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The FDA Workshop on Improving Cardiotoxicity Assessment with Human-Relevant Platforms

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Abstract

Given that cardiovascular (CV) safety concerns remain the leading cause of drug attrition at the preclinical drug development stage, the National Center for Toxicological Research (NCTR) of the US Food and Drug Administration (FDA) hosted a workshop to discuss current gaps and challenges in translating preclinical CV safety data to humans. This white paper summarizes the topics presented by speakers from academia, industry, and government intended to address the theme of improving cardiotoxicity assessment in drug development. The main conclusion is that in order to reduce CV safety liabilities of new therapeutic agents, there is an urgent need to integrate human-relevant platforms/approaches into drug development. Potential regulatory applications of human-derived cardiomyocytes and future directions in employing human-relevant platforms to fill the gaps and overcome barriers and challenges in preclinical CV safety assessment were discussed. This paper is intended to serve as an initial step in a public-private collaborative development program for human-relevant cardiotoxicity tools, particularly for cardiotoxicities characterized by contractile dysfunction or structural injury.

Executive Summary

CV safety concerns continue to be the most common reason for drug termination during preclinical drug development. These liabilities are most often identified in animal studies and represent putative risks of significant or irreversible CV harm. However, such preclinical risks are often not confirmed in human patients because many of these drugs are terminated prior to clinical testing. Poor translational specificity of preclinical animal models and our recognized shortcomings in understanding comparative pathophysiology suggest that we may be terminating beneficial medicines for liabilities in animals that would not be liabilities in patients. Additionally, animal studies have low throughput and are conducted late in preclinical development, which eliminates the opportunity to leverage the power of molecular design to avoid liabilities that are truly human relevant.

There is a clear need to change our approach on preclinical CV safety assessment so that we can increase the specificity of assessments performed at higher throughput to optimize our ability to design safer and more effective drugs. Human-relevant platforms, including human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and adult human primary cardiomyocytes (CMs), are increasingly used in pre-clinical *in vitro* cardiac toxicity screening. The use of these new *in vitro* preclinical models has been accepted for investigational new drug (IND) applications and has great potential to reveal mechanisms of drug-drug interactions (DDIs) and personalize cardiac safety predictions. We believe that current pharmaceutical CV safety assessment would benefit from an approach that may prove to be more efficient in cost and time, mechanistically informative, and translatable to human patients. Such an approach may enable the earlier recognition of development-limiting safety liabilities, reduction of false positives that lead to premature and unnecessary development termination, discovery of more relevant biomarkers, and a significant decrease of late-stage attrition.

Keywords

Cardiotoxicity; cardiomyocyte; risk assessment; human; stem cell; human platforms; drug development

Subject Terms:

Basic Science Research; Stem Cells; Translational Studies

Introduction

A working group of cardiovascular scientists from academia, industry, and government assembled at the FDA/NCTR in Jefferson, Arkansas to identify gaps and challenges in translating current approaches of preclinical CV safety assessment to human patients. Technical applications and opportunities in using human-relevant platforms to improve cardiotoxicity detection and characterization were presented and the path forward was discussed. It should be emphasized that the goal of the workshop was not to seek a consensus on new approaches or platforms, but to help inform the planning process for a consortium effort to shift the CV safety pharmacology and toxicity test battery toward human-relevant platforms that are more mechanism-based and hypothesis-driven. The goal of the workshop was aligned with the mission of FDA and the NCTR strategic plan.

Background

Drug development is a long and expensive process with far more failures than successes. The aim of the process is to advance novel drug candidates with maximum benefits and minimal or manageable safety risks. The safety assessment component of the process tends to be relatively risk-averse to protect patients from unintended harm. According to the Pharmaceutical Research and Manufacturers of America (PhRMA) 2016 Biopharmaceutical Research Industry Profile,[1] the average time to develop a drug is 10–15 years with an average cost of $2.6 billion in 2000s to early 2010s. Fewer than 12% of drugs that entered clinical trials have ultimately been approved. In analyses of reasons for attrition during drug discovery and development, clinical safety concerns and non-clinical toxicity are major contributors.[2] The current paradigm for preclinical safety assessment and clinical safety prediction relies heavily on animal studies founded on the belief that animals most closely approximate the biological complexity of human patients. However, species differences are increasingly being recognized as potentially leading to false positive and false negative predictions of patient responses and risk liabilities. A recent analysis conducted by a cross-pharma working group concluded that preclinical animal *in vivo* studies generally have a high negative predictive value (NPV)[3] (i.e., if organ-specific toxicity did not occur in the animal studies, it would likely not occur in human clinical trials), which is supportive of an approach built on conservative risk/liability aversion. The potential for low specificity in animal safety studies and high rates of false positives[3] (i.e., low positive predictive value (PPV)), particularly for CV liabilities, has been less explored and may be adding to the crippling rate of safety-related attrition. Human-based *in vitro* methods (e.g., human cells,

stem cells, and organ-on-chips) and computational models have been proposed to replace animal testing, at least partially, in preclinical drug development.[4]

Gaps and Challenges to Translating Current Preclinical CV Safety Data Approaches to Humans

Gaps: Drugs can intentionally or unintentionally target many of the cellular components of the CV system as well as signaling receptors, ion channels, and fundamental cellular processes to produce cardiotoxic liabilities. These toxicities can manifest variously as functional and/or structural perturbations of the heart muscle, valves, or systemic vasculature. The integrated CV system is difficult to model effectively in reductionist systems, so animal studies are crucial to the current preclinical CV safety assessment. Traditional approaches to drug safety assessment include acute (single dose) and chronic (multiple-dose) studies of increasing durations as preclinical development progresses. At least 2 animal species are used (one rodent and one non-rodent) to assess both functional and structural liabilities. Single-dose, short-duration studies are performed using chronically instrumented animals in a Latin square design can assess drug-induced changes in CV function (e.g., arterial blood pressure (BP), heart rate (HR), electrophysiology (ECG), and cardiac contractility). Repeat-dose general toxicity studies are more enriched for morphologic, hematologic, and biochemical endpoints to evaluate a full spectrum of potential target organs. The primary purpose of preclinical safety studies is to identify potential human hazards and establish a safe starting dose for human clinical trials. The aim to identify potential hazards dictates that doses used in the animal preclinical studies generally far exceed those intended in the clinic.

Despite their general relevance to humans, preclinical animal studies have a number of potential drawbacks. Laboratory animals do not faithfully represent general human biology in every respect, to say nothing of individual variability in disease susceptibilities, co-morbidities, and polypharmacy. Additional potential issues include:

- Studies are unblinded
- Results are observational
- There is no hypothesis testing and no type-I error control
- Large doses cause clinically irrelevant effects
- Phenotypic toxicology studies provide limited mechanistic insights.

Consequently, it is not surprising that the manifestation of some toxicities in patients differ significantly from those demonstrated in traditional animal studies. A typical example of the difference in sensitivity to CV toxicity is the dog’s predilection for vascular injury induced by vasodilators and positive inotropic agents that are not always recapitulated in humans.[5] Another example is rat-specific cardiomyopathy.[6] Animals are not good model systems for safety assessment for drug classes that have high specificity for human-specific targets, such as recombinant proteins, monoclonal antibodies, oligonucleotides, and siRNAs.

False-positive findings in animals may be more common than we understand. Unfortunately, there is no database on the rate of false positives and their impact on drug development.

Recently, the IQ Consortium analyzed a blinded database of 182 molecules and animal toxicology data coupled with clinical observations from phase I human studies, finding that the PPVs (the proportion of positive nonclinical findings that had positive clinical findings) of CV toxicity in rodents, dogs, and non-human primates were only 17%, 50%, and 20%, respectively.[7] Another cross-company database for the concordance between conscious dog telemetry data and phase I clinical CV toxicity assessment found that within the 10–30x exposure range, the unadjusted PPV ranges were only 10–13% for HR and 6–8% for diastolic BP, although the NPV was high.[3] The loss of opportunities in testing these false-positive drugs in human patients may present an even bigger problem not including drug candidates that were dropped from development prior to being tested in humans in the two databases.

Challenges: Clearly, there are significant limitations for preclinical animal studies to be used in CV safety assessment. A better preclinical assay would ideally include the following attributes: 1) higher throughput; 2) sample size adequate to make reliable inferences; 3) blinded/unbiased assessment of objective endpoints; 4) formal hypothesis testing to assess false-positive/negative findings; 5) mechanistic insight leveraging toxicopathological failure modes; and 6) human-relevant biology so that findings are more likely to translate to the clinic. However, the creation of a new paradigm in shifting our testing from phenotypic to mechanistic would face challenges including the following:

- **Biology** − Transition from normal to abnormal is generally non-binary. Thresholds of biological perturbation that represent “toxicity” are difficult to define precisely and not generally well understood mechanistically. Contextualizing those perturbations in a myriad of possible individual susceptibilities is even more difficult.
- **Math** − Traditional approaches to preclinical safety assessment identify putative risks, characterize their adversity, and determine dose/exposure margins from predicted clinical exposures. Quantitatively determining the window between no-observed-adverse-effect level (NOAEL) and clinical peak serum concentration (*Cmax*) is challenging.
- **Complex pathogeneses** – Pathogenesis of toxicity can be complex. Multiple injuries can happen in the CV system coincidentally, and we need to determine whether there is a pathogenic connection among these changes, whether these changes can be replicated in humans, and whether the toxicities are manageable in patients.
- **Human element** – There is a process of building credibility and confidence for novel assays and models that needs to be accepted by regulatory decision-makers.

Opportunities and Technology Applications of Human-Relevant Platforms

Given the limitations of preclinical animal studies in translating preclinical safety assessment in the clinical setting, human-based models have been introduced in drug discovery, screening, and toxicity testing. The Comprehensive *In Vitro* Proarrhythmia Assay

(CiPA) initiative is an example of how human-relevant platforms are being used for drug-induced proarrhythmia detection.[4, 8]

The CiPA initiative: QTc interval prolongation and Torsade de Pointes (*TdPs*) were the single most common cause of withdrawal or restriction on marketed drugs in the 1990s, which led to the international regulatory guidance of ICH S7B and ICH E14 calling for careful assessment of drug effects on human ether-a-go-go-related (hERG) or I_{Kr} channel and the QTc-corrected interval in preclinical and clinical studies. This approach was taken in response to the known links between drug-induced reductions in the hERG-related current and increases in the QTc interval and the development of *TdPs*. Instead of focusing on the actual proarrhythmic risk, ICH S7B and E14 focus on surrogates, which can identify drugs with the potential to induce *TdPs* but whose PPV is low. A number of drugs inhibit hERG or prolong the QTc interval (e.g., verapamil, ranolazine, and phenobarbital) but are not associated with *TdPs*, because they inhibit other cardiac currents that are necessary for *TdPs* development (i.e., the late inward sodium current and the L-type calcium current). Consequences of developing a compound with a hERG or QTc effect include significantly increased development burden, delays in filing and/or regulatory approval, label warnings (that may not be necessary) with competitive implications, and licensing/partnering challenges. Consequently, many drugs are terminated during discovery because of preclinical findings of a hERG or QTc effect, despite their potential to have a net favorable benefit. It has been estimated that the discontinuation rate of preclinical programs due to these issues is ~60%.[9] In fact, the finding of a QTc signal during clinical development often leads to the discontinuation of a drug's development altogether.

In the last 14 years since ICH S7B and E14 were ratified, a deeper understanding of the mechanisms responsible for *TdPs* and the role of early afterdepolarizations in the genesis of the arrhythmia has evolved. This has led to a new paradigm – CiPA.[4] This approach focuses on assessing a drug's mechanistic proclivity to be proarrhythmic and cause *TdPs*. In this approach, drugs that inhibit hERG or result in QTc prolongation due to drug-induced ion channel effects, but are otherwise safe from a proarrhythmic perspective (due to inhibiting the late inward sodium current or the L-type calcium current), will be identified and labeled accordingly. Briefly, CiPA has 4 major components:

- Drug effects on **multiple individual human cardiac ion channels** in heterologous expression systems are assessed with commercial high-throughput voltage clamp systems. This effort focuses on creating uniform ion channel testing protocols that best represent the actual ion channel properties in humans.
- The data obtained from ion channel studies are integrated into an ***in silico* human ventricular myocyte computational model** to assess proarrhythmic liabilities; this is the primary determinant of proarrhythmic potential under CiPA.
- The ECGs in a Phase 1 study, including T-wave analysis, are assessed to determine if there are **unexpected electrocardiographic effects** based on the preclinical data in humans (e.g., due to a human specific metabolite or different pharmacokinetics).

- **Human stem cell-derived cardiomyocytes** will be used for unanticipated nonclinical effects, or if human ECG data are insufficient.

CiPA is a new *in vitro* paradigm for cardiac safety evaluation of drugs that is expected to provide a more accurate and comprehensive mechanistic-based assessment of proarrhythmic potential. This should improve candidate selection decisions, enhance efficiency of drug development, and permit drugs to be efficiently developed that are safe despite hERG and/or QTc effects, ultimately providing product labeling that more accurately reflects the actual risks.

Moving Beyond CiPA

As part of the commitment to **R**eplacing, **R**educing, and/or **R**efining animal studies (the "3Rs"), CiPA has established a good example of using human-based technologies to enable more robust nonclinical proarrhythmia screening of new drug candidates and reduce the use of animals for torsadogenic risk assessment. Besides proarrhythmia assessment, other aspects of cardiac safety may also be addressed through human materials in the early drug screening process, including the uncoupling in excitation-contraction (E-C), interference with force generation, interference with mitochondrial energetics, and direct cytotoxicity (Figure 1). A few examples of how the human-relevant materials and platforms may improve our understanding of mechanisms of direct cardiotoxicity and drug-induced cardiotoxicity detection (Figure 1) were presented and discussed at the workshop.

hiPSC-CMs Recapitulate Side Effects Not Revealed in Preclinical Animal Studies

Activators of Sphingosine 1−Phosphate (S1P) receptor have been used to treat multiple sclerosis. One of the major side effects of Gilenya® (fingolimod), an $S1P_1$ and $S1P_3$ agonist,[10, 11] is bradycardia. As the study with $S1P_3$ knock-out mice suggested that $S1P_3$ is a major contributor to the HR effect of Gilenya,[12] BAF-312 and CS-0777, the selective $S1P_1$ agonists, were developed. In preclinical species, the selective $S1P_1$ agonists showed no significant HR effects. However, clinically significant bradycardia was still observed in humans, suggesting that the distributions of S1P receptors in preclinical species and humans are different. $S1P_3$ is dominant in hearts of preclinical species, while $S1P_1$ plays a major role in modulating HR in humans.[13, 14] To develop a $S1P_1$-selective agonist with minimal HR effects in humans, hiPSC-CMs and multielectrode array (MEA) technologies were applied to assess the beat rate (BR) effects of S1P agonists.[15, 16] $S1P_1$ and $S1P_3$ exhibited a similar RNA expression in hiPSC-CMs as in adult human heart tissues in QPCR assay. The hiPSC-CM MEA assay recapitulated the slow HR effect of Gilenya, BAF-312, and CS-0777 at clinically relevant concentrations, and had the same rank order in BR reduction potencies found in different agents as in human bradycardia. A mechanistic study using pharmacological tool agents of the G protein-gated inward-rectifying potassium channel (I_{KACh}) inhibitor, Gi blocker, $S1P_1$, and muscarinic receptor antagonists in hiPSC-CMs indicated that the slow HR effect of $S1P_1$ is caused by activation of I_{KACh} through the $S1P_1$-coupled Gi protein. The slow BR effects of selective $S1P_1$ agonists were more substantial in hiPSC-CMs than in rat embryonic CMs, consistent with more significant bradycardia seen in humans. These data were used for internal risk assessment and IND filling.

The Use of hiPSC-CMs *In vitro* Assays to Reveal Unexpected Cardiac Drug-Drug Interaction in Clinics

Assessment of any potential ECG and dysrhythmic effects is an important part of the preclinical development process for all new pharmaceutical agents. While tachyarrhythmic ECG events including supraventricular proarrhythmias (i.e., atrial fibrillation) and ventricular proarrhythmias (i.e., *TdP*s) have always been major concerns, recent significant bradyarrhythmias resulting from drug-drug interactions (DDIs) pose a difficult safety problem.[17, 18] In 2015, the European Medicines Agency (EMA) and U.S. FDA issued warning letters in response to 9 reported, post-marketing clinical cases of severe and life-threatening symptomatic bradycardias/bradyarrhythmias in Hepatitis C-Virus (HCV)-infected patients treated with a HCV-NS5B pronucleotide inhibitor (HCV-NS5B-NI) antiviral agent, sofosbuvir (SOF), in combination with other direct-acting antivirals (DAAs) and/or the class-III antiarrhythmic drug: amiodarone (AMIO). [19, 20] Investigations were initially conducted in anesthetized guinea-pigs and conscious, chair-restrained telemetered nonhuman primate models, in which the serious, clinical SOF+AMIO cardiac DDIs were replicated convincingly to provide hypotheses for potential mechanism(s) of action.[21] However, since AMIO presents a very prolonged half-life in animals as well as in patients (average t1/2=58 days) and an uncertain elimination (t1/2=36 days for its active metabolite – desethylamiodarone), the selective cardiac electrophysiological effects were examined in a hiPSC-CM functional assay capable of providing a rapid assessment of potential cellular/molecular mechanism(s) and identification of cell types, receptors, and/or ion channels responsible for the clinical DDIs observed with SOF+AMIO. In the preliminary *in vitro* experiments, the impact of six HCV-NS5B-NI prodrug phosphoramidate diastereochemistry (D-/L-alanine, Rp-/Sp-phosphoryl) molecules (SOF, MNI-1, MNI-2, MNI-3, MNI-4 and MK-3682 or uprifosbuvir) was evaluated in hiPSC-CMs, as well as their effects when co-applied with AMIO. Interestingly, L-ala, Sp prodrugs (SOF, MNI-1, MNI-3) increased the BR or field potential (FP) rate and decreased the beat amplitude or impedance (IMP) in hiPSC-CMs, but D-ala, Rp prodrugs, including MNI-2, MNI-4 and MK-3682 (uprifosbuvir), did not have the same effects. Furthermore, as shown on Figure 2, this stereochemical selectivity on emerging bradycardia was confirmed *in vivo*.[22] Because the analogous effect was not observed with MK-3682, a different prodrug NS5B nucleotide polymerase inhibitor, this suggests that it is not a class effect. In addition, the combination of SOF+AMIO in hiPSC-CMs mirrored the phenotypic effects of Ca^{2+} channel blockers (CCBs) in this assay, but surprisingly, replacing AMIO with CCBs did not induce the cardiac DDI. Moreover, dronedarone (an AMIO analog) did not substitute for AMIO, but rather competed with AMIO in the cardiac DDI. Ryanodine and thapsigargin (depleting the intracellular Ca^{2+} stores), and SEA-400 (a Na^{+}/Ca^{2+} exchanger NCX1 inhibitor) partially alienated or diminished the DDI effects. Comparing with the respective individual effects, other FP rate-affecting compounds either showed no effects or only exerted additive or subtractive effects. The data in hiPSC-CMs indicated that the DDI between AMIO and HCV-NS5B NI agents such as SOF and MN1 is attributed to their potential interaction(s) with key intracellular Ca^{2+}-handling mechanisms. Finally, to identify whether the cardiac DDI phenotype/signature between HCV-NS5B pronucleotide inhibitors and AMIO in hiPSC-CMs was caused exclusively by AMIO's chemical structure features (Structure Activity Relationship: SAR) or from interaction with other chemical entities, 20 AMIO's chemical analogs and 72

chemical entities (marketed drugs or reference pharmacological agents) were synthesized, and their effects on FP and IMP in hiPSC-CMs were evaluated. *In vitro* SAR studies demonstrated the required "high specificity" of AMIO chemical structure features to induce this type of cardiac DDI when combined with HCV-NS5B NI agents. These data support the utility of hiPSC-CMs as a potential comprehensive yet scalable tool, which can be used to identify and examine the unexpected, cardioactive pharmacodynamic DDIs for new chemical entities.

hiPSC-CMs in Discovery Toxicology: Balancing Promise and Translational Value

The technological co-evolution of hiPSC-CMs and platforms to address their functional output has recently enabled *in vitro* CV safety testing in phenotypically-relevant cellular models. Concomitant technical advances such as incorporation of the underlying measurement technology (e.g., MEA and impedance electrodes) into standard format multi-well plates, and automated processing thereof, have increased the throughput of these assays to a level that is compatible with discovery-phase chemistry counter screening. The pilot and validation phases of CiPA myocyte studies demonstrate that occurrence of *in vitro* arrhythmias and prolongation of field potential duration (FPD) corrected for BR can have high translational value for clinical *TdPs*.[23, 24] The relatively large size of the CiPA compound sets has also provided a convenient means for confirming low well-to-well, plate-to-plate, and site-to-site variability for these translational cardiac beat metrics.

A noteworthy difference exists, however, in the origin of human cardiomyocyte beating *in vitro* vs. *in vivo*. In the intact human heart, spontaneous beating is maintained by pacing at anatomical nodes, and transmitted through the cardiac conduction system. Drug or endocrine action at these nodes is thus a primary driver of clinical chronotropic effects. In contrast, growing evidence suggests spontaneous beating in syncytial hiPSC-CMs is mainly determined by chronological ectopic expression of HCN_4, a hyperpolarization-activated cyclic nucleotide-gated ion channel representing the molecular correlate of the pacemaker (I_f) current.[25] This may lead to a complicated mechanistic interpretation of drug effects. For example, drug-induced cessation of spontaneous beating has historically been scored as an arrhythmia in hiPSC-CM assays such as those used in the Japan iPS Cardiac Safety Assessment study[26], despite the ectopic cell-intrinsic role of I_f in maintenance of beating. Similarly it is not uncommon for changes in BR to be used as a primary safety metric, without exclusion of I_f modulation as a confounding factor.[27–30] While I_f modulation would most directly confound drug effects on BR, a corollary concern is that FPD values in hiPSC-CM assays are almost universally "corrected" for the rate of this ectopic beating using clinically derived formulas.

In summary, while hiPSC-CMs have generated justifiable excitement as an *in vitro* CV safety screening tool, the following concerns should be considered when experimental outcomes are decisional: 1) cessation of spontaneous beating during drug application has a particularly dubious translational value, as it is unknown whether cardiac beating would continue when driven by an anatomical node; 2) for chronotropic effects, potentially confounding pharmacological targets (e.g. HCN_4 and M_2 muscarinic G-protein-coupled receptors) should be addressed, if not ruled out, when results are decisional; and 3)

whenever possible, spontaneous BR should be obviated using platforms capable of electrical or optogenetic pacing.[23, 31, 32] Such extrinsic pacing methods may also recover data for other metrics (e.g., FPD and presence of arrhythmias) at drug concentrations in which spontaneous beating is completely eliminated.

Stem Cells and Genomics for Precision Cardiovascular Medicine

Emerging big data technologies and advances in genetics and genomics are providing an unprecedented opportunity for precision medicine to treat patients using a more tailored approach based on their individual genetic backgrounds. However, there remains a significant gap between patients' genotypes and phenotypes (e.g., disease and drug response). Patient-specific hiPSC-CMs hold great potential for studying genotype-phenotype associations in patients with various CV diseases, if these models can sufficiently recapitulate the requisite biological function.

The concept of *in vitro* disease modeling with hiPSC-CMs is well established for inherited cardiomyopathies (e.g., hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM)) and inherited cardiac channelopathies (e.g., long QT syndrome (LQTS) and Brugada syndrome) through many studies showing their ability to recapitulate human disease phenotypes in a dish (e.g., abnormal contractile properties and arrhythmia).[33] Moreover, hiPSC-CMs can serve as a novel platform for *in vitro* cardiotoxicity testing. For example, inter-individual variation in susceptibility to doxorubicin-induced cardiotoxicity was reproduced *in vitro* using patient-specific hiPSC-CMs.[34] In another study, cardiac toxicities of 21 FDA-approved tyrosine kinase inhibitors were screened and tested systematically in a high-throughput fashion using hiPSC-CMs.[35] hiPSC-CMs were also employed to investigate mechanisms of off-target cardiotoxic effects induced by trastuzumab, by targeting the altered pathways, to evaluate it as a potential therapeutic approach in protecting CMs from the off-target toxic effects.[36] In addition, advances in genome editing tools now make it possible to precisely link genetic variations to human diseases. Recently, the pathogenicity of variants of uncertain significance in heart disease-related genes (HCM[37] and LQTS[38]) has been determined for the first time by combining genome-editing and hiPSC technologies.

Furthermore, the aforementioned findings support the emerging "clinical trial in a dish" concept, in which hiPSC-CMs from a large patient cohort can serve as a new robust platform for preclinical drug testing. Further development of this concept may enable us to identify druggable targets, predict drug response, and facilitate individualized therapeutic interventions. Ultimately, the precise information generated through hiPSC-CMs platform is expected to lead to more efficient and effective clinical trials and thereby improve patient outcomes.

Metabolic Maturation of hiPSC-CMs

hiPSC-CMs are gaining recognition for *in vitro* cardiac toxicity screening of new drug candidates and hold great promise for precision medicine. However, the immaturity and embryonic-/fetal-like nature of hiPSC-CMs can be a potential problem for drug-induced cardiotoxicity prediction that truly reflects the toxicity in adult human hearts. To maximize

the benefits of hiPSC-CMs in disease modeling and drug discovery, various techniques have been employed to improve hiPSC-CM maturation both functionally and structurally. Long-term culture,[39] enhanced expression of KCNJ2/I_{K1},[40] chronic electrical stimulation,[41] stretch or mechanical loading,[42] developmental and epigenetic cues,[43] micropatterning,[44] and 3D cultures of hiPSC-CMs as engineered heart tissues[45] are among the advances that have been reported to improve hiPSC-CM maturation in terms of electrophysiological property, metabolic signature, contractile function, and structure modeling.[33]

One of the most difficult challenges in maturing hiPSC-CMs is to promote energy metabolism shift from anaerobic glycolysis to fatty acid beta oxidation.[46] This is because hiPSC-CMs produced in current protocols show a relatively low mitochondrial density and are predominantly glycolytic, whereas adult human CMs typically depend on fatty acids, rather than glucose, as the main energy substrate. It is well known that cells that mainly rely on mitochondrial oxidative phosphorylation (OXPHOS) for energy production are more susceptible to mitochondrial insults than glycolysis-dependent cells.[47, 48] To overcome these limitations, hiPSC-CMs were exposed to a fatty acid-based maturation medium to mimic the usage of metabolic substrates in adult human CMs. The preliminary data indicate that compared to cells grown in normal maintenance media, hiPSC-CMs cultured in maturation media had increased mitochondrial membrane potential, mitochondrial mass, and oxygen consumption as they are more reliant on fatty acids for energy generation. As expected, mitochondrial function assay and cytotoxicity assay demonstrated that maturation medium-cultured hiPSC-CMs were more sensitive to mitotoxicants than cells maintained in normal maintenance media. CMs have a high energy demand for contraction and thus can be particularly susceptible to agents that impair mitochondrial function. The fatty acid-based maturation media's ability to improve hiPSC-CM maturation may be a better approach for modeling the responses of adult human CMs to cardiotoxicants with mitochondrial liabilities.[49]

Adult Human Primary Cardiomyocyte Model for the Simultaneous Prediction of Drug-Induced Inotropic and Pro-Arrhythmia Risk

Although some academic laboratories have used adult human primary CMs to conduct cardiac research,[50–53] the production of CMs has never been scaled for drug discovery purposes. Methods enabling standardized procurement protocols and utilization of high-quality viable human heart samples from organ donors have recently been established, and cardiac safety studies aiming at validating a pro-arrhythmia model based on action potential recordings in ventricular human trabeculae have been published.[54, 55] While this approach provides data highly predictive of clinical cardiac risk, the limited throughput of tissue-based assays prevents their use in early stages of preclinical development. This prompted the development and validation of an adult human primary cardiomyocyte model, amenable to high-throughput screening and reliant on non-invasive measurements of contraction using bright-field imaging.[56] By selecting endpoints measured from the contractility transients, both pro-arrhythmia as well as inotropic risks can be identified. Validation results involving 33 clinically well-characterized positive and negative controls, including 23 torsadogenic and 10 non-torsadogenic drugs, were presented at the workshop. When drug effects were evaluated at 10-fold of the free effective therapeutic plasma concentration (fETPC), the

detection of drug-induced aftercontractions led to risk assessment with excellent sensitivity and specificity of 96 and 100%, respectively. This high predictivity supports the translational safety potential of the adult human primary cardiomyocyte preparation and the predictivity of the selected endpoint. Additionally, by monitoring the amplitude of sarcomere shortening, both multi-ion channel blockers associated with negative inotropic risk[56] as well as positive inotropes can be readily identified. Furthermore, a detailed analysis of the parameters describing the contractility transient allows for the identification of the different mechanisms of action in drugs with inotropic activity.[57] In addition to the study of normal adult myocytes, adult primary CMs are now available from diseased donors, and it is now possible to assess how cardiac toxicity risk may be affected by common comorbidities. These data provide evidence that adult human primary CMs can be a valuable model for the reliable assessment of human cardiac safety.

Future Directions

Integrated Cardiotoxicity Prediction with Animal Studies and Human-Relevant Platforms—CiPA represents a paradigm shift for drug safety evaluation, by shifting the focus from a single ion channel activity to an integrated risk assessment of proarrhythmia based on multiple ion channels; it is also innovative by reducing the reliance on animal models and focusing on human-relevant cellular electrophysiology. The studies presented at this workshop and other emerging evidence strongly suggest that the integration of human cardiomyocyte-based *in vitro* assays in the drug development process may fill the gaps in current CV safety assessment; this may be particularly relevant for improving cardiotoxicity prediction, especially for arrhythmias (including non-*TdP* arrhythmia[58]) and for contractile and structural cardiotoxicity assessment.[59] However, the current human-relevant cellular platforms have limitations related to the intrinsic inability to model and predict adverse hemodynamic effects and other off-target effects in non-cardiac tissue. Therefore, the new technologies are not expected to completely eliminate the reliance on animal models for CV safety and toxicity assessment. Moreover, animal studies can provide valuable information on pharmacokinetics of therapeutics. The development of human-based organ-on-chips and microphysiological systems may offer additional opportunities in assessing drug-induced off-target effects in the vasculature[60] and other non-cardiac tissues,[61] and in advancing modeling-informed quantitative clinical pharmacology evaluation.[62]

hiPSC-CMs have been widely used in drug-induced cardiotoxicity screening and offer several practical advantages: 1) iPSC culture is scalable and the sustainable production of large numbers CMs enables drug screening campaigns for industry applications;[33] 2) hiPSC-CMs carry patient-specific genetic information and their production requires minimally invasive procedures, which allow clinicians to link patients' clinical/genetic information with *in vitro* pharmacological responses and guide the selection of a personalized therapeutic regime;[63] 3) disease-specific hiPSC-CMs models have the potential to provide platforms for investigating disease mechanisms and evaluating novel therapeutics; [33, 36] 4) hiPSC-CMs derived from a representative pool of the general population may enable patient stratification for clinical testing[34, 64] and may be employed to further assess the risk of cardiotoxicities if adverse events are reported in post-marketing surveillance; and 5) hiPSC

technology may be utilized in human organ-on-chips and microphysiological systems to evaluate DDIs and off-target effects.

As highlighted at the workshop, important limitations related to the biology of hiPSC-CMs cannot be overlooked and, undoubtedly, additional work needs to be done to fully understand the predictive value of this system in the context of preclinical cardiac safety risk assessment. The issues related to metabolic, structural, and functional maturation remain incompletely resolved, and any one of these factors could result in inconsistent predictive value across diverse classes of drug candidates.

Isolated adult human primary CMs provide a fully mature system that is physiologically and pharmacologically relevant to the assessment of cardiac safety risk. Several platforms originally developed for the study of hiPSC-CMs can also be used to measure drug effects in adult human primary CMs, thereby enabling sufficient throughput to employ the adult primary CMs in early- to mid-stage discovery programs.[65, 66] The use of optical-based methods to measure contractility, intracellular calcium dynamics, membrane potential fluctuations, and mitochondrial function offers great opportunities to also employ adult human primary CMs for mechanistic investigations in a system that has all the native physiological components, avoiding the potential pitfalls of artificially engineered cells. While major improvements in scaling adult primary CM isolation have been accomplished in recent years,[56, 67–69] additional work needs to be performed to further expand the availability of these cells. It will also be interesting to merge efforts in large-scale primary CM isolation with advances in biomaterials and substrate technologies, so that better platforms can be developed to support the long-term culture of primary CMs under continuous electrical stimulation.[70] These methods could greatly facilitate the study of chronic cardiac toxicities.

Understanding the advantages and limitations of adult human CMs and hiPSC-CMs (Table 1) and combining the appropriate *in vitro* assays with animal studies will lead to a better prediction of CV liabilities of new therapeutics.

From a regulatory standpoint, *in vitro* CMs assays may be used at multiple stages of drug development[59], including: 1) lead candidate selection and optimization at the early drug discovery phase; 2) assessment of cardiotoxic potential before the initiation of clinical trial, especially for first-in-human (FIH) compounds when they are of a drug class with known safety concern; 3) mechanistic assessment (e.g., mitochondrial stress, lipid metabolism, cell viability, gene expression changes, cellular biomarker release, and other cellular morphological measurements) when post-marketing monitoring indicates safety concerns or DDIs; 4) waiver of human echocardiogram monitoring if a negative profile for cardiac liability was demonstrated with qualified tests; and 5) use in discovery of noninvasive biomarkers to provide earlier detection of cardiotoxic risk before irreversible injury has occurred. Integrated cardiotoxicity prediction with animal studies and human-relevant platforms may represent a new paradigm of drug development to fill the gaps and unmet needs of the current strategy and to improve drug-induced CV liability prediction.

Summary

The take home messages from this workshop are: (i) human-relevant models can assess both structural and functional abnormalities of CMs and may avoid false-positives due to animal-specific findings; (ii) hiPSC-CMs show the potential to identify delayed responses, reveal patient-specific susceptibilities, and detect/predict some off-target effects; (iii) adult human primary CMs are a physiological system with the full complement of ion channels, receptors and contractile machinery that are critical for proper cardiac function and could be considered the gold standard for cell-based cardiac toxicity studies; and (iv) to reduce CV safety liabilities of new therapeutic agents, there is an urgent need to integrate human-relevant platforms/approaches into drug development.

For a new nonclinical method to be accepted for use in CV safety assessment, there must be a systematic evaluation of the ability of the new technology to predict adverse clinical outcomes. This will involve a continuous dialogue and feedback among all relevant stakeholders, and careful qualification studies must be conducted to test the assays' precision, selectivity, sensitivity, reliability, reproducibility, and applicability. To this end, NCTR sponsored the workshop as an initiative step in building a consortium to emphasize the importance and feasibility of integrating human-relevant platforms into the drug development process. Further collaborations among participants from academia, government, and industry are discussed and will be organized by HESI Cardiac Research Safety Committee (CSRC).

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Nonstandard Abbreviations and Acronyms:

CMs	cardiomyocytes
IND	investigational new drug
DDIs	drug-drug interactions
PhRMA	Pharmaceutical Research and Manufacturers of America
NPV	negative predictive value
PPV	positive predictive value
TdPs	Torsade de Pointes
hERG	human ether-a-go-go-related gene
fETPC	free effective therapeutic plasma concentration
FIH	first-in-human

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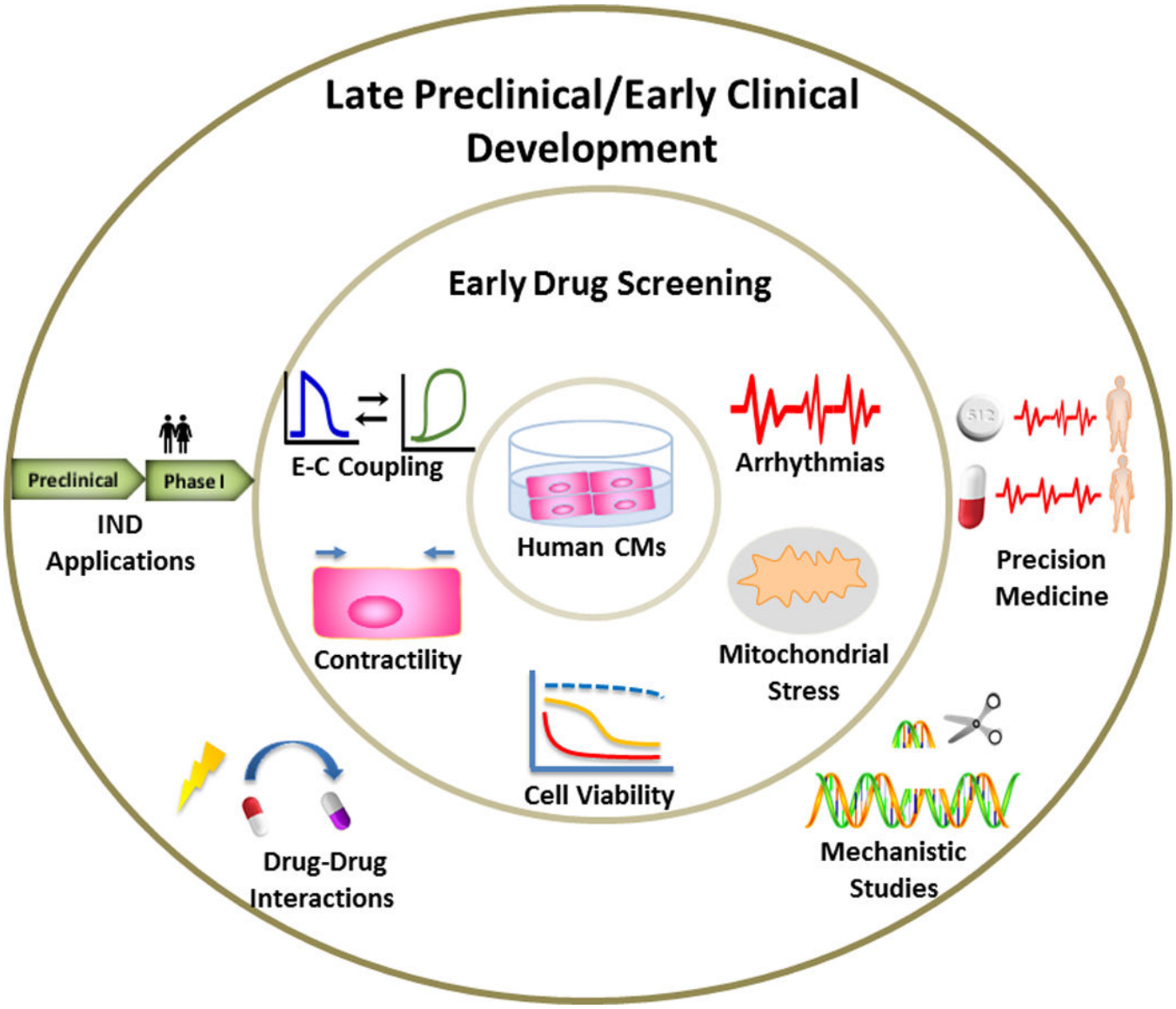

Figure 1.

Phenotypic changes that can be assessed with human cardiomyocytes in early drug screening and applications of human-relevant platforms in late preclinical and early clinical drug development process.

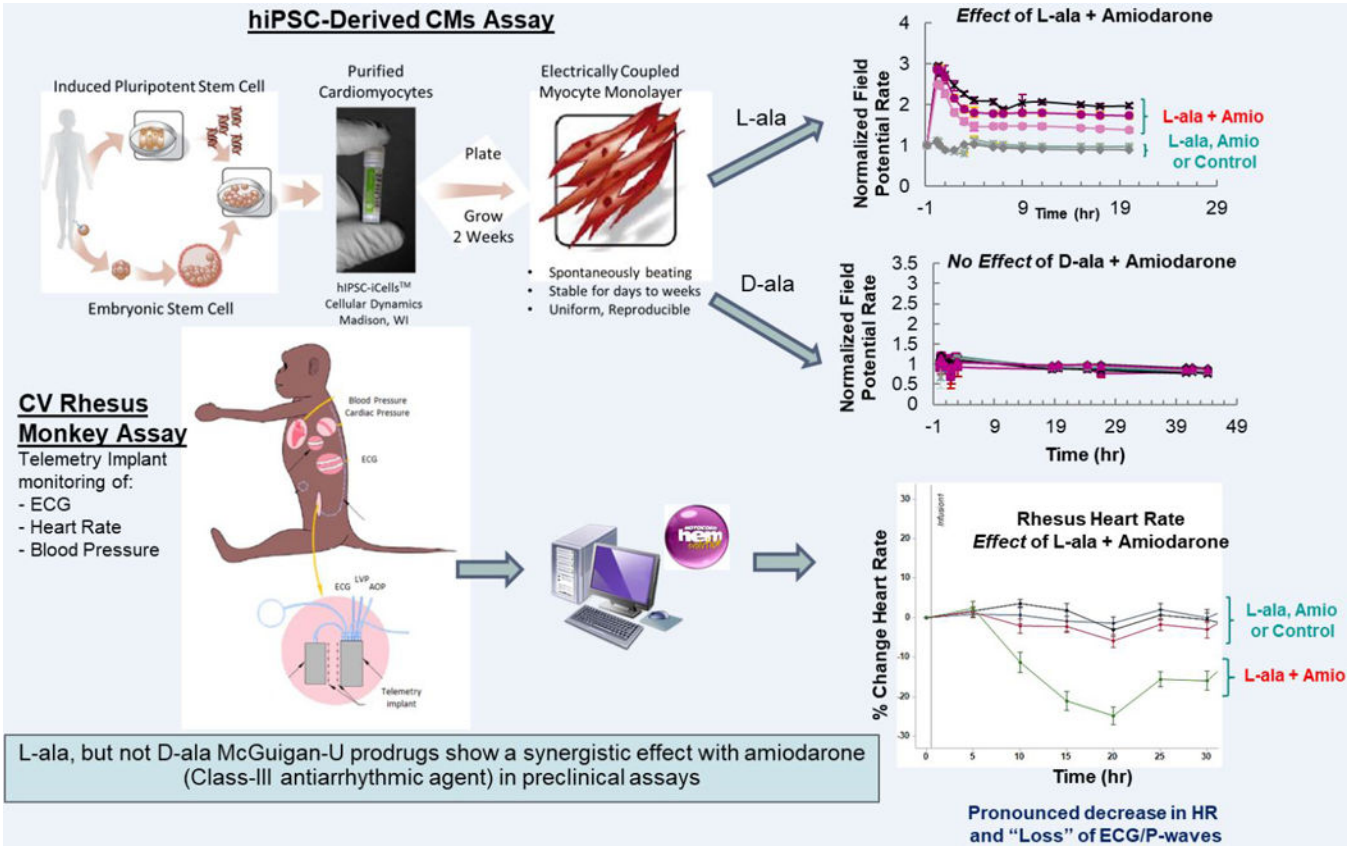

Figure 2.

hiPSC-CMs assay can replicate the HCV-NS5B NI/Amiodarone cardiac DDIs observed in clinic and preclinical models, provide a competitive advantage for early screening/de-risking of new HCV-NI candidates, and rapidly assess the putative mechanism(s) responsible of DDIs.

Table 1:

Comparison of Adult Human CMs and hiPSC-CMs for Drug-Induced Cardiotoxicity *In Vitro* Assay

	Adult Human CMs	hiPSC-CMs
Resemble of human biology		
Structure	Rod-shaped; Sarcomere highly organized; Immature: Round or polygonal; Sarcomere disorganized; T-tubules well developed[33]	Immature: Round or polygonal; Sarcomere disorganized; No T-tubes[33]
Electrophysiology	Adult and electrical-pacing-induced contraction; Physiological ion channel density; Matured Ca^{2+} handling	Immature: Spontaneous beating; Some ion channels are under- or over-expressed; Immature Ca^{2+} handling[33]
Contractility	Significant force; [33] Positive force-frequency relationship[71]	Immature: reduced force;[33] Positive force-frequency relationship can be generated in some 2D/3D cultures[72, 73]
Mitochondrial	Occupy 20-40% of cell volume; Throughout cell; Mainly rely on fatty acids[33]	Immature: Low numbers; Perinuclear; Primarily employ glycolysis[33]
CM Quality & Consistency	Single cell with known chamber information;	Single cell assay or in 2D/3D cultures; A mix population of CMs, possible for chamber-specific categorization;[33, 74] 3D cultures may enable evaluate the propagation of pacemaker signals[75]
	Reproducibility can be achieved by implementation of standardized procurement protocols and novel isolation methods[54, 56, 67]	Ensure reproducibility is critical as many issues in reprogramming, differentiation, and maintenance are not certain[33]
Scalability	Possible to scale up with the improvement in preservation of primary CMs[56] and development of immortalized CMs[68]	Easy scale-up iPSC culture, CMs can be generated in large scales[33]
Duration of exposure	Short (up to 30 minutes)[56] and long (up to few days)[70] for both acute and prolonged exposure to drugs	Long-term culture (months) is feasible,[39, 74] for both acute and chronic treatment
Ease of access to technology	Medium-throughput industrial platforms are available[65, 66, 76]	Multiple industrial platforms are commercially available for medium- and high-throughput assays[33, 76]
Autologous to "single patient" testing	No	Yes, minimally invasive
Disease Model	Hearts can be procured from patients with end-stage heart diseases; CMs can be isolated to study cardiac cellular pathophysiology and develop potential new treatments[67]	Many monogenetic disease models have been generated with patient-specific hiPSC-CMs and genome editing technology[33]; It is possible to develop disease models of polygenetic disease[74]
Population-based testing	Possible[56, 77]	Yes[64,78]
Development of MPS & human Organ-on-chips	In development[79]	Yes[72, 79]