Simultaneous Measurement of Contractility, Electrophysiology, and Troponin I Secretion to Determine Cardiotoxicity in hiPSC-Derived Cardiomyocytes

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Abstract

Many novel oncology therapeutics may induce cardiotoxicity by inhibiting survival pathways which are shared by both tumors and cardiac cells. Traditional methods to assess cardiotoxicity have relied upon in vitro overexpressing human cell lines or use in vivo animal models. These models often lack the complexity of human cardiomyocytes, whereas animal models may lack predictivity due to inherent species differences. Therefore, there is a need for more predictive and specific assays that allow for multiparametric assessment of potential cardiotoxic side effects of new drugs in humans. Using proprietary hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes), we developed a multiparametric assay to measure potential cardiotoxic drug effects in vitro. Here, both acute and long-term effects of antineoplastic drugs (nitrofen, lapatinib, doxorubicin, and ponatinib) on impedance, electrophysiology (MEA), and cardiac Troponin I (cTnl) are measured on different concentrations of cTnl (Fig. 1). cTnl release was performed at 30 min. until 64 hr. post-treatment. At the same timepoints, medium was harvested and used for the cTnl release assay. These data suggest that a multiplexed analysis is crucial to investigate short- and long-term cardiac liabilities as it provides a more comprehensive readout that generates mechanism-specific cardiotoxicity profiles, leading to better prediction of drug-induced cardiotoxicity.

Characteristics of Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium

Figure 1. Characteristics of Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium.
A: Pluricytes Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium (PCM) exhibit a high degree of ultra-structural sarcomere organization as determined by immunofluorescence (Green: alpha actinin; Red: myosin heavy chain 7). B: An overlay of the impedance (i.e. Cell Index, a surrogate marker for contractility) and field potential (i.e. ECR/MECa) signals enables simultaneous assessment of the excitation-contraction coupling and potential drug-induced functional toxicity. Waveforms are generated from a baseline measurement from one representative well. Data were obtained using the xCELLigence® RTCA CardioECR instrument (ACEA Biosciences).

Experimental approach: Multiplexing cTnl release assay with CardioECR assay (Impedance and MEA) to simultaneously assess potential structural and functional cardiotoxicity

Figure 2. Experimental approach for multiparametric analysis of potential functional and structural cardiotoxicity.
A: Cardiac Troponin I (cTnl) release in the bloodstream (left, schematic design) is a clinically relevant biomarker for the detection of myocardial damage in patients. cTnl release can also be detected in Pluricyte® Cardiomyocytes, enabling the generation of fitting dose response curves of compounds that induce structural cardiotoxicity (e.g. ponatinib, right, measurement was done at 24 hr post-addition). B: Here, we developed a multiparametric assay where our cTnl release immunoassay is multiplexed with impedance and MEA (multielectrode array) technologies. Pluricyte® Cardiomyocytes were thawed and directly seeded onto an E-Plate® at Day 0. On day 8 post-thaw compounds known to induce functional and/or structural cardiotoxicity were added at different concentrations. CardioECR measurements (Fig. 1B) were performed at 30 min. until 64 hr. post-treatment. At the same timepoints, medium was harvested and used for the cTnl release assay.

Structural toxicity: ponatinib and doxorubicin induced a dose-dependent increase in cTnl release and reduced CI values

Figure 3. Detection of potential structural cardiotoxicity by simultaneous assessment of Cell Index (CI) and cTnl release. Unlike lapatinib and nitrofen, ponatinib and doxorubicin caused a concentration- and time-dependent increase in cTnl release (right panels, represented as counts, dashed line indicates baseline cTnl levels), which correlated with reduced CI values (left panels). The CI can also be affected by changes in the morphology or contractility of the cardiomyocyte monolayer as seen after addition of nitrofen. Therefore, the CI could not be used as a direct measurement for structural cardiotoxicity.

Functional toxicity: nitrofen and lapatinib affect functionality of Pluricyte® Cardiomyocytes

Figure 4. Detection of potential functional cardiotoxicity using impedance and MEA technologies. A: The effects of 3 µM lapatinib, 3 µM nitrofen, 0.1 µM nitrendipine and 0.1 µM doxorubicin on the IBD10 (beat duration defined as the width at 10% of the CI amplitude), CI amplitude and beat rate of Pluricyte® Cardiomyocytes were quantified at 30 min., 24 hr. and 64 hr. post-addition. Data are shown as percentage change from baseline and are corrected for the negative control, 0.1% BSA. B: Lapatinib, Nitrofen, and Nitrendipine induced functional cardiotoxicity indicated by an increase in IBD10 (laptabinib), acute occurrence of arrhythmia (nitrofen) and acute decrease in CI amplitude and decrease in IBD10 (nitrendipine). As expected, doxorubicin affected the cardiomyocytes only at 24 hr. post-addition.

Concluding Remarks

- Cardiac Troponin I (cTnl) is a clinically relevant biomarker that can be used to capture potential structural toxic effects of compounds in Pluricyte® Cardiomyocytes, which cannot be detected with only MEA technology.
- Multiplexing impedance and MEA with cTnl release assay is possible and allows for a simultaneous assessment of short-term and long-term as well as structural and functional drug-induced cardiotoxicity from a single well.
- Our multiparametric analysis provides a better understanding of the wide range of drug-induced cardiotoxicities that can occur, which can further improve safety pharmacology decision-making at an early stage of drug development.

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