model variables through time. These variables represent ion channel gates/states, ion concentrations, and other quantities such as membrane voltage. We have taken five of the recent ventricular myocyte models for rabbit,<sup>22,23</sup> dog,<sup>24</sup> and human.<sup>25,26</sup> Each of these models has an ODE for the evolution of membrane voltage (*V*) through time (*t*), which takes the form

$$\frac{dV}{dt} = -\frac{1}{C_m} \left( \sum_{\text{channels}} I_j + I_{\text{stim}} \right),$$



**Figure 1** Scatter plot of  $IC_{50}$  values for the drugs against the risk categories. For all three channels and the EFTPC, there is significant overlap between categories. It is evident that no single channel's  $IC_{50}$  value will allow accurate classification of a drug into its risk category.



**Figure 2** Simulation of steady-state 1 Hz pacing of the Grandi et al.<sup>26</sup> model under verapamil application when considering (left) a solely hERG block, and (right) a hERG, Na, and CaL block. Arrows indicate the effect on the AP of increasing drug concentration, displayed are: control (0 nM, solid line), low EFTPC (25 nM, dashed line), medium EFTPC (53 nM, dash-dotted line), and high EFTPC (81 nM, dotted line).

where  $C_m$  is the membrane capacitance,  $I_j$  represent the currents due to each species of ion channel ' $j$ ', and  $I_{stim}$  is the stimulus current applied to pace the cell. Channel currents take the form

$$I_j = g_j O (V - E_{ion}).$$

Here,  $g_j$  is the maximal conductance of channel ' $j$ ',  $O$  is its open probability, and  $E_{ion}$  is the reversal potential for the species of ion which flows through channel  $j$ .

There are a number of ways to modify such models to incorporate the effects of channel block by drug compounds. One of the simplest is a 'conductance-block' formulation;  $g_j$  is reduced by a factor which is a

function of the  $IC_{50}$  value of a drug for this channel, and the concentration of the drug [which we denote by  $[D]$ ]. In general, for the conductance of a channel of type  $j$  we have

$$g_j = g_{control,j} \left[ 1 + \left( \frac{[D]}{[IC_{50}]_j} \right)^n \right]^{-1}$$

Here,  $g_{control,j}$  is the drug-free maximal conductance of the  $j$  channel. For all drugs and channels in this study, we have assumed that the Hill coefficient  $n = 1$  (or equivalently, one molecule of drug is assumed to be sufficient to block one ion channel—typical values of  $n$  for hERG block are around 0.7–1.1<sup>11</sup>). Where a drug effect on a channel has been tested

and no block was observed, the original conductance  $g_{\text{control}}$  was used. We have applied this model for drug block to  $I_{K_r}$ ,  $I_{Na}$ , and  $I_{CaL}$ .

In general, drug effects at steady-state concentration can be well represented by a conductance-block model.<sup>27</sup> This assumption seems to hold even if the drug exhibits state- or voltage-dependent binding (since, at physiological cardiac pacing rates, the timescale over which binding and unbinding occur is much longer than the timescale over which voltage changes or channel–state transitions occur). One case in which a conductance block may not be an accurate model is that of an allosteric drug block, in which a compound affects the ability of a channel to transition between open, closed, or inactivated states. Since the precise mechanism of channel block is usually not known or measured at the early stages of compound development, we have modelled drug action using the conductance-block formulation.

We performed steady 1 Hz pacing, S1–S2, and dynamic restitution protocols on the models, recording quantities such as APD50, APD90, triangulation, and maximum restitution slopes. Full details of the simulation protocols and the 15-recorded quantities can be found in Supplementary material online, S2. The protocols were performed for each model at four concentrations: low EFTPC and high EFTPC values are the lowest and highest EFTPC<sub>max</sub> values, respectively, as reported in the literature, medium EFTPC was taken to be the mean of these, and the overdose to be  $10 \times$  high EFTPC<sub>max</sub>.

An example of the AP that the steady pacing protocol generates can be seen in Figure 2 for the Grandi *et al.*<sup>26</sup> model. We see the effect of adding verapamil at low, medium, and high EFTPC; both for a case where we consider only a hERG block, and for the multi-channel case. The importance of multi-channel effects becomes clear: in the case of a solely hERG block, the APD is prolonged, in the case of hERG, Na, and CaL blocks, the effect is the opposite, with a marked reduction in APD.

### 2.3.1 A note on implementation

XML format representations of the models<sup>22–26</sup> were taken from the CellML repository.<sup>28</sup> PyCML<sup>29</sup> was used to translate the CellML format into C++ code. CVODE<sup>30</sup> was then used to integrate the ODEs in a custom-made program based on the open-source Chaste library.<sup>31</sup> For the interested reader, our full code is available to download from <http://www.comlab.ox.ac.uk/chaste>.

## 2.4 Classification

For a previously unseen compound, we wish to take the continuous measures produced by the simulation protocols above, and then categorize the compound into a discrete risk category, based upon the measure's value and our prior knowledge of this measure for other compounds. This type of problem is ubiquitous in statistics, and is known as a 'classification problem'.

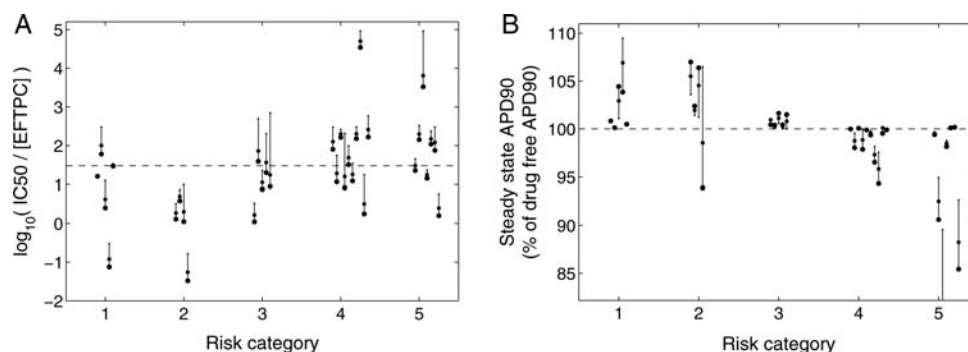
We have used one of the simpler classification methods, known as linear discriminant analysis (LDA), to place our compounds into the risk categories. LDA uses maximum likelihood estimates to calculate the probability of each point in variable space being a member of each category. To classify an unseen observation, we simply assign it to the category with the highest probability at that point. Supplementary material online, S3 provides further details of this method.

The risk of adverse cardiac events associated with drugs in risk categories 1 and 2 is similar.<sup>18</sup> Since our aim is to predict the clinical risk category associated with an early compound, we would not know whether the compound is being developed as an anti-arrhythmic or not. Since categories 1 and 2 exhibit indistinguishable TdP risks, we combine them for the LDA and subsequent classification, labelling all these drugs as 'category 2' to avoid the confusion that would arise in re-indexing the rest of the risk categories.

To quantify the predictive power of the LDA, we perform 'leave-one-out' validation. One drug is removed from our data set, leaving 30 out of the 31 drugs remaining to form the training data set. An LDA is performed on the training data set, then the 'left-out' drug is categorized accordingly. We assign an error score as follows

$$\text{Error score} = |\text{predicted category} - \text{actual category}|$$

which will be a positive integer; 0 if the predicted category is the same as the actual category, 1 if the predicted category is  $\pm 1$  away from the actual category, etc. We then repeat the analysis leaving out each of the drugs in turn. It should be noted that this analysis immediately provides a tool that can predict the torsadogenic risk of an unseen drug, according to the risk categories it has 'learnt' from a database of, in this case, 30 other drugs.



**Figure 3** (A) Current state-of-the-art measure:  $[\text{hERG IC}_{50}]/[\text{EFTPC}_{\text{max}}]$  against TdP risk categories for the different drugs in this study, as suggested and presented in Redfern *et al.*; the dotted line is their safety factor of 30. (B) Proposed *in silico* marker: simulated % change in 1 Hz steady-state APD90 relative to control for the Grandi *et al.*<sup>26</sup> human ventricular cell model. The dotted line is the 'control' case—i.e. 100%. In both (A) and (B), the measure is evaluated for low, middle, and high  $[\text{EFTPC}_{\text{max}}]$ ; producing three points of increasing size, which we display joined with a line. This is intended to show the sensitivity of the measure to variations in dose concentrations which may occur, for example, between individuals or between drug applications. Drugs are presented in alphabetical order within their risk categories. From left to right: 1: ajmaline, amiodarone, dofetilide, quinidine, tedisamil; 2: cisapride, prenylamine, terfenadine, thioridazine; 3: bepridil, chlorpromazine, haloperidol, pimozide, sertindole; 4: amitriptyline, desipramine, diphenhydramine, fluvoxamine, imipramine, mexiletine, mibefradil, nifedipine, propafenone, quetiapine; 5: cibenzoline, diltiazem, nitrendipine, phenytoin, propranolol, risperidone, verapamil.



### 3. Results

In Figure 3A, we plot  $[\text{hERG IC}_{50}]/[\text{EFTPC}_{\text{max}}]$  against the risk categories for the drugs in this study, for the spread of  $\text{EFTPC}_{\text{max}}$  values in the literature. The relationship appears to be of a similar quality to that shown for  $[\text{hERG IC}_{50}]$  in Figure 1. The difficulty in associating a drug with a risk category is evident, as drugs across all five risk categories exhibit overlapping values.

Simulation of the markers discussed in Section 2.3 was undertaken for each of the models, with a hERG-only or multi-channel block, at low, medium, high, and overdose EFTPCs. This resulted in 350 simulated markers for both multi-channel and hERG-only cases.

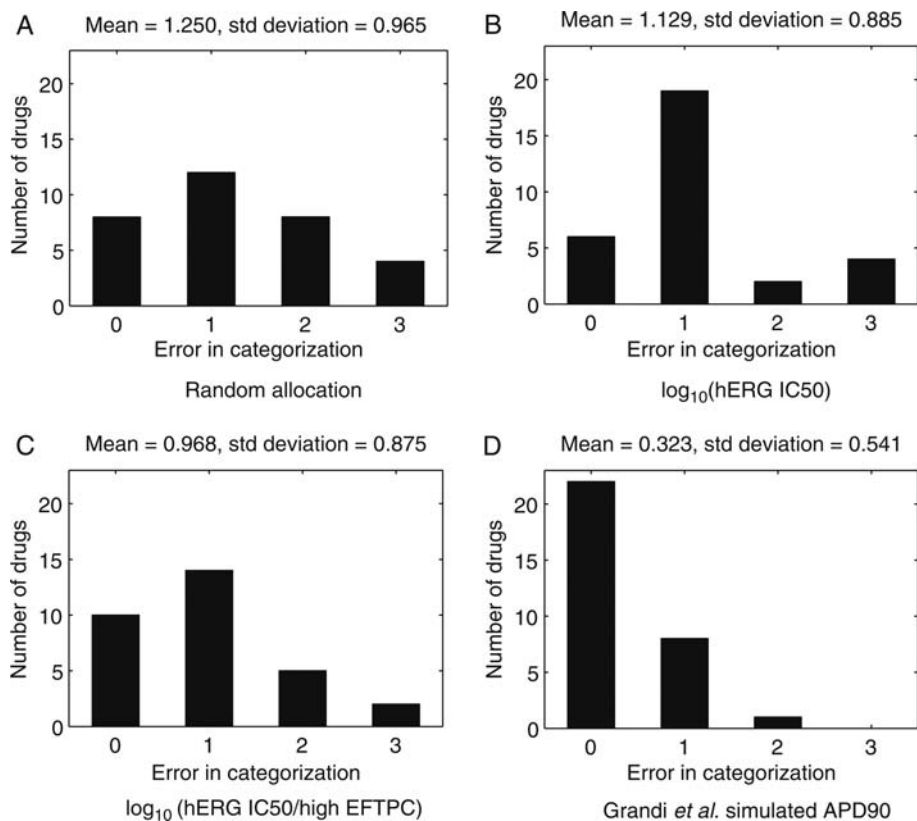
The LDA described above was then performed for the raw  $\text{IC}_{50}$  values, previously suggested risk indicators, and the simulated markers, a total of 761 possible markers. As a result of the 'leave-one-out' trial, we identified the most predictive single marker as the maximum  $\text{APD}_{90}$  at low/medium/high EFTPC under steady 1 Hz pacing as simulated by the Grandi et al. model; we present this in Figure 3B.

By comparing Figure 3A and B, it is evident that this simulated marker provides an improved association relative to that of the existing safety measure. In Figure 3B, there is one drug worthy of particular note: thioridazine (category 2) appears to have both prolongation and shortening, dependent on the EFTPC value. This situation arises because the low EFTPC is higher than the hERG  $\text{IC}_{50}$  and therefore significant prolongation occurs below (and at low) EFTPC values; as

the EFTPC increases, other channels also become significantly blocked and shorten the AP.

We now consider the errors associated with categorization based upon the different measures. Figure 4A provides a reference by displaying the mean errors when allocation of each drug into a risk category is performed at random ( $10^6$  times). Figure 4B displays the error if classification is performed with an LDA based on  $\log_{10}([\text{hERG IC}_{50}])$ ; the mean error is 1.129. The metric of  $\log_{10}([\text{hERG IC}_{50}]/[\text{High EFTPC}])$  results in the errors in classification shown in Figure 4C; the mean error is reduced to 0.968 as there is a reduction in the number of completely wrongly characterized drugs (category 2 as 5 or vice versa), relative to Figure 4B. This finding confirms that the Redfern et al. safety factor was an improvement over the hERG  $\text{IC}_{50}$ .

Errors for the most predictive marker found in this study are shown in Figure 4D, the mean error is reduced to 0.323, and no drugs are characterized to the wrong end of the risk scale. In fact, no drugs in categories 4 or 5 ('safe') are categorized as 2 or 3 ('dangerous'); we have no 'false positives'; see Supplementary material online, S5 for full results. Only one drug in category 2 or 3 is categorized as 4 or 5; this 'false negative' is amiodarone, categorized with an error of 2. Amiodarone is in classification risk category 2 (risk categories 1 and 2 being combined for classification as discussed earlier), but is classified as 4. In Figure 3B, it is the drug in risk category 1 which is very close to the control (100% line).



**Figure 4** Histograms of classification errors for (A) allocation of categories at random; (B)  $\log_{10}([\text{hERG IC}_{50}])$ ; (C)  $\log_{10}([\text{hERG IC}_{50}]/[\text{high EFTPC}])$ ; and (D) simulated marker: Grandi et al.<sup>26</sup> 1 Hz steady-state maximum  $\text{APD}_{90}$  at low/medium/high EFTPC.

In a compound development setting, the results shown in *Figure 3B* and *Figure 4D* suggest a strategy: a compound in which the simulated APD90 at any realistic EFTPC is markedly prolonged will have a strong TdP risk and should not be progressed to the market (unless the clinical benefit outweighs the safety risk, e.g. it is intended to be used as an anti-arrhythmic or an anti-cancer drug). Similarly, compounds whose simulated APD90 is shortened will have a low risk of TdP and ought to be progressed. Compounds whose APD90 is affected to a small extent fall into two types: those for whom the channel  $IC_{50}$  values are all much higher than their EFTPC and little block occurs, we would suggest these should be rapidly progressed; and those for whom channel block effects are important and to some extent may be ‘cancelling each other out’, these compounds should be referred for further cardiac safety testing.

The mean and standard deviation of the errors in categorization for all markers are shown in *Figure 5*. The markers to the bottom left of the solid lines are more predictive than random allocation, and markers to the bottom left of the dashed lines are more predictive than the Redfern *et al.* measure. We have estimated that the chance of predicting these drug risk categories with this degree of accuracy by guessing at random is  $<1$  in a million (see Supplementary material online, *Figure S2*). We also confirmed that the choice of predictive marker retained some independence from the drug data set by performing cross-validation on stratified subsets of the drugs; for details of these results, please refer to Supplementary material online, S3.2. The 30 most predictive markers (shown in bold on *Figure 5*) are all simulated markers for multi-channel effects and are listed in full in Supplementary material online, S4. None of the hERG-only block simulated markers achieved such a level of

predictive power, and the majority of the most predictive markers are the result of protocols encapsulating prolongation of APD.

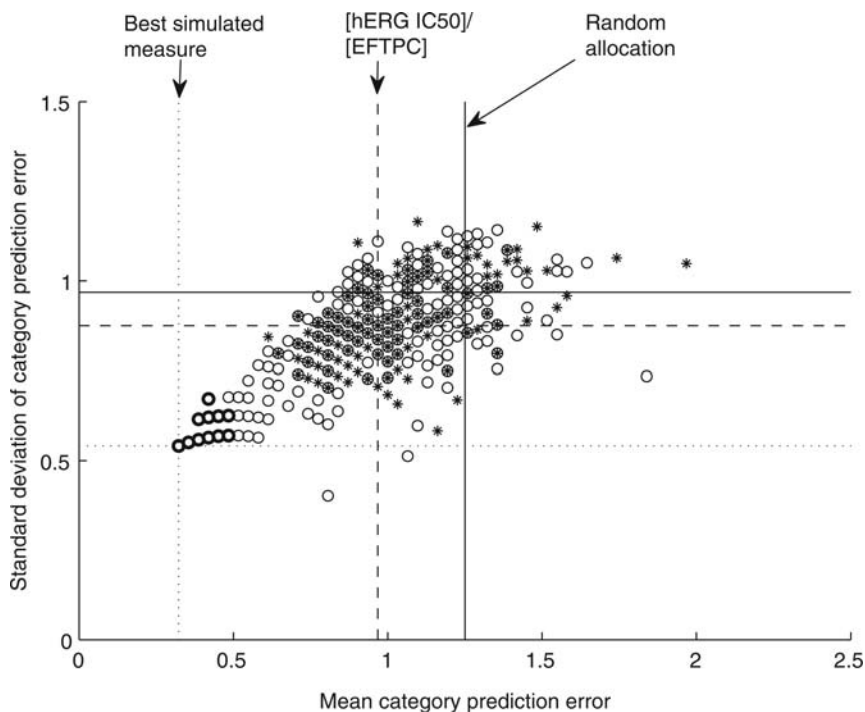
## 4. Discussion

We have developed a simulation tool that predicts a clinical torsadogenic risk category associated with a compound, based on the earliest preclinical data on its role in multiple-ion-channel blockade. Simulated markers based on EFTPC and the hERG, sodium, and L-type calcium channel  $IC_{50}$  values provide an improved prediction of a compound's torsadogenic risk, beyond that provided by the ratio of  $[hERG IC_{50}]/[EFTPC_{max}]$ .

By including these multi-channel effects, severe mis-classifications of other metrics can be correctly predicted: verapamil and propafenone, both potent hERG blockers, have their TdP risks predicted accurately. Our work confirms that consideration of hERG block is necessary, but not sufficient, to predict torsadogenic risk.<sup>11,12</sup>

Amiodarone was the only drug we could not characterize to within one risk category. There are many possible reasons for this, as it has intricate effects, being involved in membrane trafficking interference, chronic changes to ion channel expression, late sodium block, and has metabolites that are also active hERG inhibitors. Where compounds affect currents other than  $I_{Kr}$ ,  $I_{Na}$ , or  $I_{CaL}$  (e.g. ranolazine which also targets the late sodium channel), one would expect to be less able to predict their risks correctly.

The mathematical models of ventricular myocytes we have used are evidently capturing something of the channel interactions that are at work in determining cell behaviour. These cell models have now been developed for 50 years,<sup>32</sup> and as the models advance, we



**Figure 5** Scatter plot of classification error for all of the different markers. Simulated markers from a hERG-only block are denoted with stars while multi-channel block markers are denoted by circles. Bold circles highlight the 30 ‘most-predictive’ markers. Solid lines indicate the expected values if classification was performed at random, dashed lines are the values given by  $\log_{10}([hERG IC_{50}]/[high EFTPC])$ , and dotted lines are the values given by the longest APD90 at low/med/high EFTPC as shown in *Figure 3B*.

hope this will refine the predictions provided by the techniques we have proposed. Of our many simulated markers, it is those related to an increase in APD which correlate best with the TdP risk; this is in keeping with current thinking which links QT prolongation to TdP initiation.

We did not find strong predictive power from the *in silico* markers associated with steep restitution curves or APD triangulation (as suggested by the TRlaD screen<sup>33</sup>), although this may be due to the mathematical models replicating this behaviour less accurately than the simpler changes to APD. However, it is important to note that no assumptions about the accuracy of the models' APD predictions, or the mechanisms leading to TdP, are needed to show the predictive power of this method.

Our study has limitations; to maximize the number of drugs, our IC<sub>50</sub> data were taken from a multitude of different sources, and as such contain the associated variations inherent in such a data set. There are insufficient numbers of drugs to evaluate the impact of manual vs. high-throughput automated patch clamp; to ascertain this the whole study should be repeated based solely on high-throughput data. We included 31 drugs, for which we could ascertain the effects on the three channels, EFTPC, and the clinical TdP risk; as the data set expands, we expect to provide improved predictions. The mathematical model is not a perfect predictor: the model of a drug block is very simplistic and could be improved by measurement of dose–response curve hill coefficients. Kinetics of channel block have been ignored (voltage-/state-dependence, and allosteric effects); however, it would be difficult to include these effects: early safety testing does not measure such intricate details of compound–channel interactions as standard, and each compound would need associated channel block models developing for each ion channel. Interestingly, and perhaps surprisingly, despite these limitations, we have shown a marked improvement in the early prediction of torsadogenic risk.

In further work, we hope to address some of the limitations, adding more drugs to the data set, which is used as the basis of predictions. Further currents such as late sodium and  $I_{Ks}$  are known to be targets of many drugs, and inclusion of these into the study may also yield improved results. Inclusion of longer-term drug-induced changes to membrane protein trafficking and expression into the model may also improve predictions. As the mechanisms behind the initiation of Torsade-de-Pointes are elucidated, there is hope that simulation of these mechanisms directly will provide improved prediction of risk.

This type of simulated test has the potential to complement and extend existing safety tests; facilitating earlier detection of torsadogenic compounds, and preventing potentially safe compounds being discarded unnecessarily. Moreover, as the database of drugs expands, and confidence grows in the accuracy of model predictions, simulations may have the potential to replace some safety tests and thereby have a beneficial impact on replacement and reduction of animal models, cost, and time.

## Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

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