High Throughput Chronic Cardiotoxicity Evaluation of Human iPSC-Derived Cardiomyocytes in a Pro-Maturation Environment

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Abstract
In pre-clinical 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 gain importance in the assessment of cardiotoxic effects on cardiac contractility. However, acute testing within limited timescales (min to h) after compound application remains the primary application of hiPSC-CMs partly due to the inability of common cell-based assays to analyse cellular behaviour reliably over prolonged periods of time.

The aim of this study was to evaluate the applicability of hiPSC-CM contractility measurements for chronic toxicological assessment using the high-throughput FLEXcyte 96 system. We selected 15 kinase inhibitors and 3 anthracyclines with well-known cardiotoxic profiles to evaluate the reproducibility of clinical data.

Cells from commercial sources were cultured on hyperelastic silicone membranes. The resulting beat patterns were expressed using essential intrinsic parameters including amplitude, frequency, shapes of contractions and relaxation, area under curve and arrhythmic events. For the assessment of chronic compound effects, intrinsic properties of the cells were recorded daily for five days.

Figure 1: Flexcyte 96 technology

In the FLEXcyte 96-well plate (Fig.1B), the cells adhere as monolayers on flexible substrates. While being deflected by the weight of the culture medium, rhythmic contraction of the cardiomyocytes lifts the membranes in the 96-well upwards. These changes in deflection are quantified by means of capacitive distance sensing (Fig.1C).

The unique Mean Beat Function of the software automatically visualizes the CM average beat of traces from one well per experiment, enveloped by the standard deviation. Additional important parameters like contraction force (nN/m²), AUC, rising and falling times as well as beat duration are determined via the obtained mean beat while the beat rate is examined separately (Gossmann et al., 2018 & 2020).

Figure 2: Flexcyte 96 workflow

Method
Human iPSC-CMs (GoVit CM, Fujifilm Cellular Dynamics) were cultured on FLEXcyte 96 well plates at 10k per well according to manufacturers’ guidelines in 20% FCS/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 4x concentrated compound, resulting in the desired final compound concentration. Measurements were performed over a period of 5 days. (Fig.2)

Figure 4: Chronic cardiotoxic effects of kinase inhibitors and anthracyclines. Amplitude of hiPSC-CMs (GoVit-CM, FID) cultured on FLEXcyte 96 well plates after treatment with kinase inhibitors erlotinib (B), Vandetanib (C) and anthracycline idarubicin (D). Graphs show dose and time-dependent effects on hiPSC-CMs over the five days incubation period. Graphs represent mean ± SEM. Asterisks represent statistical significance with p < 0.05 (‘’) or p < 0.01 (‘’’’’). (Wilcoxon-Mann-Whitney test, n = 6). Figure 3: Heatmap of chronic cardiotoxic effects of kinase inhibitors and anthracyclines. hiPSC-CMs analysed after 1, 3, 5 and 7 days of compound treatment on the FLEXcyte 96. Shown parameters are amplitude, beat rate and beat duration. The heat map colours indicate increasing effects (green) of hiPSC-CMs, stable conditions (yellow) as well as decreasing reactions (red) up to causing effects (deep red). Erlotinib, imatinib, everolimus, temsirolimus and idarubicin show significant side toxic effects on super-therapeutic concentrations in a time-dependent manner (Fig 3).

Results
In total, 15 kinase inhibitors and 3 anthracyclines were analysed upon cardiotoxic side effects using human iPSC-CMs on the FLEXcyte 96.

Known cardiotoxic anthracyclines such as doxorubicin and epirubicin show 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 erlotinib, imatinib, everolimus, temsirolimus and idarubicin only showed toxic side effects at super-therapeutic concentrations in a time-dependent manner (Fig 3).

Conclusion
The displayed time and dose-dependent cardiotoxic progression profiles of anthracyclines and TKIs assessed with the FLEXcyte technology indicate the suitability of this technology for substractive safety and toxicity evaluation of new drug candidates.

The combination of human iPSC-CMs and the FLEXcyte 96 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 contractile cardiotoxicity, replace/minimize animal use in drug development, and reduced risk of adverse cardiac side effects in clinical trials.

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References

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