Refining the Human iPSC-Cardiomyocyte Arrhythmic Assessment Risk Model

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Human induced pluripotent stem cell–derived cardiomyocytes (hiPS-CMs) are capable of detecting drug-induced clinical arrhythmia, Torsade de Pointes (TdP), and QT prolongation. Efforts herein employ a broad set of structurally diverse drugs to optimize the predictive algorithm for applications in discovery toxicology and cardiac safety screening. The changes in the beat rhythm and rate of a confluent monolayer of hiPS-CMs by 88 marketed and 30 internal discovery compounds were detected with real-time cellular impedance measurement and quantified by measures of arrhythmic beating (IB), lowest concentration inducing ≥ 20% arrhythmic [irregular, atypical] beats in 3 consecutive 20-s sweeps, and predicted proarrhythmic score [PPS]-IB or changes in beat rate (BR), the lowest concentration inducing a reduction in beat rate of ≥ 20% at 3 consecutive sweeps compared with the time-matched vehicle control group, and PPS-BR. Drug-induced arrhythmic beats and reductions in beat rates are predictive of clinical arrhythmia and QT prolongation, respectively. A threshold of ≤ 10 μM for class determination results in 82% in vitro-in vivo concordance for TdP prediction and 91% sensitivity for non-TdP arrhythmia detection, or 83% and 91% if clinically efficacious plasma (effective serum therapeutic concentration [C₄₅₀]) values are incorporated. This human cardiomyocyte arrhythmic risk (hCAR) model provides greater predictivity for torsadogenicity in humans compared with either human ether-a-go-go-related gene (hERG) inhibition (75%) or QT prolongation (81%). The concordance of beat rate reductions to predict clinical QT prolongation is 86%, or 87% when C₄₅₀ is considered, which is greater than a hERG signal (80%). Further, arrhythmic beats resulting from cytotoxicity were identified by a distinct arrhythmic beating pattern, which occurred after the onset of cytotoxicity. This hCAR assay showed increased performance over existing preclinical tools in predicting clinical QT prolongation, arrhythmia, and TdP. Thus, hiPS-CMs are a relevant cell system to improve evaluating cardiac safety liabilities of drug candidates.

Key Words: cardiotoxicity; arrhythmia; stem cells; cardiomyocytes; label-free technology; investigative toxicology.

Cardiovascular liabilities of new chemical entities continue to be a significant source of attrition across the entire drug discovery and development process (Lavery et al., 2011). Among the most common drug-induced cardiovascular findings encountered are disturbances in the electrical activity of the myocardium. Cardiac arrhythmias, particularly Torsade de Pointes (TdP), can have serious and sometimes fatal consequences. Accordingly, detection of arrhythmia in the drug discovery process is mandated through a panel of in vitro and in vivo tests outlined by regulatory agencies prior to entry into clinical trials (ICH S7A and S7B) and again during clinical development (ICH E14). A positive signal for human ether-a-go-go-related gene (hERG) channel inhibition and/or QT prolongation in preclinical telemetry studies are adequate albeit not perfect predictors of clinical QT prolongation (Redfern et al., 2003). To this point, QT prolongation in and of itself does not always progress to serious ventricular arrhythmias such as TdP; thus, despite the heavy reliance on QT prolongation as a predictor of arrhythmia, the predictive performance of QT prolongation for arrhythmia in humans is poor (Carlsson, 2006; Hoffmann and Warner, 2006). Hence, novel screening models that are capable of incrementally improving the ability to assess a drug’s arrhythmogenic potential are needed (Chi, 2013). Recent advances in pluripotent cell technology have established procedures for consistently manufacturing high purity, functional human cardiomyocytes derived from inducible pluripotent stem cells (iPSCs) on an industrial scale (Kattman et al., 2011). Human iPSC-derived cardiomyocytes (hiPS-CMs) express the major cardiac ion channels, receptors, transporters, and contractile proteins, beat in a rhythmic and constant manner, and have been shown to respond to known...
selective modulators similarly to a human heart (Babiarz et al., 2012; Khan et al., 2013; Ma et al., 2011). Therefore, hiPS-CMs offer an opportunity to establish a biologically relevant in vitro model for early detection of cardiac liabilities.

The hiPS-CMs arrhythmic risk (hCAR) assay was developed using beating hiPS-CMs treated with a small training set of known cardiac active compounds and analyzed via rapid cellular impedance technology cross validated with microelectrode arrays (Guo et al., 2011). This model directly detects arrhythmic events in vitro and enables prediction of arrhythmia in vivo. The impedance fingerprint of drug-treated hiPS-CMs is attributed to the physical movement of the layer of beating cardiomyocytes. This property is a consequence of the movement of the cell membrane and is a consequence of the action potential, but is not a direct measurement of the voltage-dependent action potential properties. Importantly, impedance measurement is an accurate means to predict drug-induced arrhythmia (Guo et al., 2011), and integration of this model into early screening strategy in a drug development setting required additional optimization. Thus, segregation of the ability of the hCAR assay to predict QT prolongation and arrhythmia was sought by analyzing beat rate and arrhythmic beats as surrogate biomarkers, respectively. In addition, the potential confounding effects due to cytotoxicity on beat rhythm were evaluated, as it could be hypothesized that arrhythmogenic signals could in part be due to cellular injury/death resulting in the disruption of the cellular monolayer, rather than a true clinically meaningful alteration in cardiac channel activity. The predicted proarrhythmic score (PPS) estimates a drug’s risk level of arrhythmogenesis relative to the known cardioactive compounds and analyzed via rapid cellular impedance technology cross validated with microelectrode arrays (Guo et al., 2011), which requires an accurate knowledge of drug’s therapeutic exposure (effective serum therapeutic concentration [Ceff]) that often can only be roughly estimated with early discovery compounds. Therefore, a parameter more readily applicable to compounds with limited pharmacology information needed to be devised. In this study, the model validation was extended to include 88 marketed drugs and 30 Roche (RO) discovery compounds that have well-documented cardiac functional and/or overt cardiac toxicity profiles. Using a threshold of 10 or 30 μM as a level of concern for clinical risk confirmed our hypothesis that the induction of arrhythmic beats in hiPS-CMs is predictive of clinical arrhythmia (Guo et al., 2011). Further, a reduction in the spontaneous beating rate (BR) observed in this model was correlated with QT prolongation and other electrocardiogram (ECG) abnormalities, such as QRS (QRS duration) widening or atrioventricular block. Finally, arrhythmia caused primarily by cardiac ion channel modulation, eg. hERG inhibition, or by cytotoxicity each exhibited distinctive characteristic changes in the impedance waveforms. A threshold for cell index (CI) loss of ≥ 10% was identified as a threshold capable of delineating “false” cytotoxicity-induced arrhythmic beats from arrhythmia caused primarily by cardiac ion channel modulation. Collectively, these results demonstrated the utility of hiPS-CMs to enhance a comprehensive assessment of a drug candidate’s proarrhythmic potential by accurately predicting clinical response.

**MATERIALS AND METHODS**

**Cell culture.** Cryopreserved hiPS-CMs (iCell Cardiomyocytes, Cellular Dynamics International [CDI], Madison, Wisconsin) were thawed in Plating Media supplied by the vendor and plated onto 0.1% gelatin-coated, 6-well tissue-culture plates at approximately 1.5 × 10⁷ cells/well. Cells were cultured for 5–10 days at 37°C, 7% CO₂ to ensure synchronous batches of cells prior to replating onto 0.1% gelatin-coated 96-well E-Plate Cardio (Roche Applied Sciences, Mannheim, Germany, and ACEA Biosciences, San Diego, California) at approximately 50 × 10⁴ cells/well. The maintenance media (CDI) was changed every 2–3 days after plating and 24h prior to dosing.

**Impedance measurements.** Cell contraction and viability were monitored by impedance using xCELLigence RTCA (real-time cellular analyzer) Cardio system (Roche Applied Sciences/ACEA Biosciences). Impedance measurements (a 20-s sweep was recorded at a sampling rate of 77 Hz), reported as CI, were performed at selected time points. Drug treatment was initiated once a stable waveform and beat rate of 40–60 beats/min was established, which required 5 ± 2 days after cell plating on E-Plates. Drug stocks, prepared in dimethylsulfoxide or dH₂O at 1000-fold above the highest tested concentration, were serially diluted in maintenance media in a separate 96-well plate at 2× target concentration. The drug plate was equilibrated to 37°C before diluting 1:1 into the E-Plate to give a final medium volume of 200 μl/well. All compounds were tested at n ≥ 3 wells on ≥ 2 E-Plates seeded with cells from 2 or more different lots.

**Data analysis.** Data are expressed as mean ± SE. Ibₙo is defined as the lowest concentration that induced ≥ 20% arrhythmic (irregular, atypical) beats in 3 consecutive 20-s sweeps (Guo et al., 2011). The Brₙo is defined as the lowest concentration that induced a reduction in beat rate of ≥ 20% at 3 consecutive sweeps compared with the time-matched vehicle control group. Summary data for Ibₙo and the Brₙo were calculated from the raw impedance data. CI generated from the impedance tracings was used to determine cell viability and cytotoxicity (Xing et al., 2006).

The clinical QT/TdP, target organ toxicity, and Cₜₐ were compiled from the literature (Bagnes et al., 2010; Guengerich, 2011; Hamid et al., 2004; O’Brien et al., 2006; Redfern et al., 2003; Shaikh and Shih, 2012; Slordal and Spigset, 2006; Svoboda et al., 2012; Xu et al., 2008), the Arizona Community Emergency Response Team (CERT) list (http://www.qtdrugs.org/), or the Food and Drug Administration (FDA) package inserts. A positive hERG liability for a drug was defined as its hERG/IC₅₀ ≤ 30x the maximal free therapeutic exposure (Redfern et al., 2003). The hERG/IC₅₀ was used as a threshold obtained from the literature or generated internally (see Supplementary Data) using the patch clamp technique described previously (Guo and Guthrie, 2005). In instances where multiple hERG values were available, the lowest value was used.

The sensitivity or specificity was determined as the number of positive or negative identifications in preclinical models divided, respectively, by the number of drugs known to have or lack the liabilities of clinical QT prolongation or arrhythmia/TdP. Receiver operator characteristic (ROC) curves were plotted by sensitivity against (1 – specificity) of the assay at all possible thresholds. The predictive performance (ie, in vitro to in vivo concordance) was defined as the number of drugs that demonstrated a correct correlation on results from preclinical models to their known outcomes for clinical QT prolongation or arrhythmia/TdP divided by the total number of drugs under the investigation.

**Materials.** Roche compounds were synthesized in-house. All other chemicals were purchased from Sigma-Aldrich, Tocris, Toronto Research, MP Biomedicals, Enzo Life Sciences, Acros, or Alfa Aesar.

**RESULTS**

**Induction of Arrhythmic Beats by Arrhythmogenic Compounds**

Ib₂₀ values were determined for 88 drugs, of which 32 were known to be arrhythmogenic in humans (21 torsadogenic and
A Model for Cardiac Arrhythmia Prediction
d was also at 0.01 for drugs associated with human
tosadogenicity was assessed by comparing ROC curves and the
level of in vitro-in vivo concordance at varying thresholds for
IB_{20} between 0.003 and 100µM in a half-log interval (Fig. 1).
The sensitivity (ie, true positive identification) increased with
the increase in threshold IB_{20} and accompanied by a concomi-
tant decrease in specificity (ie, negative identification, see
Fig. 1A). A threshold IB_{20} of 10µM gave rise to a highest level
of concordance (82%), with 81% sensitivity and 82% specific-
ity, reflecting correct identification of 17 out of 21 torsadogenic
and 42 out of 51 nonarrhythmogenic drugs (Fig. 1C). For
comparison, when a positive hERG or QT prolongation
was used as the predictor, the number of positive calls (sensitiv-
ity) was 18 (86%) and 21 (100%), and the number of negative
identification (specificity) was 36 (71%) and 37 (73%), corre-
sponding to a concordance of 75% and 81%, respectively (Figs.
1A and 1C). Out of 11 drugs causing non-TdP arrhythmia in
humans, a threshold IB_{20} of 10µM detected 10 (91% sensitiv-
ity), whereas a positive hERG or QT prolongation detected 0
(0%) or 5 (45% sensitivity), respectively.

The PPS, a summary metric to assess arrhythmogenic risk (Guo et al., 2011), is calculated by C_{et}/IB_{20} referred as PPS-IB_{20}
for this expanded drug set (Table 1). The prediction performance
of PPS-IB_{20} was evaluated by setting the threshold at various values between 30 and 30 000 (Fig. 1). The increased
sensitivity was also accompanied by the decreased specificity
(Fig. 1A). The greatest concordance (83%) was achieved at
PPS-IB_{20} = 100, with a balanced sensitivity (81%) and specifi-
ity (84%) that were corresponding to 17 positive torsadogenic
and 43 negative identification, respectively (Fig. 1C). Using the
PPS-IB_{20} threshold of 100 identified 10 out of 11 drugs associ-
ating with non-TdP arrhythmia in the clinic (91% sensitivity).

The same metric was applied to assess the predictive power of
IB_{20} and PPS-IB_{20} to identify drugs with QT prolongation liabil-
ity (Figs. 1B and 1D). The greatest concordance was observed as
73% at 30µM for IB_{20} and as 66% at 30µM for PPS-IB_{20}.

Induction of Beat Rate Reduction by QT Prolongation
Inducers

Rapid onset, dose- and time-dependent reductions in beat
rates were a characteristic, albeit not exclusively, response to
drugs known to directly inhibit the hERG channel. Arrhythmi-
beats occurred for drugs known to induce clinical TdP either
concurrently with, or subsequent to, the reduction in beat rate
of regular beats. The arrhythmic beats exhibited a unique small
waveform, which was the hallmark of the delayed repolariza-
tion-mediated arrhythmia as described previously (Guo et al.,
2011). As a representative example of direct-acting hERG chan-
nel blockers, sotalol dose dependently reduced beat rate at the
first measurement after dosing, and onset of arrhythmic beats
occurred within 5 min after dosing (Fig. 2). Both the reduced
beat rate and the arrhythmic beat pattern persisted throughout
the sweeps evaluated over the 72 h drug exposure evaluation
period. The IB_{20} was determined as 30µM at 0.6±0.2 h (n = 3)
postdose, and BR_{20} was 10µM. Of note, high concentrations of
test compound would sporadically and occasionally induce a
complete, but reversible, arrest of beating, in a compound and
time-dependent manner, like that with terfenadine or thiori-
dazene (Guo et al., 2011).

Effects of hERG channel expression blockade were also ex-
amined. Arrhythmogenic drugs such as pentamidine, As_{2}O_{3},
and geldanamycin that lack acute effects on cardiac ion chan-
nels but decrease hERG channel function through inhibition
of hERG channel assembly (Dennis et al., 2007) displayed
delayed onset of both beat rate reduction and lengthened reper-
olarization-mediated arrhythmic beats. As shown in Figure 3
for pentamidine, no drug-induced changes were observed until 20 h
postdose, after which beat rate was reduced at a concentration
of 3µM. Arrhythmic beating was first observed at 24 h postdose
at the highest drug concentration tested (3µM) and after 72 h
at 1µM. The beat rate remained reduced and arrhythmic beats
increased in incidence throughout the remaining hours of
drug treatment. The IB_{20} for pentamidine was identified as 1µM
at 62±19 h (n = 3) postdose, and the BR_{20} was also determined to
be 1µM. These results are consistent with the time delay required
to result in a meaningful inhibition of hERG protein traffick-
ing as 24 h of pentamidine at 10µM has been shown to reduce
hERG surface expression by 30% (Kuryshiev et al., 2005).

Compounds that can induce arrhythmia via the inhibition of
cardiac ion channels other than hERG were also tested. Several
cardiac glycosides including ouabain, digoxin, and digitoxin
produce an acute effect on cardiomyocytes by inhibiting the
plasma membrane Na+/K+-ATPase, followed by a delayed
effect due to blocking hERG protein trafficking (Wang et al.,
2007). Thus, at earlier time points after ouabain application
(Supplementary Figure 1), a tachycardia-like arrhythmia was
observed, and this was followed by a time- and dose-dependent
arrest in beating. At the lower concentration of 0.01µM, the
beat rate was gradually reduced by 20h with subsequent de-
velopment of repolarization-mediated arrhythmic beats at 38 h.
The IB_{20} of this delayed arrhythmia was determined as 0.01µM
at 33±8 h (n = 5) postdose. The BR_{20} was also at 0.01µM.

The BR_{20} was determined for each of 88 drugs (Table 1, 5
experimental cytotoxic drugs lacking clinical data were not in-
cluded), of which 40 were known to cause QT prolonga-
tion in the clinic and 43 were demonstrated not to do so. To
assess the performance of BR_{20} for identifying QT prolonga-
tion risk, the in vitro-in vivo concordance was tested by setting
the threshold BR_{20} at various concentrations between 0.003 and
100µM (Fig. 1B). The optimal concordance (86%) occurred at
the threshold concentration of 10µM, whereas 35 QT positive
(88% sensitivity) and 36 QT negative (84% specificity) were
identified. If a positive hERG signal was used as the predictor,
30 positive (75% sensitivity) and 36 negative (84% specific-
ity) were correctly identified (80% concordance).
## TABLE 1
Summary of IB<sub>20</sub>, PPS-IB<sub>20</sub>, BR<sub>20</sub>, and PPS-BR<sub>20</sub> Determined in hiPS-CMs

<table>
<thead>
<tr>
<th>Drug</th>
<th>C&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>IB&lt;sub&gt;20&lt;/sub&gt; (µM)</th>
<th>PPS-IB&lt;sub&gt;20&lt;/sub&gt;</th>
<th>BR&lt;sub&gt;20&lt;/sub&gt; (µM)</th>
<th>PPS-BR&lt;sub&gt;20&lt;/sub&gt;</th>
<th>tERG</th>
<th>QT</th>
</tr>
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<tr>
<td>Acetamidophenol</td>
<td>130 000</td>
<td>&gt; 100</td>
<td>NA</td>
<td>&gt; 100</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Aconitine</td>
<td>77</td>
<td>0.03</td>
<td>2567</td>
<td>(†) 0.01</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Ajmaline (IV)</td>
<td>105</td>
<td>10</td>
<td>11</td>
<td>0.3</td>
<td>350</td>
<td>(−)</td>
<td>(+)</td>
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<td>Alfuzosin</td>
<td>56</td>
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<td>56</td>
<td>0.3</td>
<td>187</td>
<td>(−)</td>
<td>(+)</td>
</tr>
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<td>Alpidem</td>
<td>284</td>
<td>&gt; 100</td>
<td>NA</td>
<td>30</td>
<td>9</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>3874</td>
<td>&gt; 100</td>
<td>NA</td>
<td>0.3</td>
<td>12 913</td>
<td>(+)</td>
<td>(+)</td>
</tr>
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<td>Amitriptyline</td>
<td>793</td>
<td>&gt; 30</td>
<td>NA</td>
<td>3</td>
<td>264</td>
<td>(−)</td>
<td>(−)</td>
</tr>
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<td>Amoxicillin</td>
<td>17 036</td>
<td>&gt; 1000</td>
<td>NA</td>
<td>&gt; 1000</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Amphetamine B</td>
<td>89 818</td>
<td>3</td>
<td>29 939</td>
<td>3</td>
<td>29 939</td>
<td>(−)</td>
<td>(+)</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>12 132</td>
<td>3</td>
<td>4044</td>
<td>1</td>
<td>12 132</td>
<td>(−)</td>
<td>(+)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10 000</td>
<td>&gt; 1000</td>
<td>NA</td>
<td>&gt; 1000</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
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<td>267</td>
<td>0.01</td>
<td>800</td>
<td>(+)</td>
<td>(+)</td>
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<td>&gt; 100</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
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<td>Bepridil</td>
<td>3298</td>
<td>&gt; 30</td>
<td>NA</td>
<td>0.03</td>
<td>109 933</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Captopril</td>
<td>2466</td>
<td>&gt; 100</td>
<td>NA</td>
<td>&gt; 100</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
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<tr>
<td>Cetirizine</td>
<td>800</td>
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<td>NA</td>
<td>&gt; 30</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
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<tr>
<td>Chlorpromazine</td>
<td>2630</td>
<td>10</td>
<td>263</td>
<td>3</td>
<td>877</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Cibenzoline</td>
<td>2168</td>
<td>&gt; 30</td>
<td>NA</td>
<td>&gt; 100</td>
<td>217</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Cisapride</td>
<td>129</td>
<td>0.3</td>
<td>430</td>
<td>0.317 pt</td>
<td>430</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>6029</td>
<td>10</td>
<td>603</td>
<td>30</td>
<td>201</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Clozopine</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Colchicine</td>
<td>16</td>
<td>&gt; 100</td>
<td>NA</td>
<td>100</td>
<td>0</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>153 200</td>
<td>&gt; 100</td>
<td>NA</td>
<td>&gt; 100</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>1498</td>
<td>100</td>
<td>15</td>
<td>100</td>
<td>15</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Dalfampridine</td>
<td>493</td>
<td>&gt; 30</td>
<td>NA</td>
<td>&gt; 30</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Dantrolene</td>
<td>7900</td>
<td>10</td>
<td>790</td>
<td>100</td>
<td>79</td>
<td>(−)</td>
<td>(−)</td>
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<tr>
<td>Desipramine</td>
<td>601</td>
<td>10</td>
<td>60</td>
<td>3</td>
<td>200</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Dextoxarine</td>
<td>136 052</td>
<td>&gt; 100</td>
<td>NA</td>
<td>&gt; 100</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>33</td>
<td>0.003</td>
<td>10 900</td>
<td>(†) 0.1</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>3</td>
<td>0.01</td>
<td>300</td>
<td>(†) 1</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>552</td>
<td>&gt; 30</td>
<td>NA</td>
<td>(†) 0.1</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
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<td>Diphenhydramine</td>
<td>157</td>
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<td>NA</td>
<td>10</td>
<td>16</td>
<td>(−)</td>
<td>(+)</td>
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<tr>
<td>Dobutamine</td>
<td>3819</td>
<td>1</td>
<td>3819</td>
<td>(†) 1</td>
<td>NA</td>
<td>(+)</td>
<td>(−)</td>
</tr>
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<td>Doletidide</td>
<td>6</td>
<td>0.003</td>
<td>2000</td>
<td>0.003</td>
<td>2000</td>
<td>(+)</td>
<td>(+)</td>
</tr>
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<td>15 344</td>
<td>1</td>
<td>15 344</td>
<td>10</td>
<td>1534</td>
<td>(−)</td>
<td>(+)</td>
</tr>
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<td>E-4031</td>
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<td>0.03</td>
<td>433</td>
<td>0.03</td>
<td>433</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
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<td>1604</td>
<td>3</td>
<td>5345</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Erythromycin</td>
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<td>30</td>
<td>1135</td>
<td>30</td>
<td>1135</td>
<td>(+)</td>
<td>(+)</td>
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<td>1931</td>
<td>1</td>
<td>1931</td>
<td>(+)</td>
<td>(+)</td>
</tr>
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<td>Fluorouracil</td>
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<td>&gt; 30</td>
<td>NA</td>
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<td>(−)</td>
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<tr>
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<td>(+)</td>
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<td>3</td>
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<td>(−)</td>
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<tr>
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<td>56 000</td>
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<td>(+)</td>
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<td>Imatinib</td>
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<td>118</td>
<td>10</td>
<td>354</td>
<td>(−)</td>
<td>(−)</td>
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<td>35 667</td>
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<td>(+)</td>
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<tr>
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<td>30</td>
<td>590</td>
<td>(−)</td>
<td>(+)</td>
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<td>Levosimendan</td>
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<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Lidocaine (IV)</td>
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<td>100</td>
<td>360</td>
<td>30</td>
<td>1200</td>
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<td>(−)</td>
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<td>(−)</td>
<td>(−)</td>
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<tr>
<td>Mechlorethamine</td>
<td>?</td>
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<td>&gt; 100</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
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<td>Mexiletine</td>
<td>11 161</td>
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<td>372</td>
<td>3</td>
<td>3720</td>
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<td>(−)</td>
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<td>Mitoxantrone</td>
<td>3311</td>
<td>3</td>
<td>1104</td>
<td>0.3</td>
<td>11 037</td>
<td>(+)</td>
<td>(+)</td>
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<td>100</td>
<td>103</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>4898</td>
<td>10</td>
<td>490</td>
<td>10</td>
<td>490</td>
<td>(±)</td>
<td>(−)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>194</td>
<td>&gt; 3</td>
<td>NA</td>
<td>(†) 0.1</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
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<td>Nimesulide</td>
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<td>NA</td>
<td>&gt; 100</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>Nitrendipine</td>
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<td>NA</td>
<td>(†) 0.3</td>
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<td>(−)</td>
<td>(−)</td>
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<tr>
<td>Olanzapine</td>
<td>74</td>
<td>&gt; 30</td>
<td>NA</td>
<td>&gt; 30</td>
<td>NA</td>
<td>(−)</td>
<td>(−)</td>
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</table>
When $C_{eq}$ was taken into account for predicting the QT risk, a PPS could be calculated with $C_{eq}$ divided by BR$_{20}$ referred as PPS-BR$_{20}$ (Table 1). The greatest concordance (87%) was observed when PPS-BR$_{20}$ 100 was selected as a threshold of concern, 36 QT prolongation positive (90% sensitivity) and 36 negative (84% specificity) drugs were identified (Fig. 1B).

An attempt was also made to assess the predictive power of BR$_{20}$ and PPS-BR$_{20}$ to clinical torsadogenicity. The greatest prediction performance was observed as 79% at BR$_{20}$ 1µM or as 82% at PPS-BR$_{20}$ 800 (Fig. 1B).

Ca$^2+$ channel blockers, such as verapamil, diltiazem, nifedipine, and nitrendipine, with a high selectivity on Ca$^2+$ channels over other type of ion channels always induced a dose-dependent decrease in the beat amplitude but an increase in the beat rate (Table 1). These compounds were accordingly all scored as negative for QT prolongation (Table 1).

### Table 1—Continued

<table>
<thead>
<tr>
<th>Drug</th>
<th>$C_{eq}$ (nM)</th>
<th>IB$_{20}$ (µM)</th>
<th>PPS-IB$_{20}$</th>
<th>PPS-BR$_{20}$</th>
<th>hERG</th>
<th>QT</th>
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<tr>
<td>Oubain</td>
<td>170</td>
<td>0.01</td>
<td>17 000</td>
<td>(†) 0.03</td>
<td>NA</td>
<td>(−)</td>
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<td>Pemoline</td>
<td>6925</td>
<td>&gt; 100</td>
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<td>&gt; 100</td>
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<td>(−)</td>
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<tr>
<td>Pentamidine</td>
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<td>1</td>
<td>2181</td>
<td>1</td>
<td>2181</td>
<td>(−)</td>
</tr>
<tr>
<td>Phenotamine</td>
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<td>10</td>
<td>3</td>
<td>3</td>
<td>NA</td>
<td>(−)</td>
</tr>
<tr>
<td>Pimobendan</td>
<td>164</td>
<td>10</td>
<td>2</td>
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<td>2</td>
<td>(−)</td>
</tr>
<tr>
<td>Pimozide</td>
<td>217</td>
<td>0.1</td>
<td>2170</td>
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<td>(+)</td>
</tr>
<tr>
<td>Preynylamine</td>
<td>70</td>
<td>6</td>
<td>23</td>
<td>1</td>
<td>70</td>
<td>(+)</td>
</tr>
<tr>
<td>Propafenone</td>
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<td>161</td>
<td>3</td>
<td>1609</td>
<td>(+)</td>
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<tr>
<td>Propranolol (IV)</td>
<td>193</td>
<td>30</td>
<td>6</td>
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<td>643</td>
<td>(±)</td>
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<tr>
<td>Quinidine</td>
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<td>10</td>
<td>2158</td>
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<td>(+)</td>
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<tr>
<td>Ranolazine</td>
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<td>100</td>
<td>60</td>
<td>10</td>
<td>601</td>
<td>(+)</td>
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<td>Rosfexoxib</td>
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<td>&gt; 100</td>
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<td>(−)</td>
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<tr>
<td>Rosiglitazone</td>
<td>1673</td>
<td>&gt; 30</td>
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<td>(†) 1</td>
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<td>(−)</td>
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<td>Semntide</td>
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<td>(+)</td>
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<tr>
<td>Sotalol</td>
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<td>Sunitnib</td>
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<td>(−)</td>
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<td>Tefenednine</td>
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<td>(+)</td>
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<tr>
<td>Thioridazine</td>
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<td>594</td>
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<td>(+)</td>
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<tr>
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<td>220</td>
<td>(−)</td>
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<td>&gt; 30</td>
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<td>&gt; 30</td>
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<td>(−)</td>
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<td>Verapamil</td>
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<td>(†) 0.03</td>
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<td>(−)</td>
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<td>Zalcitabine</td>
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<td>3</td>
<td>109</td>
<td>1</td>
<td>328</td>
<td>(+)</td>
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</table>

The therapeutic exposure ($C_{eq}$), hERG inhibition, clinical QT prolongation, and TdP/Arrhythmia for each of 83 drugs tested are compiled from the literature. hERG inhibition data for some drugs are generated in-house. (+) and (−) represent positive or negative observations in the clinic, whereas (±) means equivocal results are reported, or the positive events are observed only in overdose. Abbreviation: NA, not applicable.

*Non-TdP type arrhythmia.

*hERG trafficking inhibition.

when $C_{eq}$ was taken into account for predicting the QT risk, a PPS could be calculated with $C_{eq}$ divided by $BR_{20}$ referred as PPS-BR$_{20}$ (Table 1). The greatest concordance (87%) was observed when PPS-BR$_{20}$ 100 was selected as a threshold of concern, 36 QT prolongation positive (90% sensitivity) and 36 negative (84% specificity) drugs were identified (Fig. 1B).

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**Arrhythmic Beats Caused by Cardiac Functional Alteration Versus General Cytotoxicity**

Overt cytotoxicity has the potential to produce confounding effects on beat parameters in hiPSC-CMs. Therefore, the ability to distinguish between beat changes due to functional inhibition of cardiomyocyte contractions versus those due to nonspecific cytotoxicity was examined. To identify the origin of arrhythmic beats, the beat waveform and pattern, the CI values, and onset time associated with beat rate and beat rhythm changes were compared between the cardiac ion channel modulators and compounds that cause structural cardiotoxicity or general cytotoxicity. Three drugs were selected as examples: (1) E-4031, potent hERG blockers without structural toxicity, (2) doxorubicin, a structural cardiotoxicant without acute cardiac ion channel (especially hERG) liability, and (3) sunitinib, a drug linked to both functional and structural cardiotoxicity (Fig. 4).

E-4031 caused a rapid onset of beat rate reduction of regular beats and occurrence of arrhythmic beats (Figs. 4A and 4B) with no change in CI. The arrhythmic beat began as a single, doubles, or triples with smaller amplitude and duration between regular beats and progressed dose dependently into fibrillation-like arrhythmia or a reversible beat arrest. The IB$_{20}$ for E-4031 was determined as 0.03µM at 0.3 ± 0.08 h postdose, and the associated CI was 1 ± 0.01 (n = 3). Doxorubicin induced a time- and dose-dependent decrease in CI without an immediate effect on beat parameters (Figs. 4C and 4D). Arrhythmic beats occurred concurrently with substantial CI reduction and were often followed by transient tachycardia/fibrillation-like pattern, ultimately progressing to irreversible arrest of beating. For doxorubicin, the IB$_{20}$ was 1µM at 19 ± 0.4 h and CI 0.6 ± 0.1 (n = 3). Sunitinib (30µM) induced a rapid decrease in CI and at lower concentrations, a rapid onset and sustained decrease in beat rate, followed by arrhythmic beats that often...
progressed to a fibrillation-like pattern (Figs. 4E and 4F). The corresponding IB\textsubscript{20} and CI for sunitinib were 3\(\mu\)M (4 ± 3 h) and 1 ± 0.1 (n = 3), respectively. Based on these observations, caution should be used before declaring drugs and chemicals truly arrhythmic when the IB\textsubscript{20} values are accompanied by a reduction in CI by ≥ 10% because the beat pattern disruptions may be due to general cytotoxicity and not a cardiomyocyte or cardiac ion channel—specific event.

The relationship between the onset time of cytotoxicity and arrhythmia was further investigated in 50 drugs including 5 well-known cytotoxic chemicals (benzalkonium, benzethonium, digitonin, oligomycin B, and Triton X-100). Figure 5 summarizes the onset time and CI at which the IB\textsubscript{20} was identified for compounds in this test set. Drugs associated with structural toxicity and/or hERG trafficking inhibition such as doxorubicin, epirubicin, mitoxantrone, pentamidine, As\textsubscript{2}O\textsubscript{3}, and geldanamycin tend to have a delayed onset of IB\textsubscript{20}. Among 22 drugs that cause structural organ toxicity or cytotoxicity, 20 had an IB\textsubscript{20} ≥ 1.5 h after drug application, whereas all 6 hERG trafficking blockers had an IB\textsubscript{20} ≥ 23 h (Fig. 5A). For the majority of drugs, IB\textsubscript{20} was not associated with a remarkable change in CI. However, all 15 drugs with a reduction in normalized CI ≥ 10% when IB\textsubscript{20} was identified were known structural toxicants and 6 of these were also hERG trafficking blockers (Fig. 5B), Together, this suggests that the hCAR model can provide significant insight into distinguishing broad mechanisms in which a drug can yield cardiac beat irregularity.

**Validation Using Internal Discovery Compounds**

Table 2A lists the IB\textsubscript{20} and BR\textsubscript{20} of 30 internal Roche compounds, along with their hERG IC\textsubscript{20} and ECG (QTc [QT interval corrected by the heart rate], QRS, and PR interval) measurements collected from telemetry dog or monkey studies (see Misner et al., 2012 for the telemetry study protocol). Using IB\textsubscript{20} ≤ 30\(\mu\)M as the threshold for a positive response, 14 out of 20 compounds that had altered ECG waveforms (QTc, QRS, or PR) and all 10 compounds without any ECG changes were identified. When BR\textsubscript{20} ≤ 30\(\mu\)M was used instead, all 20 compounds causing an abnormal ECG, and 9 out of 10 compounds with unaltered ECG were correctly identified.

**FIG. 1.** Performance analysis for prediction of clinical TdP/arrhythmia and QT prolongation by IB\textsubscript{20}, PPS-IB\textsubscript{20}, BR\textsubscript{20}, and PPS-BR\textsubscript{20}. ROC curves for prediction of clinical TdP/arrhythmia (A) and QT prolongation (B) are plotted by the sensitivity against specificity that were determined at various possible thresholds, or at single determination of hERG inhibition and/or QT prolongation. The dotted lines in (A) and (B) illustrate the result of a random guess. The in vitro-in vivo concordance of IB\textsubscript{20}, PPS-IB\textsubscript{20}, BR\textsubscript{20}, and PPS-BR\textsubscript{20} to clinical TdP/arrhythmia (C) and QT prolongation (D) is plotted as a function of threshold concentration (in \(\mu\)M) or PPS (in × 10\textsuperscript{3}). Note the clear better performance of IB\textsubscript{20} and PPS-IB\textsubscript{20} to predict clinical TdP/arrhythmia than BR\textsubscript{20}, PPS-BR\textsubscript{20}, hERG inhibition, and QT prolongation (A and D), and the better performance of BR\textsubscript{20} and PPS-BR\textsubscript{20} to predict clinical QT prolongation than IB\textsubscript{20}, PPS-IB\textsubscript{20} and hERG inhibition (B and C).
To evaluate the prediction of hERG inhibition for QT alteration, the hERG IC_{20} values for each of the 30 internal compounds were grouped by potency (Table 2B). For 21 compounds with a potent or moderate hERG inhibition (IC_{20} < 1 or 9 μM, internal criteria for hERG liability assessment), nearly half of them were linked to a QT prolongation in telemetry animals. Among 9 weak hERG blockers (IC_{20} ≥ 10 μM), 2 showed QT prolongation, and none of 3 compounds with a hERG IC_{20} ≥ 30 μM

**FIG. 2.** Induction of beat rate reduction and arising arrhythmic beats by sotalol. A, Representative beating traces captured at selected time points illustrate the rapid onset of beat rate reduction and development of arrhythmic beats, which were denoted by arrowheads. B, Changes in beat rate were normalized to that of the time-matched vehicle control group and plotted as a function of time to demonstrate dose-dependent beat rate reduction that persisted throughout the entire 72 h drug treatment. Each line represents an average of 3 wells from 3 separate E-Plates, the error bars are omitted for clarity. The dosing time is marked by the arrow. Horizontal scale bar: 4 s, vertical scale bar: 0.01 unit.

**FIG. 3.** Induction of beat rate reduction and arising arrhythmic beats by pentamidine. A, Representative beating traces captured at selected time points illustrate the delayed onset of beat rate reduction and slow development of arrhythmic beats (denoted by arrowheads). B, Changes in beat rate were normalized to that of the time-matched vehicle control group and plotted as a function of time. Wells were dosed twice at the time (indicated by arrows) and were observed up to 120 h. Each line represents an average of 3 wells from 3 separate E-Plates, the error bars are omitted for clarity. Horizontal scale bar: 4 s, vertical scale bar: 0.01 units.
was associated with QT prolongation or any other ECG abnormalities.

**DISCUSSION AND CONCLUSION**

Application of high-resolution, rapid-sampling impedance measurement with functional, beating hiPS-CMs has provided a novel *in vitro* model for evaluation of drug-induced QT prolongation and arrhythmia (Abassi et al., 2012; Jonsson et al., 2011; Nguemo et al., 2012; Peters et al., 2012). The current study expands upon our earlier work (Guo et al., 2011) by including additional well-characterized compounds, improving the prediction metric, and increasing the understanding of cytotoxicity when studying the risk of drug-induced cardiotoxicity.

Arrhythmic beats were induced by drugs known to be arrhythmogenic in the clinic and are illustrated clearly by ROC curves and *in vitro-in vivo* concordance plots (Figs. 1A and 1C); IB$_{20}$ and PPS-IB$_{20}$ were superior to BR$_{20}$, PPS-BR$_{20}$ hERG inhibition, and QT prolongation to predict drug’s arrhythmogenesis. For torsadogenicity prediction, using IB$_{20}$ ≤ 10μM as the decision metric resulted in the highest level of *in vitro-in vivo* concordance (82%), with well-balanced sensitivity (81%) and specificity (82%). Using IB$_{20}$ ≤ 30μM could achieve nearly identical concordance level (81%) with a greater sensitivity (95%) but a less optimal specificity (75%). When the drug therapeutic exposure ($C_{eff}$) was taken into account, a slightly higher concordance level (83%) was achieved at PPS-IB$_{20}$ 100, with sensitivity (81%) and specificity (84%). Though a positive hERG or QT prolongation signal could predict clinical TdP with high sensitivity (86% and 100%), they displayed high false positive calls, with 71% and 73% specificity, respectively. For detecting drugs associated with non-TdP arrhythmia, IB$_{20}$ and PPS-IB$_{20}$ exhibited a sensitivity of 91% positive identification, whereas as expected, a positive hERG or QT prolongation signal was incapable of predicting non-TdP arrhythmia.

Taken together, these results demonstrated that with this hCAR model, the clinical arrhythmogenesis of a discovery compound could be identified with a high predictive accuracy even before its clinical therapeutic exposure has been fully defined.

**FIG. 4.** Effects of E-4031, doxorubicin, and sunitinib on CI and beating patterns. Screenshots were taken from wells treated with E-4031 (A), doxorubicin (C), and sunitinib (E) at various concentrations over 72h. CI in each well was normalized to the time point immediately prior to dosing. The black arrows mark the time point of 30min and 30h postdose when the recording traces are zoomed in to display individual beats as shown in (B), (D), and (F), respectively. The red arrows mark the traces representing the concentration identified as IB$_{20}$. Recording traces of the selected concentrations (B, D, and F) are presented to demonstrate the time- and dose-dependent change of beating pattern. Arrowheads indicate the presence of arrhythmic beats; * indicates tachycardia or fibrillation-like arrhythmia. Horizontal scale bar: 5 s, vertical scale bar: 0.01 unit.
Similarly, the reduced beat rate observed in the hCAR model was highly correlated with the QT prolongation seen in the clinic. Using $\text{BR}_{20} \leq 10\mu M$ at the threshold for a positive response resulted in the highest level of in vitro-in vivo concordance (86%), with 88% sensitivity and 84% specificity (Fig. 1D). The same level of concordance was also achieved at $\text{BR}_{20} \leq 30\mu M$, with a higher sensitivity (95%), but a less optimal specificity (77%). When including the therapeutic exposure for analysis, PPS-BR$_{20} < 100$ achieved the highest level of in vitro-in vivo concordance (87%), with 90% sensitivity and 84% specificity. The prediction of clinical QT prolongation by BR$_{20}$ or PPS-BR$_{20}$ is superior to predictions made based on positive hERG signals alone (80% concordance), or on IB$_{20}$ (73%) and PPS-IB$_{20}$ (66%) as shown in Figures 1B and 1D.

Inhibition of hERG current will cause delayed myocardial repolarization, resulting in a lengthened beat-to-beat cycle interval that leads to a reduced beat rate. For compounds that inhibit $I_{Kr}$ current by direct hERG channel blockade, the reduction of beat rate in the hCAR model occurs almost instantly upon drug application (Fig. 2). In contrast, for compounds inhibiting hERG function via interruption of translocation and/or assembly of hERG channel protein in the plasma membrane exhibit a corresponding delayed onset of beat rate reduction (Fig. 3). For drugs such as cardiac glycosides, which have both acute effects on cardiac ion channels, receptors, or transporters and delayed effects on hERG trafficking, time-dependent biphasic early and delayed beat rate changes were observed (Supplementary Figure 1).

Clinical QT prolongation may also be a consequence of structural injury to the myocardium (Bagnes et al., 2010). Though the direct inhibition of $I_{Ks}$ (a slowly activating component of delayed rectifier K+ channel) has been proposed recently responsible for doxorubicin-induced QT prolongation (Ducroq et al., 2010), the mechanistic role of $I_{Ks}$ inhibition in the reduction of
beat rate caused by doxorubicin in this hCAR model is questionable because either Chromanl 293B or JNJ303, two selective $I_{ks}$ inhibitors, did not induced detectable beat rate reduction at concentrations that blocks $I_{ks}$ significantly (data not shown). This might suggest that the contribution of $I_{ks}$ to the membrane repolarization in these cells was not significant enough to influence the rate of spontaneous beating. This was indeed in line with the report that complete inhibition of $I_{ks}$ by 300$\mu$M 293B only resulted in a marginal (approximately 6.5%) prolongation of action potential duration (APD$_{90}$) in human cardiomyocytes differentiated from embryonic stem cells (Peng et al., 2010). The beat rate reduction caused by imatinib, sunitinib, and tacrine was likely related to their direct inhibition of hERG current, as their hERG IC$_{50}$ was determined by the automated
patch clamp as 20, 1.4, and 2.1 μM, respectively, and occurs at concentrations lower than the changes in CI. Further, cyclophosphamide induces QT alterations, is genotoxic, and causes heart failure chronically after high doses, which may explain the lack of response for cyclophosphamide in the hCAR model (Nakame et al., 2000; Senkus and Jassem, 2011).

Similarly, cell death was in some cases observed to coincide with arrhythmic beats, thus the contribution of cytotoxicity was further examined. The level of cell injury/death was quantified by CI (Xing et al., 2006). Three attributes appeared to be useful in differentiating the origin of arrhythmic beats: (1) the onset time when the IB_{20} was identified, (2) the CI associated with the IB_{50}, and (3) the specific pattern (waveform) of arrhythmic beats. Ion channel or receptor modulators often induce a rapid onset of changes in the beat rate and/or beat amplitude, followed by appearance of arrhythmic beats shortly after drug application (Figs. 2, 4A, and 4F). In contrast, pure structural cardiotoxicants often induced a delayed onset of beat rate changes and arrhythmic beats. Structural toxicants that also had acute effects on cardiac ion channels, receptors, or integrity of the plasma membrane exhibited a rapid effect on the beat rate or amplitude and arrhythmic beats as well (Fig. 4F). However, observation of arrhythmic beats may be delayed if inhibition of multiple ion channels or hERG trafficking occurs (Guo et al., 2011), thus onset time alone is not necessarily indicative of cytotoxicity-induced rhythm changes. As expected, noncytotoxic arrhythmogenic drugs did not decrease CI, while the changes in beat rate, amplitude, or rhythm caused by structural cardiotoxicants generally occurred only in the presence of substantial decreases in CI (Fig. 6B), eg, ≥ 10%. It is worth to note that all 6 hERG trafficking blockers tested in this study exhibited cytotoxic effects (Fig. 5B), whereas hERG-inhibition signature arrhythmic beats developed at low concentrations as shown in Figure 3B; cytotoxicity-induced arrhythmic beats similar to those shown in Figure 4D were observed at higher concentrations.

Selective ion channel blockers produce characteristic waveform changes in the hCAR model that can be used to differentiate among major ion channels targeted and between functional and cytotoxic mechanisms of action. For example, highly selective Ca^{2+} channel blockers such as nifedipine, nitrendipine, and diltiazem induced a dose- and time-dependent increase in the beat rate and a decrease in beat amplitude, which recovers over time (Supplementary Figure 2). It is important to mention that amplitude changes using impedance are an indirect, at best, assessment of contractility. Coupling micropatterning approaches that align cells in vitro with direct force measurement devices such as minicantilevers will be necessary to accurately measure changes in contractility and force (Natarajan et al., 2011; Wu et al., 2012). Like hERG inhibition, blockade of Na^{+} channel current also caused a rapid dose-dependent reduction in the beat rate (Guo et al., 2011). However, the beat rate reduction resulting from a “pure” Na^{+} channel blocker was not associated with hERG-inhibition signature arrhythmic beats (see below), but typically exhibited an oscillation in beat-to-beat interval or between slowed, stopped, and run cycles, such as a “short-long-short” beating interval, or a short or long pause followed by a train of beats with gradually increasing beat rate and amplitude. The signature pattern of arrhythmic beats induced by “pure” hERG inhibitors is characterized by a single, double, or a short train of small beats occurring in the lengthened intervals between large (regular amplitude) beats (Figs. 2, 3, 4A, and 4E). The arrhythmic beats induced by structural cardiotoxicants often showed a train of high frequency and low amplitude beats with slightly larger amplitude in between (Fig. 4B, doxorubicin). These arrhythmic beats usually occur transiently before the beating was irreversibly arrested.

Interesting case studies within the data set not only identify follow-up experiments but also underlie the challenges for obtaining rigorous predictive performance. For example, detecting the arrhythmogenic property of bepridil and fluorouracil was challenging as IB_{50} could not be determined for either compound at the highest concentration (30μM) because testing at high concentrations led to arresting of beating. Bepridil, a potent Ca^{2+} channel blocker, may suppress arrhythmic beats induction by hERG blockade in this model, like that did by nifedipine (Guo et al., 2011). Bepridil is used clinically for the treatment of angina and hypertension and has shown efficacy in drug-resistant atrial and ventricular arrhythmias as well, thus it could be speculated that the instances of torsades induced by bepridil occur uniquely on a sensitized genotypic background (Prystowsky, 1992). It would be an interesting experiment to determine if alterations in potassium concentrations would exacerbate bepridil arrhythmia in vitro as hypokalemia is a risk factor for bepridil arrhythmia. The inability to identify the arrhythmogenic property of fluorouracil is postulated to be due to its indirect mechanism of cardiovascular injury, eg, myocardial infarction or coronary vasospasm, rather than a direct cytotoxic effect (Saif et al., 2009; Shah et al., 2012). Fluorouracil does not inhibit hERG current at concentrations ≤ 100μM (Supplementary Table 1), whereas its direct effect on other cardiac ion channels was not reported in the literature. Further, using PPS-IB_{20} ≤ 100 score led to three proarrhythmic drugs (ajmaline, desipramine, prenylamine), being miscategorized as nonproarrhythmic drugs. The relatively low exposure concentrations (ie, highly potent molecules leading to a small CI_{eff}) and inhibition of Ca^{2+} or Na^{+} channels may contribute to the false negative predictions for these drugs (Cohen et al., 2011; Mirams et al., 2011). Nine “safe” drugs, all except tacrine, dantrolene, and phenolamine with a positive signal for either hERG or QT, were incorrectly identified as proarrhythmic when IB_{20} ≤ 10μM was used. The number of false positives was reduced to 8 when PPS-IB_{20} ≤ 100 score was used, increasing the assay specificity to 84%. Thus, in general, the use of PPS-IB_{20} appears to be a more robust metric than IB_{20} 10μM yet requires an understanding of the pharmacological target, an aspect that may not be relevant for all tested compounds (eg, environmental contaminants). Regardless of the prediction metric used, identification
of arrhythmogenic compounds by the hCAR model was superior to hERG or QT prolongation, primarily by reducing the incidents of false positive prediction for TdP and enabling the detection of non-TdP arrhythmogenesis.

The validation study included internal drug development candidates, which required an alteration in the evaluation process. These 30 internal drug development candidate compounds were selected from 19 projects covering diverse therapeutic targets and had thorough understanding of the safety pharmacology profiles in preclinical in vivo test studies. However, because TdP and arrhythmia are not common phenomena and are not the intended endpoint in preclinical telemetry studies, the predictive value of IB20 or BR20 in hiPS-CMs to changes in preclinical models for any of ECG measurements; QT interval, QRS duration, and PR interval were evaluated. Although the prediction by IB20 had an acceptable level of accuracy (70% sensitivity; 100% specificity), the addition of BR20 improved the prediction performance of the assay. Using a change in BR20 as the prediction metric increased the sensitivity to 100% with only one false positive (90% specificity) and provides a framework for assessing early molecular drugs. Unlike the marketed drugs where a threshold of 10μM for either IB20 or BR20 produced the best prediction, 30μM was required to achieve the best correlation between risk signals determined in this hCAR model and ECG abnormalities observed in telemetry studies. This discrepancy might attribute to the fact that animals were often overdosed in an attempt to define the no-observed-adverse-effect-level. Thus, using 30μM as a threshold for marketed drugs resulted in a high rate of false positive identification as illustrated in Figure 1.

In line with earlier reports (Lu et al., 2008), the analysis of relationship between the potency of hERG inhibition and the observed QT prolongation (Table 2B) revealed a poor correlation, even for potent hERG inhibitors. For discovery compounds without a well-defined C50, the free concentration inhibiting hERG current by 20% (hERG IC20) was used internally to assess hERG liability as an IC50 is more relevant to the exposure level to cause a detectable QT prolongation in vivo than an IC50, and is more likely achievable with poorly soluble compounds. Among compounds with a potent (IC20 < 1μM) or moderate (IC20 < 9μM) hERG inhibition, only half (50%) induced QT prolongation in animals. This translation rate reduced to 22% for weak hERG blockers (IC20 ≥ 10μM) and to zero for compounds with hERG IC20 ≥ 30μM. An approximately 50% hERG to QT translation rate for a potent hERG inhibitor and a positive QRS/PR signal frequently observed in telemetry studies strongly suggested that a model system with integrated multiple functional cardiac ion channels is needed to assess the true QT liability or arrhythmogenicity of a potent hERG blocker (Chi, 2013).

The hCAR model shares common limitations inherent with other cell-based models, such as the absence of supporting cell types and autonomic/hormonal influence, uncontrolled cell-cell contact or alignment, and limited drug-metabolizing capability, to name a few. Specifically, this model appears lacking the capability of detecting IKs inhibition and although the unique patterns of beating alteration could aid in pinpointing the target responsible for observed risk signals, the mixed ion channel effect, multitarget interaction together with cytotoxic potentials that are frequently associated with compounds in early discovery stages could confound this effort, thus, mechanistic studies with assays of more focused applications, eg, patch clamp, channel trafficking, mitochondrial function, and general cytotoxicity, would be needed to profile compounds selected for further development. The spontaneous beating in hiPS-CMs is regulated by automaticity of the fetal phenotype of cell rather than through autonomic and hormonal organ input and stimulation from specific nodal cells. In addition, the hiPS-CMs used in this study are predominantly ventricular-like cells with smaller fractions of atrial- and nodal-like cell types and thus would require additional investigations to determine whether a certain subgroup of cells is the primary target of a drug effect. Lastly, the data handling aspects of the system need to be improved, as the arrhythmic beats could be identified only manually with the current version of waveform analysis module. Lessons could be learnt from existing ECG analysis tools for developing a more sophisticated waveform analysis software for automatic quantification of arrhythmic beats and altered beating pattern.

Based on the high degree of accuracy in prediction of clinical QT prolongation and arrhythmia including TdP, the hCAR model was best positioned as an assay to bridge the gaps between hERG screening and in vivo assessments of QT and arrhythmia in preclinical and clinical settings. The hCAR model has the potential to (1) confirm the translation of a hERG (especially a marginal) signal to QT prolongation and/or proarrhythmic risk; (2) identify whether other ion channels, receptors, and transporters are affected; (3) reveal any confounding factors attributed to cytotoxicity; (4) rank-order compounds for selection of the lead scaffold or lead candidates; (5) as the first-line follow-up approach to provide a direction of selecting more specific assays to elucidate the mechanism of the findings observed in the animal or human studies; and (6) to be further validated as a means to reduce or replace current clinical QT studies.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.  

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