Chronic cardiotoxic effects of kinase inhibitors and anthracyclines on human iPSC-derived cardiomyocytes

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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) continue to increase in the assessment of toxicological 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 data.

Cells from commercial sources were cultured on hyperelastic silicone membranes. The resulting beat patterns were analysed for essential isotropic parameters including amplitude, frequency, slopes of contraction and relaxation, area under curve and arrhythmia events. For the assessment of chronic compound effects, isotropic properties of the cells were recorded daily for five days.

Method
Human iPSC-CMs (iCell CM® CMF, Fulfill Cellular Dynamics) were cultured on FLEXcyte 96 well plates at 100k per well according to manufacturers’ guidelines in 200 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 compound concentration. Measurements were performed over a period of 5 days (Fig. 2).

Figure 1. FLEXcyte 96 technology

Figure 2. FLEXcyte 96 Workflow

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 creating effects (deep red). Erlotinib, imatinib, everolimus, sirolimus and temozolomide are known compounds with low cardiotoxic potential and served as negative control. Anthracyclines are highlighted in dark grey, 10% in grey and mTOR inhibitors in light grey.

Figure 4. Chronic cardiotoxic effects of kinase inhibitors and anthracyclines. Amplitude of hiPSC-CMs (iCell CM® CMF) cultured on FLEXcyte 96 well plates after treatment with kinase inhibitors erlotinib (A), Vandetanib (B) and anthracycline idarubicin (C). 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).

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

Knocked 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, sirolimus and temozolomide only showed toxic side effects at super-therapeutic concentrations in a time-dependent manner (Fig. 3).

Erlotinib, generally regarded as non-cardiotoxic, had a minor dose and time-dependent effect on hiPSC-CMs only at concentrations in the micromolar range, probably based on general rather than cardiac-specific functional toxicity (Sharma et al., 2018) (Fig.4A). Vandetanib, with known cardiac safety issues (black box FDA cardiotoxicity warning), showed a dose-dependent effect on the contractility of hiPSC-CMs from 2 h of incubation, most probably due to its GT-proliferating properties (Le et al., 2018) (Fig.4B). The effect of idarubicin, an anthracycline chemotherapy agent, was both time and dose-dependent with progression profiles of different concentrations (Fig.4C).

Conclusion
The displayed time and dose-dependent cardiotoxic progression profiles of anthracyclines and 5Ts assessed with the FLEXcyte technology, indicate the suitability of this technology for (cardio)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 technology’s 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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