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Review

Predicting cardiac safety using human induced pluripotent stem cell-derived cardiomyocytes combined with multi-electrode array (MEA) technology: A conference report

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ABSTRACT

Safety pharmacology studies that evaluate drug candidates for potential cardiovascular liabilities remain a critical component of drug development. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have recently emerged as a new and promising tool for preclinical hazard identification and risk assessment of drugs. Recently, Pluriomics organized its first User Meeting entitled '*Combining Pluricyte® Cardiomyocytes & MEA for Safety Pharmacology applications*', consisting of scientific sessions and live demonstrations, which provided the opportunity to discuss the application of hiPSC-CMs (Pluricyte® Cardiomyocytes) in cardiac safety assessment to support early decision making in safety pharmacology. This report summarizes the outline and outcome of this Pluriomics User Meeting, which took place on November 24–25, 2016 in Leiden (The Netherlands). To reflect the content of the communications presented at this meeting we have cited key scientific articles and reviews.

1. Introduction

Current preclinical (ICH S7B; (Anon, 2005a)) and clinical (ICH E14; (Anon, 2005b)) safety guidelines require first *in vitro* human ether-a-go-go-related gene (hERG) repolarization screening of new drug entities followed by an *in vivo* QT measurement. Drugs that successfully pass preclinical testing, are subjected to a thorough QT clinical study (TQT) (Gintant, Sager, & Stockbridge, 2016; Wallis, 2010). Although the implementation of these guidelines has largely eliminated new drugs entering the market with unanticipated arrhythmia risk, limitations do exist; for instance, changes in the QT interval are highly sensitive but not necessarily specific for predicting proarrhythmia risk in humans, potentially causing promising drug candidates to be discarded (Gintant et al., 2016). Therefore, a new initiative called 'comprehensive *in vitro* proarrhythmia assay' (CiPA) was launched in 2013 by the Cardiac Safety Research Consortium (CSRC), Health and Environmental Sciences Institute (HESI) and Food and Drug Administration (FDA) (Fermini et al., 2016). This proposal shifts the emphasis away from QT prolongation and focuses on predicting TdP hazard through an expansion of the *in vitro* component of nonclinical safety evaluation using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Data on drug-induced TdP in hiPSC-CMs have already been successfully collected by using several functional readouts, such as field potential traces using multi-electrode array (MEA) technology (Ando et al., 2017; Clements, 2016; Qu & Vargas, 2015), action potentials *via* voltage-sensitive dyes (VSD) (Asakura et al., 2015; Blinova et al., 2017; Lu et al., 2017) or automated patch-clamp techniques (Obergrussberger, Brüggemann, et al., 2015), calcium transients using kinetic plate readers (Abi-Gerges et al., 2017; Grimm, Iwata, Sirenko, Bittner, & Rusyn, 2015), and cellular impedance (Obergrussberger

Abbreviations: APD, action potential duration; APC, automated patch clamp; CiPA, Comprehensive *in vitro* Proarrhythmia Assay; CSRC, Cardiac Safety Research Consortium; DAD, delayed after-depolarizations; EAD, early after-depolarization; FDA, Food and Drug Administration; FPD, field potential duration; GP-CMs, guinea pig cardiomyocytes; HESI, Health and Environmental Sciences Institute; hERG, human Ether-a-go-go-Related Gene; hiPSC-CMs, human-induced pluripotent stem cell-derived cardiomyocytes; ICH, International Conference of Harmonization; MEA, multi-electrode array; TQT, Thorough QT clinical study; TdP, Torsade de Pointes

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[1] As of September 2017, Pluriomics officially announced the establishment of Ncardia following the merger of Pluriomics and Axiogenesis.

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et al., 2017; Peters, Scott, Ochalski, & Dragan, 2012; Scott et al., 2014). However, along with great potential come challenges related to fully characterizing and standardizing these hiPSC-CM-based models (Pugsley et al., 2017).

During the Pluriomics User Meeting, we have discussed which approaches can be used to assess drug safety on hiPSC-CMs, what the value could be of using hiPSC-CMs electrophysiology data for predicting cardiotoxicity in the clinic, and how we can further optimize hiPSC-CM-based *in vitro* assays for acute and chronic cardiotoxicity assessment. In line with CiPA, the focus during this meeting was on the use of hiPSC-CMs (Pluriomics' Pluricyte® Cardiomyocytes) with MEA technology. However, combining multiple functional (and structural) read-outs as described before would probably be the most optimal way to provide more insight into the complete potential cardiotoxicity profile of a test compound. The practical use of hiPSC-CMs in drug safety screening was also emphasized during live demonstrations on site.

The user meeting was attended by representatives from pharmaceutical and biotechnology companies, contract research organizations and academia. This publication incorporates the key challenges highlighted during the user meeting and identifies key areas where a concerted effort contributes to reducing cardiovascular safety liabilities of new medicines.

1.1. Pluricyte® cardiomyocytes as a predictive model for preclinical safety assessment – Dr. Maria LH Vlaming, Pluriomics B.V. (Leiden, The Netherlands)

Current safety models employ cell lines which overexpressing specific ion channels and animal models that may differ from clinical responses in terms of sensitivity, specificity and predictive value. Although the currently existing models appear sufficient to identify compounds that inhibit the hERG channel, other potential safety risks may be missed, for example due to differences in species, morphology, metabolism, or electrophysiology properties, all of which can confound the translation of *in vitro* cardiotoxicity findings to humans (Force & Kolaja, 2011). hiPSC-CMs addresses many of these concerns by providing a human model that is functionally active. Recent research supports that hiPSC-CMs are a valuable tool to study both structural (Pointon, Abi-gerges, Cross, & Sidaway, 2013; Talbert et al., 2015) and functional (Guo et al., 2013; Pointon et al., 2015; Talbert et al., 2015) drug-induced toxicities. To provide a relevant *in vitro* model for cardiotoxicity screening, Pluriomics has developed commercially available hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) and a serum-free medium (Pluricyte® Cardiomyocyte Medium) that is designed to improve the functionality and maturity of Pluricyte® Cardiomyocytes. Improved maturation of hiPSC-CMs was demonstrated by an increased contraction profile, as well as electrophysiological properties (negative resting membrane potential, well defined action potential plateau and rapid depolarization) and distinct protein and gene expression patterns (Ribeiro et al., 2015). In addition, gene expression profiling has shown that Pluricyte® Cardiomyocytes have gene expression patterns that are very similar to those of adult cardiomyocytes *(unpublished results)*.

The potential of Pluricyte® Cardiomyocytes for application in cardiac safety and toxicity testing was investigated in relevant safety screening assays, such as MEA technology, impedance technology and calcium transient assays. MEA technology can sensitively detect electrophysiological parameters, such as the field potential duration (FPD), which reflects cellular de- and repolarization and is suggested to provide an indication of the QT interval. The assessment of impedance provides information on monolayer integrity and is a surrogate measurement of cardiomyocyte contractility. Combined impedance and FPD recording devices, capture and give information on both contractility and MEA-like signals. Calcium transient measurements allow for the analysis of cardiotoxicity on cardiomyocyte electrophysiology and in particular calcium handling. Importantly, all these assays can also detect arrhythmia-like waveforms that may serve as a torsadogenic biomarker.

MEA analysis of Pluricyte® Cardiomyocytes showed reproducible field potential signals with pronounced de- and repolarization peaks in all MEA platforms used. Impedance measurements showed a profile that was in line with the calcium transient data of the cells. The pharmacological responses of Pluricyte® Cardiomyocytes to cardioactive reference compounds (*e.g.* dofetilide, nifedipine, diltiazem, Bay K8644 and isoproterenol), were as expected and were comparable between MEA analysis and calcium transient data. For example, for dofetilide, MEA analysis with Pluricyte® Cardiomyocytes showed increases in FPD from 3 nM and TdP-like arrhythmias could be observed from 30 to 100 nM. TdP-like arrhythmia was also observed from 10 to 100 nM in calcium transient data. These data show that Pluricyte® Cardiomyocytes provide a relevant model for testing cardioactive effects of drugs at various stages of drug development. The combination of hiPSC-CMs with various electrophysiology- and contractility-based assays, enables early cardiotoxicity screening with the potential to reduce the use of animal experiments in preclinical development to the most promising drug candidates having the highest likelihood of success. This will in the future contribute to more efficient, and therefore more cost- and time-effective decision making at early stages of drug discovery and development.

1.2. Pluricyte® Cardiomyocytes as a predictive model for preclinical safety assessment, a comparison with isolated guinea pig ventricular cardiomyocytes – Richard Printemps, Physiostim (Lautrec, France)

To maximize the potential of using hiPSC-CMs in drug development, it is essential that these cells recapitulate the electrophysiological properties of adult cardiomyocytes (Honda, Kiyokawa, Tabo, & Inoue, 2011). Using manual patch-clamp analysis (Hamill, Marty, Neher, Sakmann, & Sigworth, 1981), the electrophysiological properties of hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) were characterized. While the resting membrane potential of Pluricyte® Cardiomyocytes was close to adult cardiomyocytes (~ − 78 mV), for the current patch clamp experiments they were imposed to approximately − 80 to − 85 mV. The experiments were performed at room temperature and the cells were paced at 0.5 Hz.

Recordings showed that Pluricyte® Cardiomyocytes exhibit a typical ventricular action potential waveform, indicating a relatively high level of maturity (Lundy, Zhu, Regnier, & Laflamme, 2013; Peng, Lacerda, Kirsch, Brown, & Bruening-Wright, 2010). Five reference compounds were selected and systematically tested in Pluricyte® Cardiomyocytes for their effects on the action potential duration (APD) at 20% and 90% of repolarization (APD_{20} and APD_{90}). To investigate the potential of this *in vitro* model to detect compound effects on ventricular action potential, the findings were compared to results from a well-established model for cardiac safety studies, the freshly dissociated guinea pig cardiomyocytes (GP-CMs).

In Pluricyte® Cardiomyocytes, the selective hERG channel blockers E-4031 and dofetilide (both at a concentration of 0.1 μM) induced larger APD_{90} prolongations (+ 35% and + 30%, respectively) than APD_{20} prolongations (+ 10% and + 4% respectively), resulting in increased triangulation. Action potential recordings from GP-CMs also resulted in an increase of APD_{90} in the presence of E-4031 and dofetilide (+ 30% and + 21%, respectively, at a concentration of 0.1 μM), although to a slightly lower extent than in Pluricyte® Cardiomyocytes. For both Pluricyte® Cardiomyocytes and GP-CMs the response to the hERG channel blockers was less sensitive than expected based on literature (E4031 hERG IC50 = 7 nM (Harris et al., 2013), dofetilide hERG IC50 = 5 nM (Redfern et al., 2003)) and compared to studies described herein using Pluricyte® Cardiomyocytes in MEA and calcium transient assays. A potential explanation might be the differences in assay set-up. The current patch clamp assay is making use of single