A physiological environment for human iPSC-derived cardiomyocytes facilitates maturation for reliable preclinical cardiac risk evaluation on high throughput level

Matthias Gossmann, Bettina Lickiss, Elena Dragicevic, Peter Linder, Ulrich Thomas, Sonja Stoelzel-Feix, Michael George and Niels Fertig

iNnoVitro, Jueltich, Germany and Nanion Technologies, Munich, Germany

Abstract
In preclinical drug development, cardiac contraction analysis of potential drug candidates is one of the crucial steps to ensure a successful and reliable transition to clinical stages. The use of human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) continues to increase in the assessment of safety and toxicological side effects of newly developed compounds, due to their reproducibility and low ethical concern. However, their preclinical physiotherapy remains an ongoing non-physiological response for predicting cardiac development. Acute testing within limited timelines (hr to min) after compound application remains the far safest way due to the inability of common cell-based assays to analyze cellular behavior reliably over prolonged periods of time.

The aim of this study was to evaluate the applicability of hiPSC-CM contractility measurements for acute safety and chronic toxicological assessment using the high-throughput FLEXcyte 96 system. Treatment of hiPSC-CM with positive inotropic compounds exhibited physiological responses when plated on FLEXcyte plates confirming the pro-maturation effect of the native-like environment given by the membranes. Additionally, 15 kinase inhibitors and 3 arrhythmias with well-known cardiotoxic profiles were selected to evaluate the reproducibility of clinical data.

Summary of the Result

Method
Human iPSC-CMs (CATi CamP, Fujifilm Cellular Dynamics) were cultured on FLEXcyte 96 well plates at 1:560 per well according to manufacturers’ guidelines in 20 μL maintenance medium. Cells were seeded 6 days before compound treatment to allow proper monolayer and network formation. A final media change was conducted 4-6 hours before drug application. For the experiments, 50 μL of the cell culture medium was removed and replaced with 50 μL medium containing 4 concentrated compound, resulting in the desired final concentration compound. Measurements were performed over a period of 5 days.

Figure 1. FLEXcyte 96 Technology

Figure 2. FLEXcyte 96 Workflow

Figure 3. Heatmaps of chronic cardiotoxic effects of ion channel inhibitors and arrhythmias. hiPSC-CMs analyzed after 1, 2, 3 and 5 h of compound treatment on the FLEXcyte 96. Shown are for each compound the pro-maturation effect (red) and cardiotoxicity (green) response in the treated condition. These are normalized to control (yellow) as well as decreasing reactions (red) up to crossing effects (deep red). Erlotinib, maraviroc, everolimus, simvastatin and temsirolimus all known compounds with few cardiothropic potential and served as negative control. Arrhythmias are highlighted in dark grey. Thio in grey and mTOR inhibitors in light grey.

Results
In total, 15 kinase inhibitors and 3 arrhythmias were analysed upon cardiotoxic side effects using human iPSC-CMs on the FLEXcyte 96. Adult-like positive inotropic compound reactions of hiPSC-CMs cultured on physiological FLEXcyte plates were shown isoperiodically, S-Bay 8644 and omeprazole marked.

Known cardiotoxic arrhythmias such as drosomycin and epigallocatechin showed expected toxic effects, ranging from the reduction in contractility at nanomolar concentrations to ceased beating at micromolar concentrations (deep red). Negative controls with known low cardiotoxic risk such as etoricoxib, maraviroc, everolimus and temsirolimus only showed toxic side effects at super-therapeutic, concentration dependent manner (Fig 3).

Isoproterenol increased the contraction amplitude in a dose-dependent manner to approx. 2:105% of control at 1 μM. At the same time, the durations of contraction and relaxation phases were shortened, indicating an increase in acceleration and deceleration of the contraction. In contrast, S-Bay 8644 elicited a symmetry change in the beat shape. While the contraction phase was unaffected, the increase in intracellular calcium concentration resulted in a prolongation of the relaxation phase. As a result, the total duration of the contraction-relaxation cycle was increased to 120% of control. The amplitude rose to 100% of control at 30 nM. Omeprazole mescarbil increased both the contraction amplitude as well as the duration of the contraction-relaxation cycle, indicating the positive inotropic effect based on activation of the myosin phosphorylation cycle. The symmetry of the contraction-release-cycle was not changed. The mature physiological responses of hiPSC-CMs are triggered by compounds acting at different target levels, underlying the pro-maturation effects of the FLEXcyte 96 which cannot be elicited with other assays commonly used for drug development purposes (Fig 4).

Conclusion
The displayed adult-like hiPSC-CM responses upon positive inotropic compound treatment underline the pro-maturation effect of the physiological environment created by the flexible membranes. The time and dose dependent cardiotoxic progression profiles of arrhythmias and TKIs assessed, indicate the suitability of the FLEXcyte technology for (sub)chronic safety and toxicity evaluation of new drug candidates.

The combination of human iPSC-CMs and the FLEXcyte technology allows for cardiac risk assessment using a predictive human cell model on a high-throughput format. The FLEXcyte technologies comprehensive goal on a larger scale is to advance translational studies for contract cardiotoxicity, replace/minimize animal use in drug development, and reduce risk of adverse cardiac side effects in clinical trials.

Acknowledgments
This research was supported by Johns Hopkins Center for Alternatives for Animal Testing