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REVIEW

Application of cardiac electrophysiology simulations to pro-arrhythmic safety testing

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Concerns over cardiac side effects are the largest single cause of compound attrition during pharmaceutical drug development. For a number of years, biophysically detailed mathematical models of cardiac electrical activity have been used to explore how a compound, interfering with specific ion-channel function, may explain effects at the cell-, tissue- and organ-scales. With the advent of high-throughput screening of multiple ion channels in the wet-lab, and improvements in computational modelling of their effects on cardiac cell activity, more reliable prediction of pro-arrhythmic risk is becoming possible at the earliest stages of drug development. In this paper, we review the current use of biophysically detailed mathematical models of cardiac myocyte electrical activity in drug safety testing, and suggest future directions to employ the full potential of this approach.

LINKED ARTICLE
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Abbreviations
APD, action potential duration; hERG, human ether-a-go-go-related gene, encoding the major I_{Kr} channel protein; HH, Hodgkin–Huxley; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; I_f, hyperpolarisation-activated depolarising (pacemaker) current, called 'funny current'; I_{Kr}, rapidly activating potassium current; I_{Ks}, slowly activating potassium current; I_{Na}, fast sodium current; $I_{Na,p}$, late/persistent sodium current; I_{CaL}, L-type calcium current; MM, Markov model; QT, the Q to T interval of the ECG; QSAR, quantitative structure activity relationship; TdP, Torsade de Pointes

Introduction

The role of safety pharmacology is to evaluate whether any off-target drug interactions may cause unwanted, in particular dangerous, side effects. In this review, we examine the role of computational simulation, using biophysically detailed models of cardiac electrophysiology, in predicting the risk of drug-induced pro-arrhythmic effects.

Torsade de Pointes (TdP) is a ventricular tachycardia, which has been linked to administration of drugs that delay repolarization – the final stage of the electrical cycle underlying the heartbeat. On the ECG, such drugs give rise to a prolongation of the time between onset of ventricular excitation (Q-wave) and the end of repolarization (end of T-wave). This is referred to as QT prolongation. Even in the presence of QT prolongation, however, TdP may occur as rarely as once in

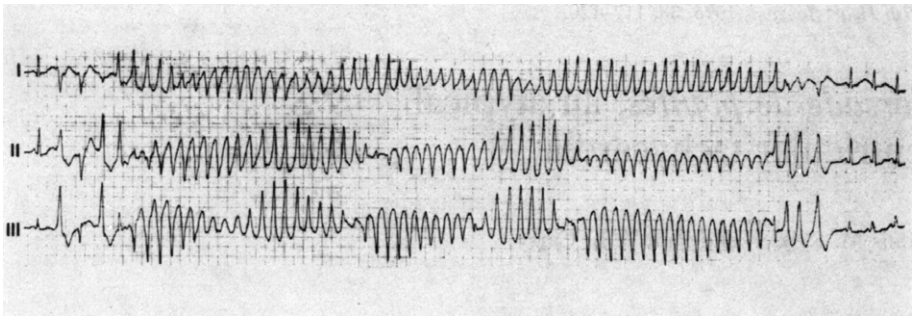

Figure 1

Onset and self-termination of TdP in a patient during ECG observation. From top to bottom: ECG leads I, II and III. Adapted by permission from BMJ Publishing Group Limited. (British Heart Journal, Krikler & Curry, 38:117–120, 1976) (Krikler and Curry 1976).

every 10 000 patient-years of exposure to a compound. Being a (potentially exceedingly) rare event in human, clinical TdP is notoriously difficult to assess during the pharmaceutical compound development process. Prediction of 'torsadogenicity' (the likelihood of a compound initiating TdP) is of high relevance for cardiac safety assessment because, *if it occurs*, TdP often degenerates into ventricular arrhythmias that may cause sudden cardiac death (unless self-terminated; see Figure 1). TdP as a potentially drug-induced side effect on the heart has become a primary concern in drug development, so that torsadogenicity needs to be assessed and, if possible, excluded as early as possible in the compound development pipeline.

As a result of the combination of (i) the potential severity of TdP, and (ii) the difficulty in predicting TdP liability at preclinical stages, this has become a significant problem for the industry. TdP risk (both real and perceived) is responsible for failure of countless compounds during development, and it remains a leading cause of drug-withdrawal from the market [e.g. of the antihistamine Terfenadine, and Cisapride (Gottlieb, 1999; Henney, 2000)].

Human ether-a-go-go-related gene (hERG), action potential duration (APD), QT, and Torsade de Pointes

TdP liability has been linked to block of a particular ion channel, expressed in cardiac cells. In humans, the channel's major subunit is encoded by the hERG (Kv11.1), which encodes a protein that forms part of a potassium channel which carries the rapidly activating potassium current (I_{Kr}), one of the major repolarizing currents in cardiac tissue. In the following, we use 'hERG-channel' to refer to this potassium channel. Unfortunately, the hERG-channel is particularly prone to interaction with a huge range of pharmaceutical compounds (Vandenberg *et al.*, 2001). Block of hERG-channels causes a reduction in repolarizing currents, and, correspondingly, an increase in the length of time that membrane voltage remains at elevated levels. This manifests itself in a prolongation of the cell's APD, as shown in Figure 2. Prolonged APs at the cellular level are one of the mechanisms that give rise to prolongation of the QT interval of the ECG (see Figure 2). Increases in APD, in particular if regionally heterogeneous, are believed to make early after-depolarizations more likely, and these are thought to serve as potential triggers for pro-arrhythmic events (Madhvani *et al.*, 2011).

As torsadogenicity can be associated with prolongation of the QT interval, it has been concluded that any block of I_{Kr} or QT prolongation should be taken as an indication of pro-arrhythmic risk. To address this, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH; http://www.ich.org) has introduced two assays: (ICH, 2005a)

- ICH-S7B (ICH, 2005a): suggests *in vitro* I_{Kr} and *in vivo* QT measurements. These take the form of patch clamp experiments using a hERG expression system, and an *in vivo* conscious animal QT study;
- ICH-E14 (ICH, 2005b): suggests a human phase II 'thorough QT' trial. If the 95% confidence interval for prolongation of QT interval, corrected for heart rate, is equal or greater than 10 ms (this generally indicates a mean prolongation of as little as 5 ms), then the compound will be of concern to regulatory bodies (Recanatini *et al.*, 2005; Pollard *et al.*, 2010). Should this occur, extensive further trials are necessary to establish whether a pro-arrhythmic liability exists, and subsequent product warning labels may restrict the market access of a drug. In practice, pharmaceutical companies spend considerable effort on avoiding this, as further trials are expensive and may not result in a positive outcome for the company (unless the clinical benefits of a compound outweighs its TdP risk – i.e. for life-threatening conditions with no existing treatment, or with poor prognosis).

Both guidelines were produced jointly by regulators and pharmaceutical industry, with the effect that the suggested assays are effectively mandatory for regulatory approval to register and market a drug. As a result, pharmaceutical companies have developed a range of earlier and cheaper in-house assays, designed to evaluate whether compounds are likely to run into problems with the regulatory requirements mentioned earlier (Pollard *et al.*, 2008). We outline a 'typical' pharmaceutical cardiac side effect screening strategy in Figure 3. This approach has been relatively effective in preventing potentially torsadogenic compounds from reach-

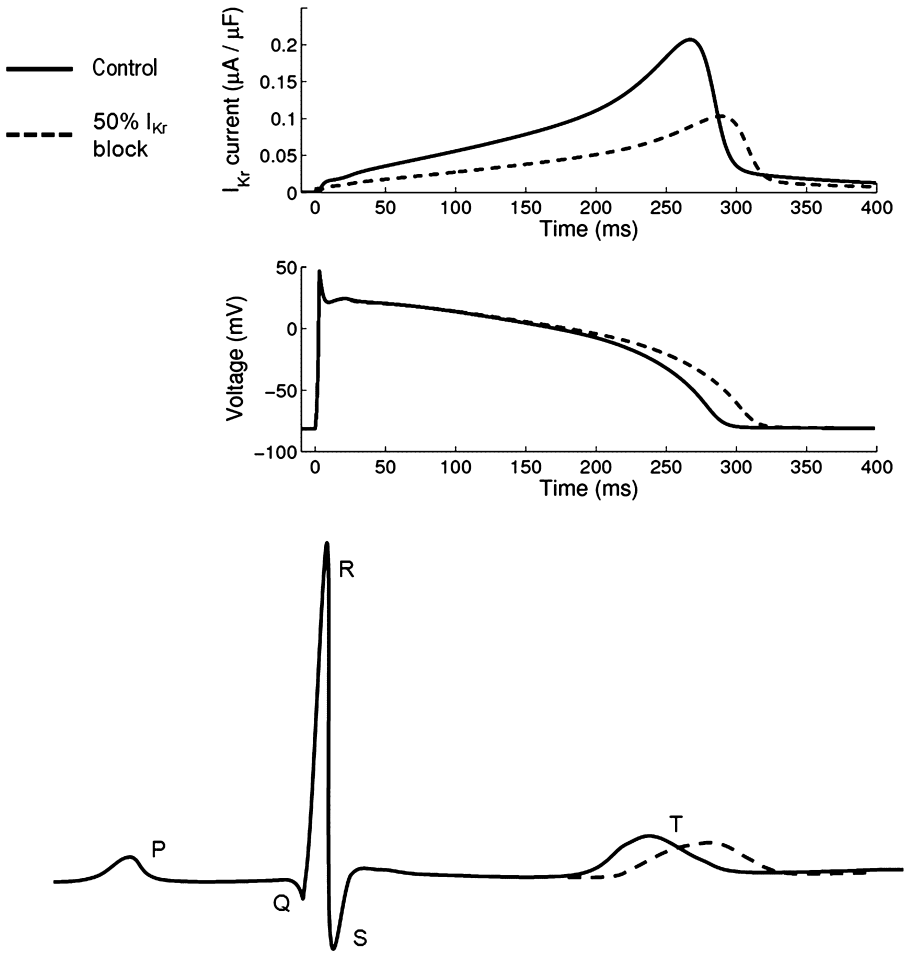

Figure 2

Schematic representation of electrophysiological changes caused by 50% block of the hERG-channel in healthy tissue. Top: hERG current (I_{Kr}); middle: action potential (voltage); bottom: ECG annotated with standard labels for characteristic parts of the curve. The QT interval is the period of time from the Q to the T waves. Top and middle panels are from a 1-Hz steady-state simulation of the (Grandi *et al.*, 2010) model, bottom is a schematic. In all panels, control is indicated by a solid line, and the effect of 50% I_{Kr} conductance block by a dashed line; simulations illustrating steady-state responses.

ing the market. However, this process is likely to include false-positive termination of otherwise promising candidate drugs. In addition, a sizeable number of compounds still fail the expensive human 'thorough QT' trial, as their QT prolongation risk remains undetected until that late stage (Gintant, 2011).

However, the sequence that we have outlined so far, hERG-channel block → APD prolongation → QT prolongation → TdP, is far from a definitive description of the problem (Hoffmann and Warner, 2006). Many compounds that would fail the (relatively recently introduced) ICH-S7B and E14 guidelines have reached the market before and are *not* associated with TdP. Many other possible factors have been suggested (Corrias *et al.*, 2010), including AP triangulation (Hondeghem *et al.*, 2001), reverse-rate dependence (Hondeghem and Hoffmann, 2003), dispersion of repolarization (Valentin *et al.*, 2004), rate adaptation (Green *et al.*, 2011) and beat-to-beat variation in QT (Abrahamsson *et al.*, 2011; Jacobson *et al.*, 2011), among others. Therefore, hERG-channel block and QT prolongation are neither necessary nor sufficient conditions for a torsadogenic risk, despite a strong association (Straus *et al.*, 2005). As the precise mechanisms that lead to initiation of TdP remain undetermined, hERG-channel block and QT prolongation remain the most widely employed biomarkers for detecting TdP risk.

Drug actions on multiple cardiac ion channels may explain some of the discrepancies highlighted earlier (Martin *et al.*, 2004). A drug may block both I_{Kr}, which carries a current contributing to repolarization, and other channels, such as fast sodium (I_{Na}) or L-type calcium (I_{CaL}), which carry currents that *oppose* repolarization. A compound therefore may be a hERG-channel blocker, but not markedly prolong AP duration or QT (see Figure 4). This is believed to be the case for Vernakalant [which also blocks I_{Na} (Schmitt *et al.*, 2008)]. Similarly, if a drug blocks the hERG-channel only mildly (or not at all), but impedes another AP-shortening channel (e.g. the slowly activating potassium current, I_{Ks}), then AP lengthening may be present in spite of an apparently

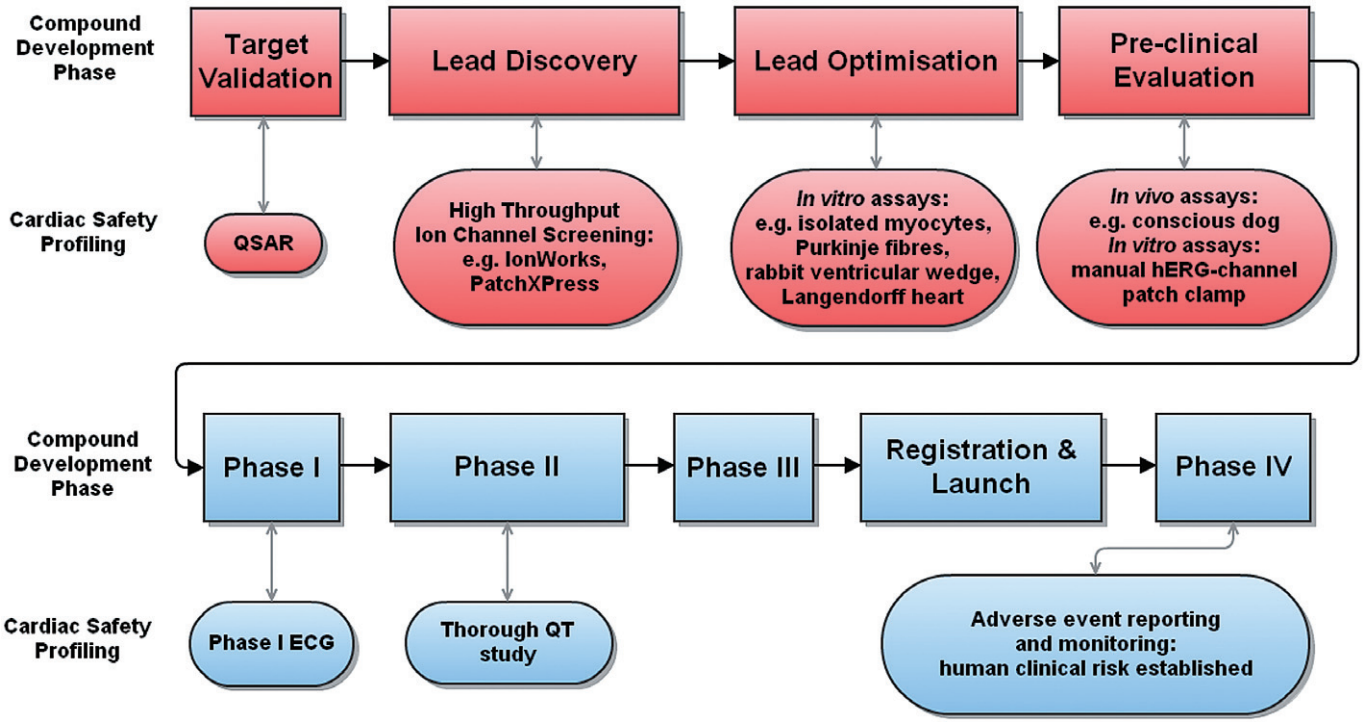

Figure 3

A generic progression strategy for evaluation of QT prolongation risk during pharmaceutical compound development. Top rows (red): preclinical assays, performed on a large number of candidate compounds; bottom rows (blue): clinical assays performed in human. Rectangular boxes: key stages of drug development; oval shapes: profiling activities conducted to assess pro-arrhythmic risk.

safe hERG assay, potentially causing QT prolongation that exceeds what would have been expected from examining hERG action alone.

Such potential for multiple ion-channel effects is recognized by pharmaceutical companies. Most, if not all, have started to employ high-throughput screens on a range of cardiac ion-channel targets (typically three to six), early in drug development (see Figure 3). The individual ion-channel targets are not yet standardized across the industry, and the results of screens are rarely used quantitatively and in context with one another.

High-throughput screens for multiple targets provide large amounts of data, and novel means are needed for turning this into meaningful information to support flagging-up of potential negative side effects that warrant further investigation in subsequent studies, or indeed to identify drug candidates that should not be progressed further.

Evaluation of compounds in an integrated biological system (e.g. animal-based models) is often a key to understanding the implications of multichannel block. However, these model systems are costly, demand primary tissue and data gathering is low-throughput. For these reasons, they are used later on in the drug development pipeline, after considerable investment has already been made, and when alternative chemical leads have already been deselected. *In silico* approaches offer the opportunity of improving the decision making at a time when alternative lead compounds are still in scope, and before considerable expense and time has been committed. In Figure 5, we outline the benefits associated with *in silico* approaches, supporting early and improved decision making.

In silico tools

A number of approaches are used to quantitatively describe the interactions of a drug compound with cardiac ion channels, including molecular dynamics simulations and quantitative structure activity relationship (QSAR) models.

In molecular dynamics studies, drug and ion channels are represented at an atomic level, and affinity is established in terms of energy minimization [notable recent work includes the study by Silva *et al*. (2009) linking molecular dynamics to biophysical models]. A drawback is that this approach is computationally very expensive. QSAR modelling is a statistical approach, based on evaluating properties of the molecular structure and estimating target affinity based on a compound's similarity to others contained in a historical dataset (Inanobe *et al*., 2008).

Because the approach to, and tools for, simulation have matured, both of these models can be used prior to any 'wet-lab' experimental work on a compound, relying only on knowledge of the chemical structure. In this review, however, we will focus on another type of *in silico* simulation: biophysically detailed AP modelling.

Since Hodgkin & Huxley's Nobel Prize winning description of AP formation in nerve cells (Hodgkin and Huxley, 1952), and its subsequent application to cardiomyocytes

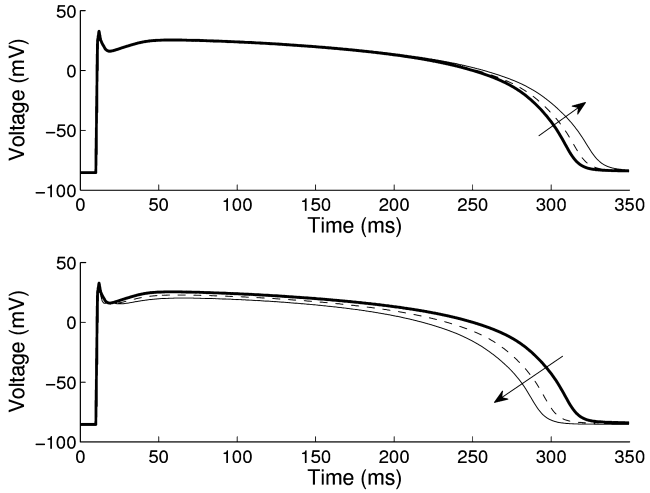

Figure 4

Changes to action potential repolarization during simulated exposure to Verapamil. Using the human epicardial myocyte model of (Ten Tusscher and Panfilov, 2006) at steady 1 Hz pacing, the application of Verapamil is modelled using a conductance-block model with hERG-channel IC_{50}=143 nM and CaL IC_{50}=100 nM [data from (Mirams *et al.*, 2011)]. Top: effect of hERG-channel block only; bottom: combined block of hERG and L-type calcium channels. Arrows indicate changes caused by increasing drug concentrations from 0 nM as control (bold) to 25 nM (dashed) and 81 nM (thin solid). 81 nM is the maximum effective free therapeutic plasma concentration.

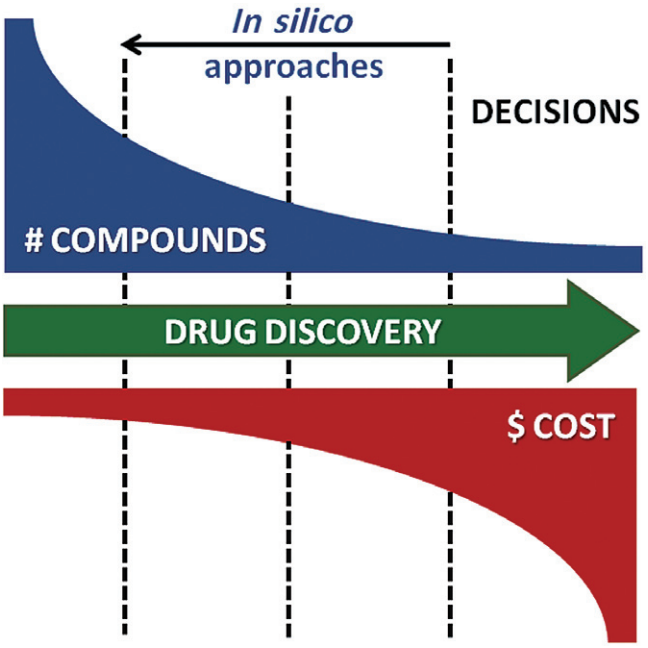

Figure 5

In silico approaches offer the possibility of bringing forward decisions on the viability of compounds for further development. *In silico* models can identify potentially unsafe compounds earlier in the pipeline, when alternative chemical leads still exist, and before expensive and time-consuming safety testing has occurred. The resulting chemical leads are more likely to succeed in later safety tests. This will reduce costs and improve the chances of developing a successful compound.

(Noble, 1960; 1962), cardiac electrophysiology insight has been aided by biophysically based computational models of AP formation [. . .], which we will refer to as 'AP models'. There is another type of AP model: a 'phenomenological model'. This represents features of an AP without modelling ion channels (Bueno-Orovio *et al.* 2008; Walmsley *et al.*, 2010). These simplified models can be helpful in tissue simulations, but they are not generally suited for the mechanistic study of drug actions. So 'AP models' in this review refers to biophysically detailed models containing descriptions of individual ionic currents. Today, these AP models represent some of the most detailed and well-tested models in systems biology (Kohl and Noble, 2009). The earliest use of such models to study the action of pharmaceutical compounds in cardiac cells dates back to the 1970s (Katzung *et al.*, 1977). In terms of drug safety testing, these models are used in the context of information on compound actions gathered from experimental data, and to integrate insight across scales of spatial complexity, from the single ion channel to cells, tissue, organ and whole body (Figure 6).

Several previous reviews have examined the role of cardiac simulation in drug discovery, design and safety assessment (Noble and Colatsky, 2000; Noble 2008; Fink and Noble, 2010; Rodríguez *et al.*, 2010; Amanfu and Saucerman, 2011), and discussed the potential of the 'systems biology approach' for modern healthcare challenges (Noble, 2002a; Hunter *et al.*, 2010; Kohl *et al.*, 2010). Here, we focus on the use of AP models in predicting drug-induced pro-arrhythmic risk.

Modelling drug/ion-channel interaction

In this section, we outline the various classes of mathematical models for the drug/ion-channel interaction studies. These were reviewed more thoroughly by Brennan *et al.*, to whom we refer the interested reader for a more complete description of the derivation of these models (Brennan *et al.*, 2009).

In the majority of cases, a drug affects cardiac ion-channel currents by direct binding. Blocking actions are usually attributed to obstruction of the flow of ions through a channel pore, either by forming a physical obstacle, or by changing the conformation of the ion channel. There are exceptions to this, for example pentamidine, which reduces whole-cell I_{Kr} by interference with hERG expression and protein trafficking (Cordes *et al.*, 2005). Such 'unexpected' mechanisms are one reason why experimental studies cannot be replaced completely by simulation. But as novel assays are developed (e.g. for hERG protein trafficking interference) such data can be included into existing models, and thereby reduce the likelihood of such unexpected results.

To date, modelling efforts have focussed on simulating direct binding of drug compounds to a channel. Ion-channel models fall into two main categories: Hodgkin–Huxley model (HH) formulations (Hodgkin and Huxley, 1952), and their generalizations, which are termed Markov models (MM).

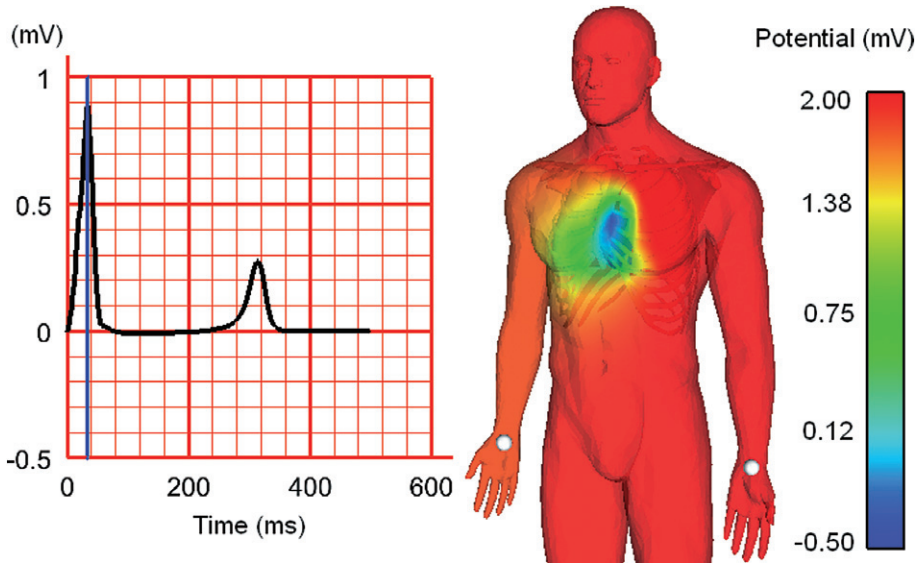

Figure 6

Simulation of human body-surface ECG, using a biophysically detailed cell electrophysiology model, embedded into an anatomically representative human heart mesh inside a whole-body mesh (containing sub-structures with distinct electrical properties). Left: simulated Lead-I ECG, as measured between the white points indicated on the human mesh (right). The time point of body surface voltage snapshot (on the right) is indicated relative to the ECG by a blue line (on the left). Image courtesy of Nejib Zemzemi, University of Oxford, using techniques developed in (Zemzemi *et al.*, 2011).

The simplest way to introduce a drug-block into a cardiac electrophysiology model is via '*conductance-block*', that is by reducing the maximum conductance of an affected ion channel or transporter, using a scaling factor. This factor follows a function for the dose–response curve, describing the effect of a compound on the maximum current flowing through the target. Typically, this scaling factor '*b*' is related to the drug concentration '[*D*]' according to:

$$b = \frac{1}{1+\left(\frac{[D]}{IC_{50}}\right)^{n}} \quad (1)$$

where IC_{50} is the drug concentration at which a 50% reduction of the peak current is observed, while n is the Hill coefficient of the dose–response curve (often assumed to be equal to one, i.e. one drug molecule is necessary and sufficient to block one ion-channel). Such models for drug action do not include 'kinetics', and drug block is assumed to reach its steady state immediately. Note that it is possible for a simple conductance-block model to exhibit frequency/use dependence at the AP scale, as the roles of a current may change depending on pacing rate. More detailed models of drug/ion-channel interaction take into account the rate at which a drug binds to/unbinds from the ion channel, leading to a differential equation for b (Starmer *et al.*, 1991). Both these types of drug/ion-channel interaction models can be reproduced in either HH or MM ion-channel formulations.

For more complex drug/ion-channel interactions, the MM approach is required (while it may be a relatively simple exercise for a mathematician to convert HH models into MM, the automation of this process is non-trivial). In MM formulations, a drug may be given access only to certain channel states (open, closed, inactivated, etc.) termed *state-dependent block*. The speed at which overall drug binding (and unbinding) occurs will therefore depend upon the proportion of time that a channel spends in each of the states. This will vary with pacing rates, and allows one to incorporate use/rate-dependence of drug actions (Barber *et al.*, 1991; Tsujimae *et al.*, 2007). The term 'voltage dependent' block is often applied to describe this, but this is potentially misleading, as drug binding and block depends primarily on the state of the ion-carrying protein (of course, different states of a channel may become available preferentially at certain voltages). True *voltage-dependent block*, as seen experimentally, occurs when the charge of a drug compound affects its likelihood of reaching a binding site in a transmembrane voltage-dependent manner [independent of/in addition to channel conformation availability effects (Tikhonov and Magazanik, 1998)]. This may be included in Markov models by making drug binding rates for individual states functions of voltage.

A further intricacy occurs when the bound drug alters the rates of transition between ion-channel states. This is termed allosteric block. Models incorporating this behaviour are complex and require large amounts of experimental data for parameterization (often more than are available).

Many other factors that can influence the binding of a drug to an ion channel are increasingly being modelled, including: temperature, pH (Cardona *et al.*, 2010), or co-administration of other compounds. In practice, however, pharmaceutical ion-channel screening does not usually record sufficient data to evaluate many of the kinetic changes caused by drug/ion-channel interaction, and it will

not do so until more advanced high-throughput screening technologies/approaches become available. Simpler dose–response curves, described by the equation above, are often the only available quantitative data. Fortunately, with the exception of drugs that exhibit allosteric effects, the 'conductance block' approach is as useful as kinetic models of drug action, when considering steady-state pacing conditions (Brennan *et al.*, 2009). However for conditions with variable pacing rates (such as in arrhythmia) the conductance block approximation may be insufficient for simulation of realistic drug actions. In addition, drug block is rarely at a true 'steady state', as compound concentration fluctuates between dosing. The incorporation of, or coupling to, pharmacokinetic/pharmacodynamic models to predict the free concentration of drug compound likely to be found at the cardiac ion channels is a desirable future development (van der Graaf and Benson, 2011).

At present all 'production simulations' that we are aware of in pharmaceutical companies take (quite reasonably) the simpler modelling approach of conductance block (Bottino *et al.*, 2006; Davies *et al.*, 2012). The increasing availability of high-throughput ion-channel screens means that required parameters for these models (IC_{50} and Hill coefficient) can be acquired at low cost for large numbers of candidate compounds, thereby effectively auto-generating the parameters for this class of models. One plea to industry in this context is to consider dose–response curve characterisation as a parameter fitting exercise for equation (1) above. There *is* information to be gained from the parameters of the dose–response curve, even where a drug effect reaches, for example, only 40% block of an ion channel at the largest concentration tested. Communication of such data is relevant, and much more informative than stating that 'no IC_{50} was attained'.

Anything other than simple conductance-block models are typically developed manually, requiring a skilled computational modelling team. The literature on such models is expanding quickly (Noble, 1980; Bean *et al.*, 1983; Gilliam *et al.*, 1989; Weirich and Antoni, 1989; Pásek and Simurda, 2004; Clancy *et al.*, 2007; Comtois *et al.*, 2008; Brennan and Tarassenko, 2010). But manual 'model building' is not scalable to the creation of models for every drug candidate and ion channel of interest. The process of 'model building', in particular for kinetics and allosteric effects of drug/ion-channel interactions, needs to be automated and established in-house inside pharmaceutical companies. This should be aided by innovative experimental designs, in order to focus on the most relevant data for parameter/model fitting techniques (Ball and Sansom, 1989; Ball *et al.*, 1999; Fink and Noble, 2009; Moreno *et al.*, 2011), and will benefit from academia/pharma consortia, such as the recent PreDiCT initiative (Fletcher *et al.*, 2011).

Modelling the effects of drugs on the AP

There has been some success in the safety pharmacology field using computational models that incorporate drug/ion-channel representations into whole-cell AP models. These models relate the change in membrane potential to the sum of the currents flowing in and out of the cell. They are composed of (typically 10–70) differential equations, with multiple parameters. To facilitate their exchange and re-use, the CellML model description language and associated tools have been vital (Lloyd *et al.*, 2008; Garny *et al.*, 2009; Cooper *et al.*, 2011). The reason for the relevance of whole-cell models is that it is at this spatial scale that the block of single or multiple ion channels may manifest itself in an alteration of AP parameters, a manifestation of key electrophysiologically relevant behaviour of cardiac myocytes (Rudy, 2007). Whole-cell integration of ion currents occurs, of course, also in 'biological model systems' of adult heart cells, such as stem-cell derived cardiomyocytes. A recent combined experimental and modelling study has highlighted that the expectation that this may soon lead to patient-specific pharmacological assays could be overly optimistic (Jonsson *et al.*, 2012), as ion current amplitudes and activation properties of stem-cell derived cardiomyocytes are probably closer to neonatal than to adult phenotypes.

In terms of cardiac drug safety assessment, two notable examples of the biophysical simulation approach are related to the development of ivabradine and ranolazine. Ivabradine blocks the so-called 'funny current' (I_f) first observed in 1968, when it was assumed to carry only potassium ions (Noble and Tsien, 1968). The I_f current was later identified as a mixed cation channel (DiFrancesco, 1981), and simulation work suggested that reduction of I_f would slow sino-atrial node pacemaker cells by a moderate amount only, due to redundancy with other ion channels (Noble *et al.*, 1992). Ivabradine, marketed by Servier as Procoralan and Corlentor for the symptomatic management of stable angina pectoris, has been reported to be highly effective and safe, causing a small but significant reduction in heart rate. The European Medicines Agency are currently moving to add 'chronic heart failure' to the indications for Corlentor, where it has been reported to significantly reduce mortality (Swedberg *et al.*, 2010). Thus, biophysically detailed AP modelling predicted essential aspects of the pharmacological profile of ivabradine, based on the description of underlying ionic current mechanisms.

Ranolazine (Ranexa; CV Therapeutics, now Gilead) is an anti-angina drug. In spite of a large affinity for the hERG-channel, it was observed to cause only moderate QT prolongation. As mentioned earlier, strong hERG-channel block would normally spell the end for a compound, but CV Therapeutics had not observed any early after-depolarizations (as one might otherwise have expected with the degree of hERG-channel block that ranolazine caused). Further studies showed that ranolazine is also a potent blocker of the late/persistent sodium current $I_{Na,p}$ (Antzelevitch *et al.*, 2004). At this point, there was still no quantitatively plausible explanation as to why the block of the relatively small sodium current $I_{Na,p}$ could curb potentially detrimental effects expected from strong hERG-channel block. The company turned to AP models. These showed that block of $I_{Na,p}$ compensated for many of the effects that would otherwise have been associated with hERG-channel block, including AP prolongation. Computer simulations further identified that the reduced sodium loading, caused by $I_{Na,p}$ block, led to reduced calcium loading of the cell. The associated smooth repolarization makes QT prolongation, which is still seen in this setting, much less proarrhythmic than usual (Noble and

Noble, 2006). Benefiting from this mechanistic insight provided by AP models, Ranolazine is now on the market for angina treatment, and it is being tested as an anti-arrhythmic agent (Belardinelli *et al.*, 2006).

Motivated by the observation that multi-channel effects may alleviate torsadogenicity, a recent modelling investigation (Mirams *et al.*, 2011) examined the effect of including into a human ventricular AP model the IC_{50} data for three ion channels: hERG/I_{Kr}, I_{Na} and $I_{Ca,L}$. The study included IC_{50} data for 31 currently marketed compounds, and assessed the extent to which AP prolongation in the model correlated with the human clinical TdP risk of each drug. By simply including three (instead of I_{Kr} only) ion-channel effects, the predictive classification of drugs into the risk categories established by Redfern (Redfern *et al.*, 2003) was substantially improved. This suggests that AP modelling of multiple ion-channel effects may improve early identification of clinical risk. It also implies that torsadogenic effects of hERG block can be eliminated by inhibiting additional channels. Of course, in certain circumstances, this may be detrimental for cardiac function via other mechanisms (e.g. reduced contractility), which represents a separate but important safety issue.

So, how many ion channels should one screen? And how about currents that cannot easily be recorded? This forms part of a conundrum that one might term the 'inverse problem': is it possible to infer how a drug affects different ionic currents by measuring an overall change to the AP (which is easier to record than ion currents)? Bottino *et al.* approached this task with some success, measuring hERG-channel block and associated AP shapes experimentally, and then attempting to fit the IC_{50} values of five other channels/exchangers present in the cell, using computational AP models (Bottino *et al.*, 2006). As pharmaceutical ion-channel assays expand, this approach may become relevant in the evaluation of drug action on channels/exchangers that cannot yet be screened efficiently in an automated setting, or that may not even be known.

One of the remaining challenges is the variability of data recorded in cardiac preparations of many different types. This can be caused by experimental errors, but more usually, data variability is an expression of genuine differences between individual cells (or hearts), termed *extrinsic variability*; or to inherent stochasticity in the behaviour of a single cell (or ion channel), termed *intrinsic variability*. Mathematical consideration of these factors is only just beginning (Dangerfield *et al.*, 2010; Walmsley *et al.*, 2010). First applications to simulating drug action on cardiac cells include the recent study by Davies *et al.*, who re-parameterized a dog ventricular AP model, fitting it to traces from different experiments, thus encapsulating *extrinsic variability* in the model. They were able to observe different levels of AP prolongation in models representing different animals, potentially explaining some of the observed variation in reaction to drugs as a consequence of natural variation in electrophysiology (Davies *et al.*, 2012). This work points towards the need to develop a spectrum of models, representing such variability as will be present in a patient population (Sarkar & Sobie, 2011). This is a concept that may become important in detecting rare side effects and working towards patient-specific prescription.

Drug effects in tissue models

Like real cells, AP models exhibit different behaviour in isolation, compared to a setting when they are coupled together in tissue, and many of the clinically used biomarkers can be observed only at the tissue level. For this reason, AP models have been coupled together to form tissue simulations (Winslow *et al.*, 1993; Silva and Rudy, 2010), and a number of advanced simulation software packages have been developed to run simulations, from basic cell models (Garny *et al.*, 2009), 1-D (string) or 2-D (sheet) tissue models that can be implemented on a personal computer, to more extensive 3-D (block or anatomical volume) simulations run on supercomputers (Bordas *et al.*, 2009; Pitt-Francis *et al.*, 2009; Niederer *et al.*, 2011a). In recent years, the simulation of drug effects has been an expanding area of application of these tissue models (Soubret *et al.*, 2009).

One reason for this trend is that the most frequently used marker for cardiac side effects in the clinical setting is the QT interval of the ECG (Figure 2), and multicellular simulations are required to reproduce these. Drug actions, incorporated into ion-channel and AP models, can be related to ECG changes at all levels of tissue complexity. Pseudo-ECG behaviour, computed using 1-D tissue strand models, is thought to reproduce certain aspects of 'real' ECGs well, such as relative changes in QT interval duration (Viswanathan *et al.*, 1999; Benson *et al.*, 2009). 2-D and 3-D simplified models (Garny *et al.*, 2005), highly structured 3-D whole ventricular representations (Rodríguez *et al.*, 2010), and body surface potential simulations (Zemzemi *et al.*, 2011), as illustrated in Figure 6, can all simulate ECG dynamics. In the remainder of this section, we will illustrate some recent efforts in studying drug actions using these tissue models.

At the tissue strand level, Obiol-Pardo *et al.* used QSAR models to predict the IC_{50} values for I_{Kr} and I_{Ks}, and then simulated conductance-block in 1-D coupled AP models (Obiol-Pardo *et al.*, 2011). This allowed them to compute drug-induced changes in a pseudo-ECG, based purely on projections between compound structure data and historical information on I_{Kr} and I_{Ks} block by similar compounds. This suggests that it may become possible to simulate ECG effects even before a compound is synthesized, particularly when reliable QSAR models are developed for further ion channels.

Of a particular interest for the prediction of arrhythmogenicity is the identification of drug-induced changes in transmural dispersion of repolarization (Müller and Dhein, 1993), as this may provide the conditions necessary for sustenance of arrhythmic behaviour. For experimental exploration of this concept, coronary-perfused ventricular tissue wedges have become popular, and a matching 'virtual ventricular wedge' model of canine tissue has been developed as a corresponding *in silico* safety screen (Benson *et al.*, 2008; Holden, 2010).

Arrhythmias can be induced in wedges (and other cardiac tissue models) by special pacing protocols, and several studies have examined how drug actions change the vulnerability of cardiac tissue to the induction of arrhythmias, including ventricular fibrillation (Weiss *et al.*, 1999; Qu and Weiss, 2005; Seigneuric *et al.*, 2005). The role of early after-depolarizations in triggering and/or sustaining arrhythmias in this context,

has been simulated (Viswanathan and Rudy, 1999; Huffaker *et al.*, 2007), as has been the hypothesis that ischemic tissue may provide a substrate for arrhythmia initiation under drug action (Trénor *et al.*, 2005).

In Moreno *et al.*, allosteric models for block of the fast sodium current, I_{Na}, were fitted to experimental data for lidocaine and flecainide (Moreno *et al.*, 2011). Simulations at the human ventricle scale highlighted differences in sustainability of ventricular fibrillation, which matched observed differences in the clinical profiles of the two drugs.

Thus, computer models of drug actions on ion channels, embedded into cell AP models that form part of multi-cellular tissue simulations, may reproduce and partially predict relevant experimental and clinical findings. This is a remarkable achievement, and illustrates the value of representing our understanding of biological structures and functions in quantitative mathematical descriptions that adhere to basic physical laws, such as conversation of mass and charge, or of reaction and diffusion behaviour.

Challenges and opportunities

The field of computational electrophysiology has made large strides towards more accurate simulation of drug-induced changes in cardiac electrical behaviour. Of course, there are many further mechanisms and interactions that need to be considered in order to allow systematic, accurate and high-throughput prediction of drug actions on the heart. Tissue and organ electrophysiological modelling still requires significant computing power that may not be easily available for routine work [although improvements in hardware and model implementation have reduced this overhead very significantly in recent years (Niederer *et al.*, 2011b)].

Present limitations include that fact that the vast majority of 'whole heart models' do not include descriptions of the atria, atrio-ventricular activation, mechano-electric coupling and feedback, tissue deformation, coronary flow, and fluid/solid interactions, let alone their combined and interactive effects. So, most of the *in silico* hearts do not actually pump, even though it is ultimately this mechanical activity of the heart that decides a patient's fate! The heart also contains more non-mycoytes than muscle cells (Camelliti *et al.*, 2005), and contributions of endothelial cells, connective tissue or intra-cardiac neurones (to name but a few) are not yet receiving due attention in modelling (and, arguably, experimental research).

Some of the mechanisms thought to be of relevance for arrhythmogenicity rely on heterogeneous cell behaviour throughout the tissue, for example regional (and temporal) dispersion in excitability, refractoriness and electrical load. There is an ongoing debate about the presence and relevance of cells in the mid-myocardium with prolonged AP duration [see the recent M-cells debate in the *Heart Rhythm* journal (Janse *et al.*, 2011; Nattel *et al.*, 2011; Wilson *et al.*, 2011)], which highlights that heterogeneity throughout the heart is poorly understood at present. It is, arguably, even more poorly modelled. We are far from a comprehensive appreciation of the physiological relevance of local heterogeneity for homogenous global cardiac function [called 'homogeneity out of heterogeneity' (Katz and Katz, 1989)], and are barely touching the surface of patho-physiological changes to this heterogeneity, whose increase – but presumably also reduction – may well underlie disturbances in organ behaviour (Markhasin *et al.*, 2003).

Spontaneous drug-induced arrhythmias at therapeutic concentrations do not tend to occur *in silico*. But, perhaps, we should not expect them, as: (i) at present our models represent healthy cardiac tissue, with no factors that pre-dispose towards arrhythmias, and (ii) many drug-induced arrhythmias are rare events in the clinic. Disease states, gender differences, electrolyte imbalance, energy availability and use, autonomic control, circadian changes (Jeyaraj *et al.*, 2012), etc. all need to be incorporated into the computational models of the future, and used for cardiac safety testing. This work has begun with models of the AP for patients with hereditary long QT syndromes (Clancy and Rudy, 2001; 2002; Grant *et al.*, 2002), and for models of ischemia (Noble, 2002b). Application of these models to drug safety investigations will open the door to simulating the risk for different patient subgroups. This could serve as an important step towards better stratification of therapeutic approaches, so that patients with no anticipated susceptibility to cardiac adverse events could benefit from a range of drugs that may otherwise not be accessible to them. This concept of 'rehabilitation of pharmacological compounds' also benefits from a potentially attractive economical context, as much of the testing and development work has already occurred.

To explore these possibilities, members of *preDiCT* (http://www.vph-predict.eu), a Europe-wide consortium on cardiac modelling for drug safety, have put forward state-of-the-art models and highlighted their potential use in a recent workshop that was well-attended by representatives from pharmaceutical companies and regulatory agencies, including AstraZeneca, Pfizer, GlaxoSmithKline, Roche, Health Canada, the US Food and Drug Administration, the UK Medicines and Healthcare products Regulatory Agency and the European Medicines Agency [for report see (Fletcher *et al.*, 2011)]. Some of the more important suggestions made at the meeting focussed on the issue of access to, and consistency of, datasets; and we expand upon them here.

Firstly, good-quality experimental data are needed, and we applaud the efforts of teams who are assembling and publishing large ion-channel screens. Notable examples include the University of Kraków who have put together an open-access dataset of hERG-channel IC_{50} values (Polak *et al.*, 2011), and the AstraZeneca safety pharmacology group for both hERG- and fast sodium channel data (Redfern *et al.*, 2003; Harmer *et al.*, 2011). In this context, the publication of raw datasets, rather than pre-analysed IC_{50} values and dose–response curves, would be highly desirable, as the original data allow one to glean extra parameters (for example Hill coefficients or, potentially, ion-channel kinetic parameters).

Secondly, because we want to be able to accurately predict when a drug is safe, data from studies that are suggestive of lack of effect are crucial in training and testing the models (a measure of *specificity* is only possible when such data are included). The current trend of not publishing such results needs to change, as data that is 'negative for an effect' is not 'negative data'.

Thirdly, it is evident that assays are not performed consistently between laboratories. Temperature, species, cell

lines, perfusion protocols, etc. are not standardized, and assays may change over time as new technologies become available. To tackle this problem an international group of scientists recently published a draft Minimum Information standard for Cardiac Electrophysiology Experiments (Quinn *et al.*, 2011), in an effort to, at the very least, define the differences between laboratories, including descriptions of the processing, filtering or other conditioning of data that may have occurred. Not only the reporting of ('positive and negative') raw data, but more comprehensive metadata are needed to advance this area.

Despite the limitations in models and available data, now is the time to expand the role of cardiac simulation in the pharmaceutical industry. The development costs per marketed compound are becoming prohibitively expensive, and any assays that have the potential to reduce these costs, via more accurate and/or earlier safety predictions (Figure 5), are worth investigating (Mirams and Noble, 2011).

In principle, cardiac AP models now capture a majority of relevant sarcolemmal ion channels, exchangers and pumps that a pharmaceutical compound is likely to interact with. There is no technological reason, or gap in biological knowledge, to suggest that prediction of drug actions will not be possible on this basis. Of course, simulation will not replace the role of animal-based experimental models in cardiac safety testing for the foreseeable future. There will always be novel drug (off-)target effects that are not screened for, which are important in cardiac behaviour, perhaps in genetic toxicology involving up-/down-regulation of channel expression, for example. But models can provide increasingly accurate predictions for a vast majority of compounds with simple affinity for, say, the 10 most promiscuous ion channels and exchangers. Computational models do not need to provide 99% accurate predictions to be useful, as the *in vitro* models that they complement and/or partially replace tend to be no more than ~70% specific for the human QT liability. Thus, replacement of experimental assays, including animal-based work (Figure 3) is not an impossible vision, but something that has already begun, while remaining experiments will be conducted more selectively and yield more relevant information.

In the future, we envisage that pharmaceutical companies will not store databases of IC_{50} values only, but instead create and use repositories of kinetic models that represent compound action on a particular ion channel. These models will be integrated into AP/tissue/organ simulations and analysed to provide a biophysically-based rational risk assessment. The simulation of animal and human tests will be performed in tandem with, and aided by, fewer yet more targeted experimental studies. An agreement of simulation with animal experiments, for example, will provide confidence in the computational projection towards estimation of clinically relevant effects in humans, which may be different due to species differences – yet another domain of mathematical model application. Disagreement will highlight the need for further, more thorough investigation.

Conclusions

Biophysically detailed AP models can offer a 'short cut' for cardiac safety testing in the context of QT evaluation. In addition, computational modelling at tissue and organ levels can be helpful in predicting cardiac (and, increasingly, other organ) side effects. Models are already helping to develop an understanding of the mechanisms causing, or predisposing to, cardiac side effects such as TdP. These models do not only allow one to reproduce, and increasingly predict, the result of existing tests, but through an improved understanding of the underlying mechanisms involved in the regulation of cardiac activity, they can be used to propose better safety assessments.

The various challenges outlined earlier can only be addressed via pre-competitive collaboration between industry and academia, bringing together experts with skills in electrophysiology, pharmacology, toxicology, mathematical modelling, numerical methods, computer science, data management and statistics. In the short term, cardiac electrophysiology models offer an opportunity to gain extra information from *in vitro* and *in vivo* animal-based model systems. In the medium term, they will allow us to reduce the numbers of experiments that have to be performed, and speed-up the process of safety assessment. In the longer term, simulation offers the hope of replacing certain assays, and suggesting novel ones with greater predictive power.

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Conflicts of interest

GRM has received research support from GlaxoSmithKline Plc, YC is employed by GlaxoSmithKline Plc, and MRD is employed by AstraZeneca UK Ltd.

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