Engineered Cardiac Tissues Generated in the Biowire II: A Platform for Human-Based Drug Discovery

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ABSTRACT

Recent advances in techniques to differentiate human induced pluripotent stem cells (hiPSCs) hold the promise of an unlimited supply of human derived cardiac cells from both healthy and disease populations. That promise has been tempered by the observation that hiPSC-derived cardiomyocytes (hiPSC-CMs) typically retain a fetal-like phenotype, raising concern about the translatability of the in vitro data obtained to drug safety, discovery, and development studies. The Biowire II platform was used to generate 3D engineered cardiac tissues (ECTs) from hiPSC-CMs and cardiac fibroblasts. Long term electrical stimulation was employed to obtain ECTs that possess a phenotype like that of adult human myocardium including a lack of spontaneous beating, the presence of a positive force-frequency response from 1 to 4 Hz and prominent postrest potentiation. Pharmacology studies were performed in the ECTs to confirm the presence and functionality of pathways that modulate cardiac contractility in humans. Canonical responses were observed for compounds that act via the β-adrenergic/cAMP-mediated pathway, eg, isoproterenol and milrinone; the L-type calcium channel, eg, FPL64176 and nifedipine; and indirectly effect intracellular Ca2+ concentrations, eg, digoxin. Expected positive inotropic responses were observed for compounds that modulate proteins of the cardiac sarcomere, eg, omecamtiv mecarbil and levosimendan. ECTs generated in the Biowire II platform display adult-like properties and have canonical responses to cardiotherapeutic and cardiotoxic agents that affect contractility in humans via a variety of mechanisms. These data demonstrate that this human-based model can be used to assess the effects of novel compounds on contractility early in the drug discovery and development process.

Key words: cardiomyocytes; contractility; engineered cardiac tissue; drug discovery; drug safety; in vitro models.

Cardiovascular disease (CVD) remains a leading cause of morbidity and mortality worldwide (Benjamin et al., 2018). Patients with heart failure (HF) comprise a growing percentage of the CVD population (Benjamin et al., 2018). HF is instigated by both genetic, eg, inherited cardiomyopathy and acquired risk factors eg, coronary heart disease or hypertension (Stienen, 2015). Cardiotoxicity and HF can also be an unwanted consequence of treatment with a range of drugs in clinical usage (McNaughton et al., 2014; Onakpoya et al., 2016; Siramshetty et al., 2016). There remains the need for both novel therapies to prevent and treat HF as well as improved ways to assess the cardiac safety liabilities of candidate drug therapies.

Cardiac contractility is a consequence of a precise series of events known as excitation-contraction coupling (Eisner et al., 2017). This highly orchestrated process links electrical excitation of the surface membrane (the action potential) and changes...
in the cytoplasmic calcium concentration ([Ca^{2+}]) to muscle contraction and relaxation (Eisner et al., 2017). Aberrant contractility is a hallmark of HF and of numerous drugs with cardiotoxic effects and contributes to symptoms including dyspnea, fatigue, arrhythmia, and ischemia (Guth et al., 2015). Historically, contractility measurements have been performed using cardiac tissue isolated from patients or animals. The use of isolated tissues poses significant challenges, including limited availability and variability for the former, and species-to-species translation for the latter. These challenges have restricted the utility of these methods and highlight the need for novel models. For in vitro models to faithfully recapitulate this process they should demonstrate functional hallmarks of the electrical, calcium handling, and contractile machinery.

The recent development of robust cardiac differentiation protocols for human induced pluripotent stem cells (hiPSCs) has provided the potential for an unlimited supply of human derived cardiac cells from both healthy and diseased sources, but this potential has been limited by the observation that hiPSC-derived cardiomyocytes (hiPSC-CMs) typically retain a more fetal-like phenotype (Denning et al., 2016; Veerman et al., 2015). This raises concerns about the predictability and translatability of results obtained in vitro to the settings of drug safety, discovery, and development.

In this study, the Biowire II platform was used to generate 3D engineered cardiac tissues (ECTs) from hiPSC-CMs and cardiac fibroblasts and to make nondestructive contractility measurements of the ECTs. We present data demonstrating that ECTs without spontaneous activity that exhibited a positive force-frequency relationship (FFR) were assessed for automaticity (ie, spontaneous beat rate), the FFR (active force at 1 Hz through 4 Hz), and postrest potentiation. ECTs without spontaneous activity that exhibited a positive force-frequency relationship were used for compound testing.

TESTS were conducted in a 37°C, 5% CO₂ environmental chamber under field stimulation at 1 Hz. Tissues were incubated for 30 min in the environmental chamber after which a baseline contractility video was acquired for 30 s. One-third of the tissue culture medium volume (6 ml total) was pipetted from the well containing the ECTs twice to equilibrate the ECTs to the shear stress induced by the procedure. A video of 30 s duration was acquired 10 min after the pipetting (baseline). The test article was added to the well to provide the desired final concentration and one-third of the media volume was pipetted twice to gently mix. The test article stock solutions were prepared in either dimethyl sulfoxide or water as appropriate, and serially diluted in medium. Following 10–30 min of incubation, a video of 30 s duration was acquired. The same procedure was followed for all subsequent doses (lowest to highest) such that 1 ECT was incubated with all doses. Contractility videos were analyzed using a custom analysis software. The force at each dose was normalized by dividing the force values in the presence of compound by the baseline force values of the same tissue.

Statistics. Statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc, La Jolla, California). Data are presented as mean ± SEM. Results were considered statistically significant (#) if p < .05 using one-way ANOVA followed by a Tukey’s post hoc multiple comparison test or where appropriate repeat measure one-way ANOVA followed by Dunnett’s post hoc multiple comparison test. The replicate numbers are indicated in the figure captions. For EC50/IC50 calculation, the nonlinear fit function of GraphPad Prism (Sigmoidal with 3 parameters) was used to find the best fit for the data.
ie, the FFR. FFR is a property of the adult heart where a stepwise increase in force is observed when the frequency of stimulation is increased within a physiologically relevant range (Buckley et al., 1972). Prior to electrical stimulation ie, 1-week postseeding, the ECTs were spontaneously beating at approximately 1.5 Hz and had limited capture at 3 and 4 Hz impairing the ability to assess the FFR. After 7 weeks of culture (6 weeks of electrical stimulation), a positive FFR was observed ie, increased force of contraction with increasing stimulation frequencies (Figs. 1B and 1D).

We further investigated the contractile machinery of the ECTs by assessing postrest potentiation of force, an indicator of the capacity of the sarcoplasmic reticulum (SR) to store and release Ca\(^{2+}\). In large mammals and humans, short periods of cardiac rest give rise to an increased force of contraction of the first beats following restimulation. This results from an increased uptake and subsequent release of Ca\(^{2+}\) from the SR (Pieske et al., 1996). Due to spontaneous beating, we could not assess postrest potentiation at 1-week postseeding. After 6 weeks of electrical stimulation, we observed a significant postrest potentiation of force following 10 s of rest (Figs. 1C and 1D).

In aggregate the data demonstrate that the ECTs manifest contractile parameters that approach that seen in adult human myocardium.

**ECTs Generated via Biowire II Display Positive Inotropic Responses to Drugs That Modulate Messenger 3'-5'-Cyclic Adenosine Monophosphate Production and Degradation**

β-adrenergic signaling is an important physiological regulator of cardiac contractility (de Lucia et al., 2018). It results in an increase in the production of the intracellular second messenger
cyclic adenosine monophosphate (cAMP) and a positive inotropic response (Bers, 2008). ECTs treated with the \( \beta \)-agonist isoproterenol showed a maximal 6.6 ± 0.8-fold increase in contractile force at a concentration of 10 nM with a half maximal effective concentration (EC\(_{50}\)) of 0.5 nM (Figure 2A, time constants and rates of contraction and relaxation are summarized in Supplemental Table 1). Similarly, addition of dobutamine, a predominantly \( \beta_1 \) agonist increased the maximal contractile force by 4.4 ± 0.6-fold at 360 nM with EC\(_{50}\) of 16 nM (Figure 2B).

Further, we investigated the intracellular pathways involved in adrenergic signaling, using small molecule modulators that effect distinct signaling pathways. Degradation of cAMP is mediated by a cAMP-dependent enzyme, phosphodiesterase-3 (PDE3). We observed a maximal 4.3 ± 1.2-fold increase in the force of contraction when ECTs were treated with the PDE3 inhibitor milrinone at a concentration of 100 \( \mu \)M with an EC\(_{50}\) of 1.6 \( \mu \)M (Figure 2C, Supplemental Table 1).

Pituitary adenylate cyclase-activating polypeptide (PACAP27) is a 27-amino acid peptide that activates adenyl cyclase via binding to its cognate G-protein-coupled receptor. We observed a maximal 3.4 ± 1.1-fold increase in force of contraction when ECTs were treated with 10 nM PACAP27 with an EC\(_{50}\) of 1.1 nM (Figure 2D, Supplemental Table 1).

**ECTs Have Functional L-Type Ca\(^{2+}\) Channels, SR, and Na\(^{+}-\)Ca\(^{2+}\) Exchangers**

The voltage-gated L-type Ca\(^{2+}\) channels (LTCCs) are the primary mediators of Ca\(^{2+}\) influx into the cardiomyocyte, which is essential for initiation of cardiac excitation-contraction coupling (Benitah et al., 2010; Bers, 2002). Treatment of ECTs with the LTCC blocker nifedipine completely inhibited contraction at a concentration of 1 \( \mu \)M with a half maximal inhibitory concentration (IC\(_{50}\)) of 62 nM (Figure 3A, Supplemental Table 2), whereas treatment with the LTCC activator FPL 64176 induced a maximal 8.5 ± 0.6-fold increase in the force of contraction at a concentration of 10 \( \mu \)M with an EC\(_{50}\) of 502 nM (Figure 3B, Supplemental Table 2).

The Na\(^{+}-\)Ca\(^{2+}\) exchanger (NCX) is the primary means by which cardiomyocytes regulate intracellular Ca\(^{2+}\) and hence a critical modulator of excitation-contraction coupling. To assess the functionality of the NCX in this model we treated ECTs with digoxin. Digoxin is a cardiac glycoside, a class of compounds that directly inhibits the Na\(^{+}/K\(^{+}\) ATPase and requires a functional NCX for its inotropic effect (Altamirano et al., 2006; Ozdemir et al., 2008). The inhibition of the Na\(^{+}/K\(^{+}\) ATPase results in increased intracellular Na\(^{+}\) accumulation, which reduces the electrochemical drive for Ca\(^{2+}\) efflux through NCX. In this setting excess intracellular Ca\(^{2+}\) is primarily removed from the cytosol by the sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA), which loads the SR with Ca\(^{2+}\) leading to an increase in cardiac contractility (Ottolia et al., 2013). Treatment of ECTs with digoxin induced a maximal 9.7 ± 1.7-fold increase in contractile force at a concentration of 100 \( \mu \)M with an EC\(_{50}\) of 21 nM (Figure 3C, Supplemental Table 2). Digoxin at concentration of 1 \( \mu \)M induced ectopic activity and significantly prolonged the duration of contraction (Supplemental Figure 1). At concentrations above 10 \( \mu \)M, ECT contractility was significantly diminished and contraction could not be elicited in the majority of the ECTs using external field stimulation.

Thapsigargin, a noncompetitive inhibitor of SERCA activity reduces the SR calcium load and leads to increased cytosolic calcium and reduced contractility. Treatment of ECTs with thapsigargin completely inhibited contraction at...
concentrations above 1 \mu M with IC_{50} of 165 nM (Figure 3D, Supplemental Table 2).

ECTs Respond to Sarcomere Modulators

In the cardiac sarcomere, energy derived from myosin-mediated ATP hydrolysis is used to drive contraction (Malik et al., 2011). Omecamtiv mecarbil (OM) is a novel positive inotrope that binds to and stimulates the ATPase activity of myosin resulting in an increased force of contraction in humans (Planelles-Herrero et al., 2017). Treatment of ECTs with OM elicited a maximal 2.6 ± 0.3-fold increase in contractile force at a concentration of 10 \mu M with an EC_{50} of 370 nM (Figure 4A). OM at 10 \mu M significantly increased the time of contraction and relaxation and had no effect on the rates of contraction and relaxation (Supplemental Table 3). In contrast, treatment of ECTs with MYK-461 (mavacamten), an inhibitor of cardiac myosin ATPase, completely abolished contraction at concentrations above 10 \mu M with IC_{50} of 287 nM (Figure 4B, Supplemental Table 3).

Cardiac troponin is a trimeric protein complex in the sarcomere (Sorsa et al., 2004). It plays a critical role in regulating sensitivity to Ca^{2+}. Levosimendan is a positive inotrope that modulates troponin-C, 1 protein of the trimer and increases its sensitivity to regulation by Ca^{2+} (Pollesello et al., 1994; Robertson et al., 2016). Treatment of ECTs with levosimendan induced a maximal 3.3 ± 0.4-fold increase in contractile force at a concentration of 2 \mu M with an EC_{50} of 234 nM (Figure 4C).

ECTs Respond to Activation of Phospholipase C

Endothelin-1 (ET-1) is a 21-amino acid peptide that binds to the ET receptor, a G_{s}-protein-coupled receptor, and activates phospholipase C (Sugden, 2003). Second messenger effects are proposed to mediate positive inotropic events that involve modulation of the intracellular Ca^{2+} transients and myofilament Ca^{2+} sensitivity. Treatment of ECTs with ET-1 induced a maximal 5.2 ± 0.7-fold increase in contractile force at 40 nM with an EC_{50} of 98 pM (Figure 4D, Supplemental Table 3).

DISCUSSION

Here we evaluated the contractile function of human 3D ECTs generated from hiPSC-CMs in the Biowire II platform. We demonstrate that the platform enables the generation of 3D ECTs that remain viable and retain their functionality for weeks. These ECTs develop properties of the adult myocardium and exhibit robust responses to a variety of inotropic compounds with distinct mechanisms of action selected to confirm the presence, and interrogate the function of, pathways known to regulate cardiac function.

G-protein coupled receptor signaling pathways play a critical role in functional adaptation in the heart by regulating inotropic and chronotropic responses. Endothelin-1, a potent vasoactive peptide signaling via the phospholipase-C/protein kinase-C pathway, has been shown to regulate cardiac contractility both in vivo and in isolated human myocardium (MacCarthy et al., 2000; Pieske et al., 1999). ECTs generated a positive inotropic response, in a concentration-dependent manner, upon exposure to ET-1; confirming the presence of a functional ET-1 receptor signaling cascade. In patients suffering from HF, dysregulation of \beta-adrenergic signaling is a common feature of cardiac pathophysiology and a target of therapy eg, \beta-adrenergic antagonists are a cornerstone of therapy (Bristow et al., 1990). We demonstrated canonical responses to the well-studied \beta-agonists isoproterenol and dobutamine. Inotropic responses are observed for PACAP27, which activates adenyl cyclase via...
receptors distinct from isoproterenol and milrinone (an inhibitor of cAMP degradation) indicating that a range of cAMP-mediated signaling cascades are also present in the ECTs. Although observation of the stimulatory effects of β-adrenergic agonism in engineered human heart tissues is not novel, previous reports have suggested little to no significant effect on contractility (Hirt et al., 2014), but rather an increase in chronotropy. Additionally, others have observed positive inotropic responses at concentrations far right-shifted and therefore, less physiologically relevant to effects observed in this study (Zhang et al., 2013). Interestingly, milrinone has been shown to produce a positive inotropic response in fibroblast containing ECTs (Ravenscroft et al., 2016), whereas when tissues were prepared without fibroblasts, there was no inotropic response to milrinone as compared to human heart tissue (Mannhardt et al., 2017); suggesting maturity in the adrenergic pathway and/or enhanced PDE3A expression in fibroblast containing ECTs. We observed a significant increase in the magnitude of force generated in response to milrinone in ECTs electrically stimulated for 6 weeks as compared to 3 weeks, demonstrating that long term electrical stimulation is also an important factor that contributes to the increased inotropic response to milrinone (Supplemental Figure 2). Further studies are required to determine the mechanism by which the inotropic response to milrinone was increased in ECTs generated in the Biowire II platform.

Calcium channels are both the direct and indirect target of various cardiac therapies. LTCC antagonists are a mainstay in the treatment of hypertension, cardiac ischemia, and arrhythmias, but can elicit cardio depressant effect. Conversely, compounds such as Bay K-8644 and the more potent FPL 64176 were designed to stimulate LTCC activity and have the consequent effect of increasing the contractile force. As such, treatment of the ECTs with the LTCC blocker, nifedipine and with FPL 64176 yielded the expected result of decreased and increased contractility respectively, indicating the LTCC of the ECT was functional and could be regulated by external stimuli. One of the oldest HF therapies is digoxin, a cardiac glycoside isolated from the foxglove plant. Digoxin induces a positive inotropic effect via its ability to promote Ca2+ loading in the SR. A biphasic increase in force was observed when ECTs were treated with digoxin at or below 1µM demonstrating that contractility can be regulated at the level of NCX and that the SR modulates Ca2+ levels in a physiologically relevant manner. Treatment of tissues with digoxin at concentrations above 1µM showed toxic effects commonly observed with this compound (Supplemental Figure 1) (Mannhardt et al., 2017; Ruch et al., 2003). The recent discovery of agents that modulate sarcomere proteins holds great promise for the development of novel treatments for HF. OM is an agent that directly regulates cardiac myosin and levosimendan is an agent that regulates the function of troponin-C, the Ca2+ sensor of the sarcomere. Data from studies in HF patients show OM and levosimendan can promote a positive inotropic effect and hemodynamic improvements (Follath et al., 2002; Teerlink et al., 2016b) without a significant increase in oxygen consumption (Lilleberg et al., 1998; Shen et al., 2010; Ukkonen et al., 2000). Consistent with data from clinical and animal studies, both compounds were able to modulate contractility in ECTs eliciting significant increases in contractile force with concentrations typical for these compounds (Nagy et al., 2015). MYK461, a small molecule inhibitor of myosin also shows potential in the treatment of HF. Tissue treated with MYK461 showed significant decrease in contractility in the range consistent with published results (Green et al., 2016).
Figure 5. Engineered cardiac tissues (ECTs) generated in Biowire II platform have canonical responses to compounds that affect contractility via physiologically relevant pathways. A, β-adrenergic/cAMP-mediated pathway and the L-type calcium channel. β-AR, β-adrenergic receptor; AC, adenyl cyclase; PACAP, pituitary adenylate cyclase-activating peptide; cAMP, cyclic adenosine monophosphate; ATP, adenosine triphosphate; PDE, phosphodiesterase; PKA, protein kinase A; RYR2, ryanodine receptor; TnI, troponin-I; PLB, phospholamban. B, Gq-protein-coupled receptor signaling. ET-R, endothelin receptor; PLC, phospholipase C; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; PKC, protein kinase C. C, Modulation of proteins of the cardiac sarcomere.

Table 1. Summary of Engineered Cardiac Tissues (ECT) Inotropic Responses

| Compound       | ECT EC50/IC50 | Effective Plasma Concentration or EC50/IC50 Reference                                                                 |
|----------------|---------------|----------------------------------------------------------------------------------------------------------------------|
| Isoproterenol  | 0.5 nM        | 27 nM<sup>a</sup>                                                                                                        |
| Dobutamine     | 16 nM         | 133–632 nM; Plasma levels of 133 nM were associated with significant increase in cardiac index with linear increase in index up to 632 nM (Leier et al., 1979) |
| Milrinone      | 1.6 µM        | 0.791 µM; Plasma concentration associated with 50% increase in cardiac index (Bailey et al., 1994)                   |
| PACAP27        | 1.1 nM        | 0.5 nM<sup>a</sup>                                                                                                        |
| Nifedipine     | 62 nM         | 10–81 nM; Steady state of 10 nM (Raemisch and Sommer, 1983); plasma levels above 81 nM were associated with decreased dp/dt<sub>max</sub> (Clifton et al., 1990) |
| FPL64176       | 502 nM        | 600 nM<sup>a</sup> Recalculated EC50 in guinea pig papillary muscle (Rampe et al., 1993)                                  |
| Digoxin        | 21 nM         | 1.5–2.6 nM; Plasma level range in patients with improved fractional shortening (Guyatt et al., 1988)                     |
| Thapsigargin   | 165 nM        | 300 nM<sup>a</sup> Complete inhibition of contraction in isolated adult rat cardiomyocytes (Wrzosek et al., 1992)          |
| Omecamtiv      | 370 nM        | 792 nM; Maximum plasma level associated with increased stroke volume (Teerlink et al., 2016a)                           |
| MYK461         | 287 nM        | 180 nM<sup>a</sup> IC50 in adult rat ventricular cardiomyocytes (Green et al., 2016)                                       |
| Levosimendan   | 234 nM        | 351 nM; Plasma level associated with increased stroke volume (Kivikko et al., 2003)                                      |
| Endothelin-1   | 0.1 nM        | 9.1 nM<sup>a</sup> EC50 in isolated human ventricular trabeculae (Saetrum Opgaard et al., 2000)                            |

The EC50/IC50 for each compound was compared to the effective therapeutic dose of the compound where data were available. For compounds where effective therapeutic dose was not available, comparison was made with published results from in vitro or animal models and is indicated by superscript letter (a).
In conclusion, human ECTs created in the Biowire II platform have adult-like canonical responses to compounds known to affect contractility via an array of physiologically relevant pathways (Figure 5, Summary data presented in Table 1). These studies can be conducted under external stimulation, providing investigators with a high degree of experimental control, and with the ability to assess compounds using a nondestructive measurement of contractility. This model can be used to assess the effects of novel compounds in a human-based model of contractility early in the drug discovery and development process. These results suggest the utility of the Biowire II platform to create disease models via the use of patient derived iPSC-CMs.

SUPPLEMENTARY DATA

Supplementary data are available at Toxicological Sciences online.

FUNDING

The authors received no financial support for the research, authorship and for the publication of this article.

DECLARATION OF CONFLICTING INTERESTS

N.T.F., I.P., D.R.B., M.G., M.P.G., and R.A.-S. are employees and shareholders of TARA Biosystems Inc.

REFERENCES

Altamirano, J., Li, Y., DeSantiago, J., Piacentino, V., 3rd, Houser, S. R., and Bers, D. M. (2006). The inotropic effect of cardioactive glycosides in ventricular myocytes requires Na\(^{\text{\textsuperscript{+}}}\)-Ca\(^{\text{\textsuperscript{2\text{\textsuperscript{+}}}}}\) exchanger function. J. Physiol. 575, 845–854.

Bailey, J. M., Levy, J. H., Kikura, M., Szlam, F., and Hug, C. C., Jr. (1994). Pharmacokinetics of intravenous milrinone in patients undergoing cardiac surgery. Anesthesiology 81, 616–622.

Benitah, J. P., Alvarez, J. L., and Gomez, A. M. (2010). L-type Ca\(^{\text{\textsuperscript{2\text{\textsuperscript{+}}}}}\) current in ventricular cardiomyocytes. J. Mol. Cell. Cardiol. 48, 26–36.

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiue, S. E., Cushman, M., Delling, F. N., Deo, R., et al. (2018). Heart disease and stroke statistics-2018 update: A report from the American heart association. Circulation 137, e67–e492.

Bers, D. M. (2002). Cardiac excitation-contraction coupling. Nature 415, 198–205.

Bers, D. M. (2008). Calcium cycling and signaling in cardiac myocytes. Annu. Rev. Physiol. 70, 23–49.

Bristow, M. R., Hersherberger, R. E., Port, J. D., Gilbert, E. M., Sandoval, A., Rasmussen, R., Yates, A. E., and Feldman, A. M. (1990). Beta-adrenergic pathways in nonfailing and failing human ventricular myocardium. Circulation 82, 112–125.

Buckley, N. M., Penefsky, Z. J., and Litwak, R. S. (1972). Comparative force-frequency relationships in human and other mammalian ventricular myocardium. Pflugers Arch. 332, 258–270.

Clifton, G. D., Booth, D. C., Hobbs, S., Boucher, B. A., Foster, T. S., McAllister, R. G., Jr., and DeMaria, A. N. (1990). Negative inotropic effect of intravenous nifedipine in coronary artery disease: Relation to plasma levels. Am. Heart J. 119, 283–290.

de Lucia, C., Eguchi, A., and Koch, W. J. (2018). New insights in cardiac beta-adrenergic signaling during heart failure and aging. Front. Pharmacol. 9, 904.

Denning, C., Borgdorff, V., Crutchley, J., Firth, K. S., George, V., Kalra, S., Kondrashov, A., Huang, M. D., Mosqueira, D., Patel, A., et al. (2016). Cardiomyocytes from human pluripotent stem cells: From laboratory curiosity to industrial biomedical platform. Biochim. Biophys. Acta 1863, 1728–1748.

Eisner, D. A., Caldwell, J. L., Kistamas, K., and Trafford, A. W. (2017). Calcium and excitation-contraction coupling in the heart. Circ. Res. 121, 181–195.

Flesch, M., Kilter, H., Cremer, B., Laufs, U., Sudkamp, M., Ortman, M., Muller, F. U., and Böhm, M. (1999). Effects of endotoxin on human myocardial contractility involvement of nitric oxide and peroxynitrite. J. Am. Coll. Cardiol. 33, 1062–1070.

Follath, F., Cleland, J. G., Just, H., Papp, J. G., Scholz, H., Peukkuri nen, K., Harjola, V. P., Mitrovic, V., Abdalla, M., Sandell, E. P., et al. (2002). Efficacy and safety of intravenous levosimendan compared with dobutamine in severe low-output heart failure (the lido study): A randomised double-blind trial. Lancet 360, 196–202.

Green, E. M., Wakimoto, H., Anderson, R. L., Evanchik, M. J., Gorham, J. M., Harrison, B. C., Henze, M., Kasas, R., Oslob, J. D., Rodriguez, H. M., et al. (2016). A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. Science 351, 617–621.

Guth, B. D., Chiang, A. Y., Doyle, J., Engwall, M. J., Guillon, J. M., Hoffmann, P., Koerner, J., Mittelstadt, S., Ottinger, S., Pierson, J. B., et al. (2015). The evaluation of drug-induced changes in cardiac inotropy in dogs: Results from a HESI-sponsored consortium. J. Pharmacol. Toxicol. Methods 75, 70–90.

Guyatt, G. H., Sullivan, M. J., Fallen, E. L., Tihal, H., Rideout, E., Halcrow, S., Nogradi, S., Townsend, M., and Taylor, D. W. (1988). A controlled trial of digoxin in congestive heart failure. Am. J. Cardiol. 61, 371–375.

Hirose, M., Furukawa, Y., Lakhe, M., and Chiba, S. (1998). Regional differences in cardiac effects of pituitary adenylate cyclase-activating polypeptide-27 in the isolated dog heart. Eur. J. Pharmacol. 349, 269–276.

Hirt, M. N., Boedinghaus, J., Mitchell, A., Schaaf, S., Bornchen, C., Muller, C., Schulz, H., Hubner, N., Stenzig, J., Stoehr, A., et al. (2014). Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. J. Mol. Cell. Cardiol. 74, 151–161.

Kivikko, M., Lehtonen, L., and Colucci, W. S. (2003). Sustained hemodynamic effects of intravenous levosimendan. Circulation 107, 81–86.

Leier, C. V., Unverferth, D. V., and Kates, R. E. (1979). The relationship between plasma dobutamine concentrations and cardiovascular responses in cardiac failure. Am. J. Med. 66, 238–242.

Lilleberg, J., Nieminen, M. S., Akkila, J., Heikkila, L., Kuitunen, A., Lehtonen, L., Mattila, S., and Salmenpera, M. (1998). Effects of a new calcium sensitizer, levosimendan, on haemodynamics, coronary blood flow and myocardial substrate utilization early after coronary artery bypass grafting. Eur. Heart J. 19, 660–668.

MacCarthy, P. A., Grocott-Mason, R., Prendergast, B. D., and Shah, A. M. (2000). Contrasting inotropic effects of endogenous endothelin in the normal and failing human heart: Studies with an intracoronary ET(A) receptor antagonist. Circulation 101, 142–147.
Malik, F. I., Hartman, J. J., Elias, K. A., Morgan, B. P., Rodriguez, H., Brejc, K., Anderson, R. L., Sueoka, S. H., Lee, K. H., Finer, J. T., et al. (2011). Cardiac myosin activation: A potential therapeutic approach for systolic heart failure. Science 331, 1439–1443.

Mannhardt, I., Eder, A., Dumotier, B., Prondzynski, M., Kramer, E., Traebert, M., Sohren, K. D., Flenner, F., Stathopoulos, K., Lemoine, M. D., et al. (2017). Blinded contractility analysis in hiPSC-cardiomyocytes in engineered heart tissue format: Comparison with human atrial trabeculae. Toxicol. Sci. 158, 164–175.

McNaughton, R., Huet, G., and Shakir, S. (2014). An investigation into drug products withdrawn from the EU market between 2002 and 2011 for safety reasons and the evidence used to support the decision-making. BMJ Open 4, e004221.

Nagy, L., Kovacs, A., Bodi, B., Pasztor, E. T., Pulop, G. A., Toth, A., Edes, I., and Papp, Z. (2015). The novel cardiac myosin activator omecamtiv mecarbil increases the calcium sensitivity of force production in isolated cardiomyocytes and skeletal muscle fibres of the rat. Br. J. Pharmacol. 172, 4506–4518.

Onakpoya, I. J., Heneghan, C. J., and Aronson, J. K. (2016). Worldwide withdrawal of medicinal products because of adverse drug reactions: A systematic review and analysis. Crit. Rev. Toxicol. 46, 477–489.

Ottolia, M., Torres, N., Bridge, J. H., Philipson, K. D., and Goldhaber, J. I. (2013). Na/Ca exchange and contraction of the heart. J. Mol. Cell. Cardiol. 61, 28–33.

Ozdemir, S., Bito, V., Holemans, P., Vinet, L., Mercadier, J. J., Varro, A., and Sipido, K. R. (2008). Pharmacological inhibition of Na/Ca exchange results in increased cellular Ca2+ load attributable to the predominance of forward mode block. Circ. Res. 102, 1398–1405.

Pieske, B., Beyermann, B., Breu, V., Löffler, B. M., Schlothauer, K., Maier, L. S., Schmidt-Schweda, S., Just, H., and Hasenfuss, G. (1999). Functional effects of endothelin and regulation of endothelin receptors in isolated human nonfailing and failing myocardium. Circulation 99, 1802–1809.

Pieske, B., Sutterlin, M., Schmidt-Schweda, S., Minami, K., Meyer, M., Olschewski, M., Holubarsch, C., Just, H., and Hasenfuss, G. (1996). Diminished post-rest potentiation of contractile force in human dilated cardiomyopathy. Functional evidence for alterations in intracellular Ca2+ handling. J. Clin. Invest. 98, 764–776.

Planelles-Herrero, V. J., Hartman, J. J., Robert-Paganin, J., Malik, F. I., and Houdusse, A. (2017). Mechanistic and structural basis for activation of cardiac myosin force production by omecamtiv mecarbil. Nat. Commun. 8, 190.

Pollesello, P., Ovaska, M., Kaiiola, J., Tilgmann, C., Lundstrom, K., Kalkkinen, N., Umlanen, I., Nissinen, E., and Taskinen, J. (1994). Binding of a new Ca2+-sensitizer, levsimendan, to recombinant human cardiac troponin C. A molecular modeling, fluorescence probe, and proton nuclear magnetic resonance study. J. Biol. Chem. 269, 28584–28590.

Raemsch, K. D., and Sommer, J. (1983). Pharmacokinetics and metabolism of nifedipine. Hypertension 5, II18–II24.

Rampé, D., Anderson, B., Rapien-Pryor, V., Li, T., and Dage, R. C. (1993). Comparison of the in vitro and in vivo cardiovascular effects of two structurally distinct Ca2+ channel activators, BAY K 8644 and FPL 64176. J. Pharmacol. Exp. Ther. 265, 1125–1130.

Ravenscroft, S. M., Pointron, A., Williams, A. W., Cross, M. J., and Sidaway, J. E. (2016). Cardiac non-myocyte cells show enhanced pharmacological function suggestive of contractile maturity in stem cell derived cardiomyocyte microtissues. Toxicol. Sci. 152, 99–112.

Robertson, I. M., Pineda-Sanabria, S. E., Yan, Z., Kampourakis, T., Sun, Y. B., Sykes, B. D., and Irving, M. (2016). Reversible cova lent binding to cardiac troponin C by the Ca2+-sensitizer lev simendan. Biochemistry 55, 6032–6045.

Ruch, S. R., Nishio, M., and Wasserstrom, J. A. (2003). Effect of cardiac glycosides on action potential characteristics and contractility in cat ventricular myocytes: Role of calcium overload. J. Pharmacol. Exp. Ther. 307, 419–428.

Saetrum Opgaard, O., Moller, S., de Vries, R., Edvinsson, L., and Saxena, P. R. (2000). Positive inotropic responses mediated by endothelin ET(A) and ET(B) receptors in human myocardial trabeculae. Clin. Sci. (Lond.) 99, 161–168.

Shen, Y. T., Malik, F. I., Zhao, X., Depre, C., Dhar, S. K., Abazrea, P., Morgans, D. J., and Vatner, S. F. (2010). Improvement of cardiac function by a cardiac myosin activator in conscious dogs with systolic heart failure. Circ. Heart Fail. 3, 522–527.

Siramshetty, V. B., Nickel, J., Omiecinski, C., Gohlke, B. O., Drwal, M. N., and Preissner, R. (2016). WITHDRAWN—a resource for withdrawn and discontinued drugs. Nucleic Acids Res. 44, D1080–1086.

Sorsa, T., Pollesello, P., and Solaro, R. J. (2004). The contractile apparatus as a target for drugs against heart failure: Interaction of levsimendan, a calcium sensitiser, with cardiac troponin C. Mol. Cell. Biochem. 266, 87–107.

Stienen, G. J. (2015). Pathomechanisms in heart failure: The contractile connection. J. Muscle Res. Cell Motil. 36, 47–60.

Sugden, P. H. (2003). An overview of endothelin signaling in the cardiac myocyte. J. Mol. Cell. Cardiol. 35, 871–886.

Teerlink, J. R., Felker, G. M., McMurray, J. J., Solomon, S. D., Adams, K. F., Jr., Cleland, J. G., Ezekowitz, J. A., Goudev, A., Macdonald, P., Metra, M., et al. (2016a). Chronic oral study of myosin activation to increase contractility in heart failure (COSMIC-HF): A phase 2, pharmacokinetic, randomised, placebo-controlled trial. Lancet 388, 2895–2903.

Teerlink, J. R., Felker, G. M., McMurray, J. J. V., Portonikowski, P., Metra, M., Filipatos, G. S., Ezekowitz, J. A., Dickstein, K., Cleland, J. G. F., Kim, J. B., et al. (2016b). Acute treatment with omecamtiv mecarbil to increase contractility in acute heart failure: The ATOMIC-AHF study. J. Am. Coll. Cardiol. 67, 1444–1455.

Ukkonen, H., Saraste, M., Akkila, J., Knutti, J., Karanko, M., Iida, H., Lehikoinen, P., Nagren, K., Lehtonen, L., and Voipio-Pulkki, L. M. (2000). Myocardial efficiency during levsimendan infusion in congestive heart failure. Clin. Pharmacol. Ther. 68, 522–531.

Veerman, C. C., Kosmidis, G., Mummery, C. L., Casini, S., Verkerk, A. O., and Bellin, M. (2015). Immaturity of human stem-cell-derived cardiomyocytes in culture: Fatal flaw or soluble problem? Stem Cells Dev. 24, 1035–1052.

Wroosek, A., Schneider, H., Gruenering, S., and Chiesi, M. (1992). Effect of thapsigargin on cardiac muscle cells. Cell Calcium 13, 281–292.

Zhang, D., Shadrin, I. Y., Lam, J., Xian, H. Q., Snodgrass, H. R., and Bursac, N. (2013). Tissue-engineered cardiac patch for advanced functional maturation of human ESC-derived cardiomyocytes. Biomaterials 34, 5813–5820.

Zhao, Y., Rafatian, N., Feric, N. T., Cox, B. J., Aschar-Sobbi, R., Wang, E. Y., Aggarwal, P., Zhang, B., Conant, G., Ronaldson-Bouchard, K., et al. (2019). A platform for generation of chamber-specific cardiac tissues and disease modeling. Cell 176, 913–927.