An Impedance-Based Cellular Assay Using Human iPSC-Derived Cardiomyocytes to Quantify Modulators of Cardiac Contractility

Clay W. Scott*,1, Xiaoyu Zhang†, Najah Abi-Gerges‡, Sarah D. Lamore*, Yama A. Abassi†, and Matthew F. Peters*

*Drug Safety and Metabolism, AstraZeneca Pharmaceuticals, Waltham, Massachusetts 02451, †ACEA Biosciences Inc., San Diego, California 92121 and ‡Drug Safety and Metabolism, AstraZeneca Pharmaceuticals, Alderley Park, SK10 4TG, UK

1To whom correspondence should be addressed at Drug Safety and Metabolism, AstraZeneca Pharmaceuticals, 35 Gatehouse Park, Waltham, MA 02451. E-mail: clay.scott@astrazeneca.com.

ABSTRACT

Cardiovascular toxicity, a prominent reason for late-stage failures in drug development, has resulted in a demand for in vitro assays that can predict this liability in early drug discovery. Current in vitro cardiovascular safety testing primarily focuses on ion channel modulation and low throughput cardiomyocyte (CM) contractility measurements. We evaluated both human induced pluripotent stem cell-derived CMs (hiPSC-CMs) and rat neonatal CMs (rat CMs) on the xCELLigence Cardio system which uses impedance technology to quantify CM beating properties in a 96-well format. Forty-nine compounds were tested in concentration-response mode to determine potency for modulation of CM beating, a surrogate biomarker for contractility. These compounds had previously been tested in vivo and in a low throughput in vitro optical-based contractility assay that measures sarcomere shortening in electrically paced dog CMs. In comparison with in vivo contractility effects, hiPSC-CM impedance had assay sensitivity, specificity, and accuracy values of 90%, 74%, and 82%, respectively. These values compared favorably to values reported for the dog CM optical assay (83%, 84%, and 82%) and were slightly better than impedance using rat CMs (77%, 74%, and 74%). The potency values from the hiPSC-CM and rat CM assays spanned four orders of magnitude and correlated with values from the dog CM optical assay ($r^2 = 0.76$ and 0.70, respectively). The Cardio system assay has $>5\times$ higher throughput than the optical assay. Thus, hiPSC-CM impedance testing can help detect the human cardiotoxic potential of novel therapeutics early in drug discovery, and if a hazard is identified, has sufficient throughput to support the design-make-test-analyze cycle to mitigate this liability.

Key words: impedance; human-induced pluripotent stem cells; cardiomyocytes; cardiac contractility; label-free
assays that measure inhibitory activity against individual ion channels and transporters that comprise the cardiac action potential (Brown, 2009; Elkins et al., 2013; Möller and Witchel, 2011). However, other aspects of cardiotoxicity are not amenable to single target screening. Cells that stably recapitulate aspects of cardiovascular physiology are needed to enable development of in vitro assays to measure functional correlates of cardiac contractility, heart rate, hypertrophy, etc.

Compounds that modulate cardiac contractility as an unintended side effect can have serious medical consequences. For example, several oncology drugs cause reduced cardiac contractility which results in cardiomyopathy that can lead to heart failure (Mellor et al., 2011). Historically, effects on cardiac contractility were evaluated in animal studies to determine the suitability of a candidate drug to continue on a development path. Repeated attrition at this phase was an impetus for developing an in vitro assay of cardiac contractility that could be used earlier in the drug discovery process (Harmer et al., 2012). This assay uses electrically paced adult dog cardiomyocytes (CM) and quantifies contraction velocity and sarcomere shortening using an optical microscopy system. The assay utilizes less compound and resource than an in vivo study and can generate potency data in low-throughput mode (e.g., 10 IC50/week/scientist) to support structure activity relationship studies to design out this liability. Although a major improvement over in vivo studies for early screening, the dog CM optical assay has insufficient throughput for use as a routine safety screen and uses animal cells to model human cardiac contractility. Thus, further improvements are required to achieve early detection of this cardiac risk.

Recent access to human induced pluripotent stem cell-derived CMs (hiPSC-CMs) that show a stable phenotype has enabled the development of human-based safety screens to monitor various aspects of cardiac cell biology (Carlson et al., 2013; Peng et al., 2010; Pointon et al., 2013; Rana et al., 2012; Sirenko et al., 2013). These cells develop a synchronized beating pattern in culture and express relevant ion channels that shape the cardiac action potential (Ma et al., 2011; Navarrete et al., 2013; Puppala et al., 2013). The xCELLigence Real-Time Cell Analyser Cardio system utilizes label-free impedance technology to quantifying beating properties of CMs and has been used in proof-of-concept studies to detect antiarrhythmics and inotropes (Abassi et al., 2012; Guo et al., 2011; Jonsson et al., 2011; Nguemo et al., 2012). A more detailed assessment of antiarrhythmics demonstrated the value of aligning this technology with hiPSC-CMs as a front-line cardiac safety screen (Guo et al., 2013). However, no systematic analysis has been performed to evaluate the sensitivity and predictivity of an impedance-based assay for detecting compounds that modulate cardiac contractility. In this study, we tested both hiPSC-CMs and rat neonatal CMs in the xCELLigence Cardio system using a set of compounds whose in vivo effects on cardiac contractility had been published. These compounds had also been tested in the dog CM optical assay, therefore results from the optical and impedance assays were compared.

MATERIALS AND METHODS

Compound sources, preparation and addition. Compounds were obtained either from the AstraZeneca compound collection or from Sigma (St. Louis, Missouri). Compounds were solubilized and serially diluted in DMSO or H2O (for chloroquine, disopyramide, and levosimendan), and then further diluted onto the assay plate to achieve a final concentration of 0.1% vehicle. Vehicle alone was included as a control condition.

Myocyte isolation and culture. Rat neonatal CMs were isolated and maintained in culture as previously described (Peters et al., 2012). hiPSC-CMs were purchased from Cellular Dynamics International (Madison, Wisconsin) and maintained in culture according to their protocol. hiPSC-CMs were typically used in impedance experiments at day 14 post-thaw.

Quantifying CM impedance values. Cells were plated in 96-well xCELLigence Cardio E-plates and maintained in culture as previously described (Lamore et al., 2013). CMs were treated with various concentrations of test compounds or vehicle, and impedance data were collected after a 20-min incubation. The beating pattern was quantified according to beat rate (the number of positive peaks per minute) and beat amplitude (magnitude of impedance change between the positive peak and negative peak) as described by Abassi et al. (2012). Impedance cell index values were used to detect cytotoxic effects of test compounds (Kustermann et al., 2013). The compounds were tested in a blinded fashion. Results were derived from at least three independent experiments. A 20% change in impedance was used as the “active” or threshold criteria, independent of test concentration, because this magnitude of effect exceeds baseline variability. Four compounds were reproducibly active but an IC50 or EC50 could not be calculated, either because they produced an enhanced signal that did not plateau (so an EC50 could not be calculated) or they produced an inhibitory signal that did not reach 50% (so an IC50 could not be calculated).

Statistical analyses. Data were graphed and potency values (EC50, IC50) were calculated using Prism (GraphPad Software). These values are expressed as pXIC50 (the negative log of EC50 or IC50 value). For compounds that enhanced impedance beat rate or amplitude but did not achieve a plateau at the highest concentration tested, the data were not quantified but reported as active. Data variability was calculated as the 95% confidence interval of pXIC50 values (pIC50). This enabled direct comparison of data variance with results published from the dog CM optical assay (Harmer et al., 2012). Statistical bias of data between assays was calculated using a Methods Comparison Tool (Altman and Bland, 1983).

RESULTS

CM Impedance Profiles

Forty-nine compounds were tested for effects on the impedance profile of hiPSC-CMs. This set of compounds contained 9 positive inotropes, 21 negative inotropes, and 19 nonactives as defined by their cardiovascular activity in either dog, cynomolgous monkey, or human (Harmer et al., 2012). Each compound was tested in concentration-response mode, with the highest test concentration equal to that used in the dog CM optical assay (Harmer et al., 2012). A compound was deemed active if it induced >20% change in impedance beat rate or beat amplitude in at least two of three experiments. Seven of the nine positive inotropes were active (Table 1). One of the inactive positive inotropes (milrinone, a phosphodiesterase-3 inhibitor) showed a concentration-dependent increase in beat rate in each of three experiments, but did not achieve a sufficiently large effect in two of the three experiments to be classified as active. Of the 21 negative inotropes tested on hiPSC-CMs, only one compound (atenolol; a β1-adrenoceptor antagonist) did not cause an impedance change. Of the 19 compounds that do not affect cardiac contractility in vivo, 14 were inactive in the hiPSC-CM impedance assay, and 5 compounds induced an impedance change.
The 49 compounds were also tested for impedance effects on rat neonatal CMs. These cells were less sensitive to the effects of positive inotropes: only four of the nine compounds were active. In contrast, the negative inotropes and in vivo inactives profiled fairly similarly across rat and hiPSC-CMs. Nineteen of the 21 negative inotropes were active against rat CMs. The two inactives were atenolol, which was also inactive with hiPSC-CMs, plus clonidine, an \( \alpha_2 \)-adrenoceptor agonist. Fourteen of the 19 in vivo inactives did not elicit an impedance response with rat CMs, whereas 5 compounds were active. Four of these five compounds were also active against hiPSC-CMs.

The results shown in Table 1 were used to calculate assay performance values for the hiPSC-CM and rat CM impedance assays (Table 2). For comparison purposes, this table also contains values for the dog CM optical assay. According to the European Centre for the Validation of Alternative Methods (ECVAM) criteria (Genschow et al., 2002), the hiPSC-CM impedance assay has excellent sensitivity, sufficient specificity, and

| Compound             | Activity in vivo | hiPSC-CM impedance | Rat CM impedance | Dog CM sarcomere shortening |
|----------------------|-----------------|--------------------|-----------------|-----------------------------|
| Milrinone            | Positive inotrope| NA                 | NA              | Active                      |
| Levosimendan         | Positive inotrope| NA                 | NA              | Active                      |
| AZ3                  | Positive inotrope| Active             | NA              | Active                      |
| Glibenclamide        | Positive inotrope| Active             | NA              | Active                      |
| AZ1                  | Positive inotrope| Active             | NA              | NA                          |
| Digoxin              | Positive inotrope| Active             | Active          | Active                      |
| Dobutamine           | Positive inotrope| Active             | Active          | Active                      |
| Epinephrine          | Positive inotrope| Active             | Active          | Active                      |
| Isoproterenol        | Positive inotrope| Active             | Active          | Active                      |
| Sildenafil            | NA               | Active             | NA              | NA                          |
| AZ7                  | NA               | Active             | Active          | NA                          |
| AZ8                  | NA               | Active             | Active          | NA                          |
| Phenotolamine        | NA               | Active             | Active          | Active                      |
| Zimelidine           | NA               | Active             | Active          | Active                      |
| Lapatinib            | NA               | NA                 | Active          | NA                          |
| Paracetamol          | NA               | NA                 | Active          | NA                          |
| Amoxicillin          | NA               | NA                 | NA              | NA                          |
| Aspirin              | NA               | NA                 | NA              | NA                          |
| AZ2                  | NA               | NA                 | NA              | NA                          |
| Captopril            | NA               | NA                 | NA              | NA                          |
| Cimetidine           | NA               | NA                 | NA              | NA                          |
| Enalapril            | NA               | NA                 | NA              | NA                          |
| Furosemide           | NA               | NA                 | NA              | NA                          |
| Lisinopril           | NA               | NA                 | NA              | NA                          |
| Pravastatin          | NA               | NA                 | NA              | NA                          |
| Ramipril             | NA               | NA                 | NA              | NA                          |
| Ranitidine           | NA               | NA                 | NA              | NA                          |
| Tolbutamide          | NA               | NA                 | NA              | NA                          |
| Atenolol             | Negative inotrope| NA                 | NA              | NA                          |
| Clonidine            | Negative inotrope| Active             | NA              | Active                      |
| Doxorubicin          | Negative inotrope| Active             | Active          | NA                          |
| AZ10                 | Negative inotrope| Active             | Active          | NA                          |
| AZ11                 | Negative inotrope| Active             | Active          | NA                          |
| Amitriptyline        | Negative inotrope| Active             | Active          | Active                      |
| AZ4                  | Negative inotrope| Active             | Active          | Active                      |
| Bepridil             | Negative inotrope| Active             | Active          | Active                      |
| Chloroquine          | Negative inotrope| Active             | Active          | Active                      |
| Flecainide           | Negative inotrope| Active             | Active          | Active                      |
| AZ12                 | Negative inotrope| Active             | Active          | Active                      |
| AZ6                  | Negative inotrope| Active             | Active          | Active                      |
| AZ9                  | Negative inotrope| Active             | Active          | Active                      |
| Cibenzoline          | Negative inotrope| Active             | Active          | Active                      |
| Diltiazem            | Negative inotrope| Active             | Active          | Active                      |
| Disopyramide         | Negative inotrope| Active             | Active          | Active                      |
| Haloperidol          | Negative inotrope| Active             | Active          | Active                      |
| Nifedipine           | Negative inotrope| Active             | Active          | Active                      |
| Quinidine            | Negative inotrope| Active             | Active          | Active                      |
| Sunitinib            | Negative inotrope| Active             | Active          | Active                      |
| Verapamil            | Negative inotrope| Active             | Active          | Active                      |

Abbreviations: hiPSC-CM, human induced pluripotent stem cell-derived CMs; NA, not active.
good accuracy for predicting in vivo effects on cardiac contractility. These assay performance values are comparable to those from the dog CM optical assay and are somewhat better than the rat CM impedance assay.

Comparing Potency Values Across Assay Platforms and Across Species

The potency values for effects on beat rate and amplitude were calculated for all active compounds (Table 3). For compounds that affected both beat rate and amplitude on hiPSC-CMs (22 of the 27 active inotropes), the potency values for both parameters were consistently within 10-fold of each other and mostly within 3-fold. Thus, compound-induced changes in beat rate and amplitude are tightly coupled for most of the inotropes evaluated. These compounds covered a 40,000-fold range in potency, from 2.5 nM (epinephrine) to 100 μM (AZ2). None of the compounds caused a decrease in cell index within the potency range where effects on beat rate or amplitude were observed, indicating that the concentration-dependent changes in these parameters were not a secondary consequence of cytotoxicity (data not shown).

With rat CMs, quantifying concentration-dependent decreased beating is difficult because typically a large spike in beat rate of variable magnitude immediately precedes beat cessation (Lamore et al., 2013). This complex two-phase behavior is not observed with beat amplitude, hence impedance amplitude IC50 values were used to compare potencies across assays. For almost all compounds that caused a decrease in beat rate, this response occurred within the same potency window as the amplitude change (see Supplementary Table 1). Thus, just as with hiPSC-CMs, beat rate and beat amplitude changes were tightly coupled in rat neonatal CMs.

The potency of compounds in the hiPSC-CM impedance assay correlated with potency in the dog CM optical assay (r2 = 0.76; Figure 1). The hiPSC-CM potency values had a 4.2-fold bias toward a more potent response compared to the dog CM assay. The rat CM assay gave potency values that also correlated with the dog CM assay (r2 = 0.70) and without potency bias. Finally, the potency values from the hiPSC-CM and rat CM impedance assays gave a correlation with r2 = 0.64 and a 3.0-fold potency bias favoring the hiPSC-CMs. As evidenced by the error bars in Figure 1, all three assays had robust reproducibility. The hiPSC-CM impedance assay produced EC50 values with an average CIR of 2.1-fold. The rat CM impedance assay gave an average CIR of 1.7-fold. These values compare favorably to the dog CM optical assay average CIR of 1.6-fold.

The predictivity and reproducibility of data generated with the 49 compounds support using the hiPSC-CM impedance assay as a routine detection screen to identify compounds with undesired effects on CM contractility. Toward this goal, the data in Table 2 were evaluated to establish a potency cutoff criterion that gives an optimal balance between detecting true positives and true negatives. As shown in Figure 2, a pXC50 cutoff value of 4.6 (i.e., XC50 of 25 μM) is optimal, as 87% of the true positives have a potency value <25 μM, whereas 75% of the true negatives have a potency value >25 μM. Therefore, compounds with XC50

### Table 2. Translational Predictivity of CM Assays

| Assay parameter | hiPSC-CM impedance | Rat CM impedance | Dog CM sarcomere shortening |
|-----------------|--------------------|------------------|-----------------------------|
| Sensitivity     | 90%                | 77%              | 83%                         |
| Specificity     | 74%                | 74%              | 84%                         |
| Accuracy        | 82%                | 74%              | 82%                         |
| Neg predictivity| 82%                | 67%              | 76%                         |
| Pos predictivity| 84%                | 82%              | 89%                         |

### Table 3. Potency Values for Effects on hiPSC and Rat CM Impedance Profiles

| Compound | Impedance bead rate | Impedance amplitude | Rat CM |
|----------|---------------------|---------------------|--------|
|          | pXC50 | pCIR | pXC50 | pCIR | pXC50 | pCIR |
| Milrinone | x     | x    | x     | x    | x     | x    |
| Levosimendan | x     | x    | x     | x    | x     | x    |
| AZ3      | 4.69  | 0.50 | 4.06  | 0.00 | x     | x    |
| Glincnclamide | 4.35 | 0.02 | 4.26  | 0.09 | x     | x    |
| AZ1      | 4.81  | 0.05 | 4.61  | 0.05 | x     | x    |
| Digoxin  | 7.29  | 0.59 | 7.26  | 0.18 | 4.38  | 0.03 |
| Dobutamine | 7.13 | 0.36 | x     | x    | 7.44  |       |
| Epinephrine | 9.07 | 0.00 | 8.61  | 0.59 | 7.70  | 0.13 |
| Isoproterenol | 10.01 | 1.51 | x     | x    | 8.90  | 0.16 |
| Sildenafil | 5.77  | 0.68 | x     | x    | x     | x    |
| AZ7      | 4.57  | 0.00 | 4.47  | 0.37 | 4.93  | 0.22 |
| AZ8      | 5.19  | 0.08 | 5.22  | 0.02 | 4.82  | 0.35 |
| Phentolamine | 4.95 | 1.61 | 4.86  | 0.00 | <4.50 | 0.00 |
| Zimeldine | 5.77  | 0.23 | 5.45  | 0.37 | 5.92  | 0.01 |
| Laptanib  | x     | x    | x     | x    | x     | x    |
| Paracetamol | x    | x    | x     | x    | x     | x    |
| Amoxicilln | x     | x    | x     | x    | x     | x    |
| Aspinin   | x     | x    | x     | x    | x     | x    |
| AZ2      | x     | x    | x     | x    | x     | x    |
| Captotril | x     | x    | x     | x    | x     | x    |
| Cimetidine | x    | x    | x     | x    | x     | x    |
| Enalapril | x     | x    | x     | x    | x     | x    |
| Furosemide | x     | x    | x     | x    | x     | x    |
| Lisinopril | x     | x    | x     | x    | x     | x    |
| Pravastatin | x   | x    | x     | x    | x     | x    |
| Ramipril  | x     | x    | x     | x    | x     | x    |
| Ranitidine | x    | x    | x     | x    | x     | x    |
| Tolbutamide | x    | x    | x     | x    | x     | x    |
| Atenolol  | x     | x    | x     | x    | x     | x    |
| Clonidine | Active | 6.57 | 0.66 | x    | x    |
| Doxorubicin | 4.75 | 0.50 | 4.75  | 0.53 | Active | x    |
| AZ10     | 4.88  | 0.40 | 4.84  | 0.45 | 4.92  | 0.24 |
| AZ11     | 6.21  | 0.38 | 6.05  | 0.48 | 4.85  | 0.22 |
| Amitrityline | 6.78 | 0.48 | 6.65  | 0.43 | 5.42  | 0.08 |
| AZ4      | 4.61  | 1.60 | 4.04  | 0.05 | 4.25  | 0.15 |
| Bepridil  | 6.43  | 0.30 | 6.29  | 0.29 | 5.57  | 0.17 |
| Chloroquine | 4.72 | 0.23 | 4.53  | 0.25 | 5.70  | 0.35 |
| Flecaidine | 6.03  | 0.34 | 5.93  | 0.34 | 5.68  | 0.40 |
| AZ12     | 5.71  | 0.16 | 5.66  | 0.19 | 4.88  | 0.30 |
| AZ6      | 4.38  | 0.20 | 3.98  | 0.02 | 4.84  | 0.36 |
| AZ9      | 5.94  | 0.47 | 5.99  | 0.22 | 4.77  | 0.03 |
| Glicazoln | 5.26  | 0.40 | 5.28  | 0.21 | 5.17  | 0.25 |
| Diltiazem | Active | 6.15 | 0.37 | 6.56  | 0.38 |
| Disopramide | 4.18 | 0.41 | 4.24  | 0.00 | 5.02  | 0.14 |
| Haloperidol | 6.50 | 0.29 | 6.37  | 0.37 | 6.68  | 0.42 |
| Nifedipine | Active | 7.37 | 0.55 | 6.42  | 0.30 |
| Quinidine | 5.07  | 0.40 | 4.96  | 0.49 | 4.78  | 0.27 |
| Sunitinib | 5.86  | 0.01 | 5.62  | 0.15 | 5.28  | 0.41 |
| Verapamil | 7.44  | 0.00 | 7.25  | 0.33 | 7.41  | 0.20 |

Abbreviation: x, not active.
would be deemed active and, if warranted, would be tested in follow-up assays to evaluate further their contractility risk.

Evaluating Impedance Profiles for Mechanistic Insight

Most of the positive and negative inotropes caused a reduction in both hiPSC-CM beat rate and amplitude (see quinidine in Figure 3a), therefore the overall impedance profile could not be used to distinguish positive inotropes from negative inotropes. Instead, impedance response might help discern distinct pharmacological activity that underpins an inotropic effect. For example, the positive inotropes and \( \beta \)-adrenoceptor agonists isoproterenol, epinephrine, and dobutamine (Figure 3b) increased impedance beat rate and had no effect or only a small decrease in amplitude. Milrinone, a phosphodiesterase-3 inhibitor that increases intracellular cAMP, gave the same profile as the \( \beta \)-adrenoceptor agonists (which also enhance cAMP), but the magnitude of the beat rate increase was not consistently above the threshold defined as active. Positive inotropes that have other molecular mechanisms of action did not enhance beat rate, but caused a decrease in both beat rate and amplitude instead.

The negative inotrope L-type calcium channel blockers represent a second example of compounds that induced a pharmacological-specific impedance profile. Diltiazem, verapamil, and nifedipine (Figure 3c) all caused an increase in beat rate and decrease in amplitude. This profile was not seen with any other active compounds. Another L-type calcium channel blocker, bepridil, did not increase beat rate, but instead reduced both beat rate and amplitude. Bepridil has a distinct and complex pharmacology profile that also includes inhibition of the inward sodium current, outward repolarizing potassium currents, and calcium binding to calmodulin (Gill et al., 1992). Presumably, these additional activities alter the overall impedance profile of this compound.

DISCUSSION

The dog CM optical contractility assay is a well-validated, robust in vitro assay that detects compounds having direct inotropic effects on the heart (Harmer et al., 2012). The disadvantages of this in vitro assay are that it requires processing of animal cardiac tissue, requires cross-species translation of the results to assess human safety risks, and has low throughput. The hiPSC-CM impedance-based assay described in this study demonstrated a similar precision, predictivity, and potencies to the dog CM optical assay, but does not share these liabilities. Utilizing the hiPSC-CM impedance assay is consistent with the global 3Rs initiative to reduce, replace, and refine the use of laboratory animals in research. In addition, it eliminates the uncertainty in translating across species to predict effects on humans and has \( >5 \)-fold higher assay throughput, which enables routine screening to build in safety during lead expansion and optimization phases of drug discovery.

When used with hiPSC-CMs, the xCELLigence Cardio system was able to detect 7 of 9 positive inotropes (78%) and 20 of 21 negative inotropes (95%). Of the 19 negative control compounds, 14 were appropriately inactive (74%). These results indicate the hiPSC-CM impedance assay has excellent sensitivity, good predictivity, and acceptable specificity (Table 2) based on ECVAM criteria (Genschow et al., 2002). These metrics are comparable to those from the dog CM optical assay. In comparison, the rat CM impedance assay had reduced sensitivity for detecting the positive inotropes tested in this study. This led to relatively poorer assay metrics, albeit “sufficient” performance as per ECVAM criteria. Importantly, compounds that were active in the rat CM impedance assay gave potency values that aligned with those obtained in the hiPSC-CM impedance assay and the dog CM optical assay and covered a potency range of \( >4 \) log units (Figure 1). These three assays incorporate four key differences: species of CMs, adult versus immature cells, spontaneous versus electrically paced beating, and techniques for quantifying
beating. The correlations observed across assays indicate that the pharmacologies modulated by these compounds are core to CM contractility and can be robustly quantified using an impedance measure of cell morphology change or an optical measure of sarcomere shortening.

Detailed analysis of the false positives and false negatives in the hiPSC-CM impedance assay provides some insight on the limitations of this assay. For example, of the three false negatives, two of them (β1-adrenoceptor antagonist atenolol and PDE-3 inhibitor milrinone) produce their inotropic effects by modulating cAMP levels. The inability to detect the receptor antagonist activity and a robust phosphodiesterase inhibitor response is likely due to low basal receptor tone resulting in low cAMP levels in these cells. Indeed, atenolol activity can be detected by pretreating the cells with the β-adrenoceptor agonist isoproterenol (Peters, unpublished data), which is consistent with this hypothesis. Some of the false positives may be due to testing the compounds in vitro at concentrations that exceeded their in vivo exposure, thereby revealing lower potency polypharmacology that could affect CM beating. As demonstrated by Lin and Will (2012), incorporating exposure data on reference compounds, when available, can improve the predictive capability of in vitro assays. Species differences may underlie some of these false positives and negatives. For example, AZ7 and AZ8 are not active in the dog CM optical assay and are not active in vivo in the dog (Harmer et al., 2012), but are active in hiPSC-CM impedance, so are listed as false positives. However, these compounds have not been tested in humans so it is unclear whether they are truly false positives or whether their activity in dog is not predictive of activity in humans. As a second example, AZ10 is active in the hiPSC-CM impedance assay and is a negative inotrope in cynomolgous monkey, but is inactive in the dog CM optical assay. Although it remains to be proven with these particular examples as to whether the dog CM optical assay is predictive of contractility changes in humans, incorporating an in vitro assay that uses human cells into a safety hazard testing cascade has potential to improve human safety risk assessment.

Inotropic responses occur in vivo through complex and multifaceted cardiovascular processes. Inotropy is typically integrated with chronotropic responses via positive feedback signaling loops. Integration can incorporate pathways at multiple levels including cellular, mechanical, organ, and neuronal that combine for a positive force frequency relationship whereby the heart beats faster and stronger in tandem. This raises a series of challenges with respect to isolating inotropy from chronotropy in a cellular assay and achieving good translation between an in vitro assay and in vivo response. For some signaling mechanisms it may not be possible to fully separate inotropy and chronotropy. In addition, the set of drugs classified as inotropes vary with respect to chronotropic actions. Thus, distinguishing inotropic from chronotropic actions may require activity beyond the isolated CM. Considering these complications, the relative success of the isolated dog CM optical assay for predicting in vivo inotropy (82% accuracy) is remarkable and may represent the upper limit. This accuracy would be acceptable for a front-line detection assay if screening throughput could be enhanced.

Moving from dog CMs in an optical assay to rat or hiPSC-CMs in impedance introduces a new set of fundamental challenges which, if tolerable, may bring new opportunities. First, both the rat and hiPSC-CMs used in this study have an immature phenotype (Ma et al., 2011; Robertson et al., 2013) with the corresponding negative force–frequency relationship (Germanguz et al., 2011). This inverted relationship relative to in vivo raises the possibility that detection of inotropy could be severely limited or poorly translate. A second fundamental difference is that, unlike the canine optical assay which uses electrically paced CMs, the impedance assays use spontaneously beating CMs. Spontaneous beating allows beat rate to vary along with beat amplitude. Simultaneous changes in two interrelated variables raise the possibility that the interaction between inotropy and chronotropy would confound detection of in vivo actives. However, the assay metrics demonstrate that these fundamental differences in assay design are not serious limitations. The hiPSC-CM impedance assay has an accuracy value (82%) in detecting true positives and negatives that is the same as the dog CM optical assay. Combining strong assay metrics with higher throughput, the hiPSC-CM impedance assay is ideally suited to be a detection assay, i.e., a front-line screen. As the impedance readout is a surrogate biomarker for contractility, hits from this assay can be tested in secondary assays (e.g., a sarcomere shortening assay, which is a more direct readout of contractile force) for further validation and mechanistic characterization.

In the hiPSC-CM impedance assay, most active compounds affected both beat rate and amplitude and did so within the same concentration range which suggests that these behaviors are linked. However, some compounds induced differential changes in beat rate and amplitude which may reveal...
mechanistic distinctions. For example, the β-adrenoceptor agonists increased beat rate with little if any effect on amplitude. The impedance response pattern showed strongest connections with cellular signaling pathways rather than in vivo classifications (positive/negative inotrope) perhaps due to the complication of chronotropic actions. These results indicate that both impedance rate and amplitude results are valuable in vitro descriptors of drug action. An increase in CM spontaneous-beating amplitude may be a primary effect or secondary to a rate change through force frequency relationship. Adding electrical stimulation to the impedance instrument would enable pacing the hiPSC-CMs to evaluate rate-independent effects on amplitude. The capacity to separate rate and amplitude may strengthen mechanistic understanding of the effects of compounds on underlying biology and thereby improve in vitro-to-in vivo translational safety. Finally, there are several additional ways to quantify the impedance beat waveform including rate of rise, rate of fall, half amplitude width, etc. We have not evaluated those metrics to know whether they would provide mechanism-distinguishing capabilities, nor are we aware of any publications that have systematically evaluated these parameters with sets of cardioactive compounds.

In summary, the impedance assay using hiPSC-CMs performs favorably as an in vitro contractility assay. Compared to the dog CM optical assay it has higher throughput and is more relevant to humans thereby enabling its use as a front-line safety screen to detect compounds that directly affect human cardiac contractility. These results expand further the scope of cardiac biology that can be assessed using label-free impedance cardiac contractility. These results expand further the scope of safety screen to detect compounds that directly affect human relevant to humans thereby enabling its use as a front-line in vitro forms favorably as an

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

FUNDING
This work was funded by AstraZeneca.

ACKNOWLEDGMENTS
The authors thank Alex Harmer, Chris Pollard, Jo Bowes, J.P. Valentin, Lois Lazor, Jennifer Sasaki, and Pete Newham for critical review of data and/or the manuscript. C.W.S., N.A.-G., S.D.L. and M.F.P. are employees of AstraZeneca Pharmaceuticals L.P. X.Z. and Y.A.A. are employees of ACEA Biosciences Inc, which manufactures the xCELLigence Cardio system used in this study.

REFERENCES
Abassi, Y. A., Xi, B., Li, N., Ouyang, W., Seiler, A., Watzele, M., Kettenhofen, R., Bohlen, H., Ehlich, A., Kolossov, E., et al. (2012). Dynamic monitoring of beating periodicity of stem cell-derived cardiomyocytes as a predictive tool for preclinical safety assessment. Br. J. Pharmacol. 165, 1424–1441.

Altman, D. G., and Bland, J. M. (1983). Measurement in medicine: The analysis of method comparison studies. J. R. Stat. Soc. Statistician 32, 307–317.

Brown, A. M. (2009). High throughput functional screening of an ion channel library for drug safety and efficacy. Eur. Biophys. J. 38, 273–278.

Carlson, C., Koonce, C., Aoyama, N., Einhorn, S., Fiene, S., Thompson, A., Swanson, B., Anson, B., and Kattman, S. (2013). Phenotypic screening with human iPS cell-derived cardiomyocytes: HTS-compatible assays for interrogating cardiac hypertrophy. J. Biomol. Screen. 18, 1203–1211.

Elkins, R. C., Davies, M. R., Brough, S. J., Gavaghan, D. J., Cui, Y., Abi-Gerges, N., and Mirams, G. R. (2013). Variability in high-throughput ion-channel screening data and consequences for cardiac safety assessment. J. Pharmacol. Toxicol. Methods 68, 112–122.

Genschow, E., Spielmann, H., Scholz, G., Seiler, A., Brown, N., Piersma, A., Brady, M., Clemann, N., Huuksenen, H., Paillard, F., et al. (2002). The ECVAM international validation study on in vitro embryotoxicity tests: Results of the definitive phase and evaluation of prediction models. European Centre for the Validation of Alternative Methods. Altern. Lab. Anim. 30, 151–176.

Germanguz, I., Sedan, O., Zeevi-Levin, N., Shtrichman, R., Barak, E., Ziskind, A., Eliyahu, S., Meiry, G., Amit, M., Itskovitz-Eldor, J., et al. (2011). Molecular characterization and functional properties of cardiomyocytes derived from human inducible pluripotent stem cells. J. Cell. Mol. Med. 15, 38–51.

Gill, A., Flaim, S. F., Damiano, B. P., Sit, S. P., and Brannan, M. D. (1992). Pharmacology of bepridil. Am. J. Cardiol. 69, 11D–16D.

Guo, L., Abrams, R. M., Babiarz, J. E., Cohen, J. D., Kameoka, S., Sanders, M. J., Chiao, E., and Kolaja, K. L. (2011). Estimating the risk of drug-induced proarrhythmia using human induced pluripotent stem cell-derived cardiomyocytes. Toxicol. Sci. 123, 281–289.

Guo, L., Coyle, L., Abrams, R. M., Kemper, R., Chiao, E. T., and Kolaja, K. L. (2013). Refining the human iPSC-cardiomyocyte arrhythmic risk assessment model. Toxicol. Sci. 136, 581–594.

Harmer, A. R., Abi-Gerges, N., Morton, M. J., Pullen, G. F., Valentin, J. P., and Pollard, C. F. (2012). Validation of an in vitro contractility assay using canine ventricular myocytes. Toxicol. Appl. Pharmacol. 260, 162–172.

Jonsson, M. K., Wang, Q. D., and Becker, B. (2011). Impedance-based detection of beating rhythm and proarrhythmic effects of compounds on stem cell-derived cardiomyocytes. Assay Drug Dev. Technol. 9, 589–599.

Kola, I., and Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? Nat. Rev. Drug Discov. 3, 711–715.

Kustermann, S., Boess, F., Buness, A., Schmitz, M., Watzele, M., Weiser, T., Singer, T., Suter, L., and Roth, A. (2013). A label-free, impedance-based real time assay to identify drug-induced toxicities and differentiate cytotropic from cytotoxic effects. Toxicol. In vitro 27, 1589–1595.

Lamore, S. D., Kamenidi, H. W., Scott, C. W., Dragan, Y. P., and Peters, M. F. (2013). Cellular impedance assays for predictive preclinical drug screening of kinase inhibitor cardiovascular toxicity. Toxicol. Sci. 135, 402–413.

Lin, Z., and Will, Y. (2012). Evaluation of drugs with specific organ toxicities in organ-specific cell lines. Toxicol. Sci. 126, 114–127.

Ma, J., Guo, L., Fiene, S. J., Anson, B. D., Thomson, J. A., Kamp, T. J., Kolaja, K. L., Swanson, B. J., and January, C. T. (2011).
High purity human-induced pluripotent stem cell-derived cardiomyocytes: Electrophysiological properties of action potentials and ionic currents. Am. J. Physiol. Heart Circ. Physiol. 301, H2006–H2017.

Mellor, H. R., Bell, A. R., Valentin, J. P., and Roberts, R. R. (2011). Cardiotoxicity associated with targeting kinase pathways in cancer. Toxicol. Sci. 120, 14–32.

Moller, C., and Witchel, H. (2011). Automated electrophysiology makes the pace for cardiac ion channel safety screening. Front. Pharmacol. 2, 73.

Munos, B. (2009). Lessons from 60 years of pharmaceutical innovation. Nat. Rev. Drug Discov. 8, 959–968.

Navarrete, E. G., Liang, P., Lan, F., Sanchez-Freire, V., Simmons, C., Gong, T., Sharma, A., Burridge, P. W., Patlolla, B., Lee, A. S., et al. (2013). Screening drug-induced arrhythmia events using human induced pluripotent stem cell-derived cardiomyocytes and low-impedance microelectrode arrays. Circulation 128, S3–S13.

Nguemo, F., Saric, T., Pfannkuche, K., Watzele, M., Reppel, M., and Hescheler, J. (2012). In vitro model for assessing arrhythmogenic properties of drugs based on high-resolution impedance measurements. Cell. Physiol. Biochem. 29, 819–832.

Peng, S., Lacerda, A. E., Kirsch, G. E., Brown, A. M., and Bruening-Wright, A. (2010). The action potential and comparative pharmacology of stem cell-derived human cardiomyocytes. J. Pharmacol. Toxicol. Methods 61, 277–286.

Peters, M. F., Scott, C. W., Ochalski, R., and Dragan, Y. P. (2012). Evaluation of cellular impedance measures of cardiomyocyte cultures for drug screening applications. Assay Drug Dev. Technol. 10, 525–532.

Pointon, A., Abi-Gerges, N., Cross, M. J., and Sidaway, J. E. (2013). Phenotypic profiling of structural cardiotoxins in vitro reveals dependency on multiple mechanisms of toxicity. Toxicol. Sci. 132, 317–326.

Puppala, D., Collis, L. P., Sun, S. Z., Bonato, V., Chen, X., Anson, B., Fletcher, M., Fermini, B., and Engle, S. J. (2013). Comparative gene expression profiling in human-induced pluripotent stem cell—Derived cardiocytes and human and cynomolgus heart tissue. Toxicol. Sci. 131, 292–301.

Rana, P., Anson, B., Engle, S., and Will, Y. (2012). Characterization of human-induced pluripotent stem cell-derived cardiomyocytes: Bioenergetics and utilization in safety screening. Toxicol. Sci. 130, 117–131.

Robertson, C., Tran, D. D., and George, S. C. (2013). Concise review: Maturation phases of human pluripotent stem cell-derived cardiomyocytes. Stem Cells 31, 829–837.

Schuster, D., Laggner, C., and Langer, T. (2005). Why drugs fail—A study on side effects in new chemical entities. Curr. Pharm. Des. 11, 3545–3559.

Shah, R. R. (2006). Can pharmacogenetics help rescue drugs withdrawn from the market? Pharmacogenomics 7, 889–908.

Sirenko, O., Cromwell, E. F., Crittenden, C., Wignall, J. A., Wright, F. A., and Rusyn, I. (2013). Assessment of beating parameters in human induced pluripotent stem cells enables quantitative in vitro screening for cardiotoxicity. Toxicol. Appl. Pharmacol. 273, 500–507.

Stevens, J. L., and Baker, T. K. (2009). The future of drug safety testing: Expanding the view and narrowing the focus. Drug Discov. Today 14, 162–167.