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

## Technology

the FLEXcyte technology is based on a special 96 well plate that contains high-precision, ultra-thin and hyperelastic silicone membranes instead of stiff plastic surfaces as basis for human iPSC-CMs. This FLEXcyte 96 plate is analysed in the FLEXcyte 96 device (Fig.1A), an add-on system for the CardioExcyte 96 (Nanion Technologies).

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 average beat of traces from one well per sweep, enveloped by the standard deviation. Additional parameters like amplitude, rising and falling times as well as beat duration are analysed via the obtained mean beat while the beat rate is examined separately (D) (Gossmann et al., 2016, Gossmann et al., 2020).

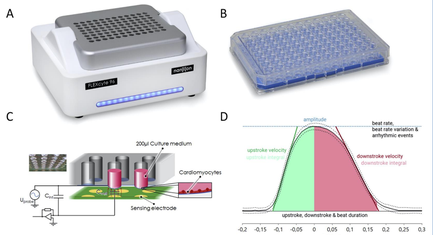


**Figure 1. Flexcyte 96 technology**

## Method

Human iPSC-CMs (iCell® CM², Fujifilm 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 4x concentrated compound, resulting in the desired final compound concentration. Measurements were performed over a period of 5 days. (Fig.2)

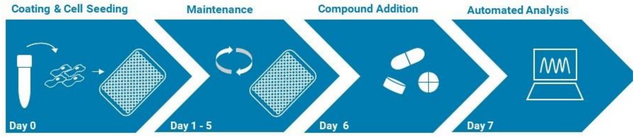


**Figure 2. FLEXcyte 96 Workflow**

## Summary of the Result

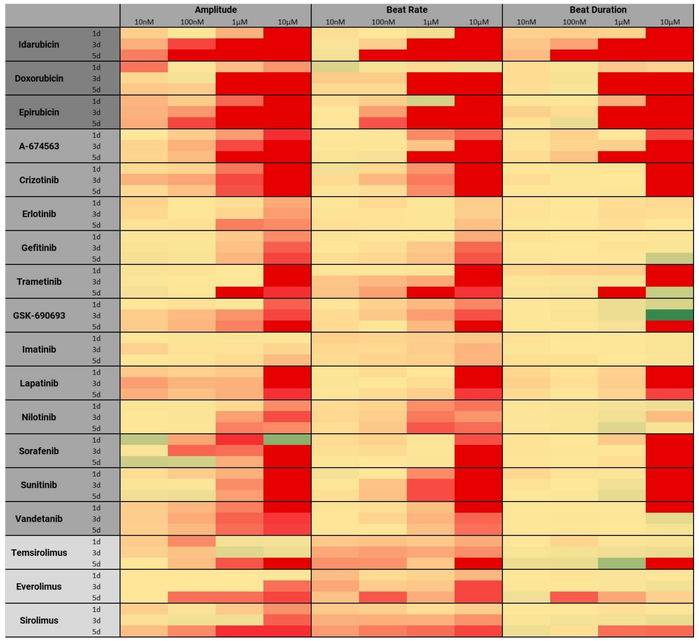


**Figure 3. Heatmap of chronic cardiotoxic effects of kinase inhibitors and anthracyclines**. hiPSC-CMs analysed after 1 d, 3 d and 5 d of compound treatment on the FLEXcyte 96. Shown parameters are amplitude, beat rate and beat duration. The heat map colours indicate increasing effects (green) of hiPSC-CMs, stable conditions (yellow) as well as decreasing reactions (red) up to ceasing effects (deep red). Erlotinib, imatinib, everolimus, sirolimus and temsirolimus are known compounds with low cardiotoxic potential and served as negative control. Anthracyclines are highlighted in dark grey, TKIs in grey and mTOR inhibitors in light grey.

## References

**Gossmann et al., 2016 Mechano-Pharmacological Characterization of Cardiomyocytes Derived from Human Induced Pluripotent Stem Cells**

**Gossmann et al., 2020 Integration of mechanical conditioning into a high throughput contractility assay for cardiac safety assessment**

**Sharma et al., 2017 High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells**

**Lee et al., 2018 Electrophysiological mechanisms of vandetanib-induced cardiotoxicity: Comparison of action potentials in rabbit Purkinje fibers and pluripotent stem cell-derived cardiomyocytes**

## Acknowledgments

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

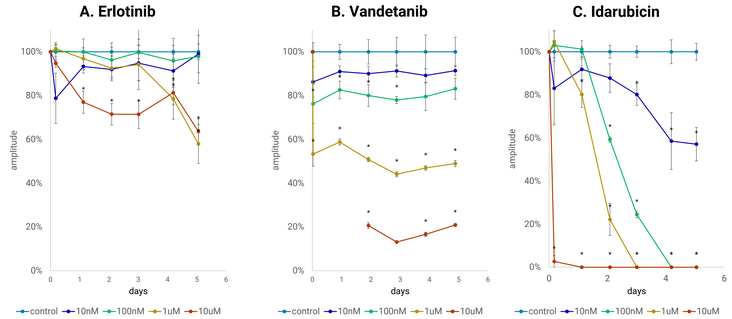


**Figure 4. Chronic cardiotoxic effects of kinase inhibitors and anthracyclines**. Amplitude of hiPSC-CMs (iCell® CM², FCDI) cultured on FLEXcyte 96 well plates after treatment with kinase inhibitors erlotinib (A), vandetanib (B) and anthracycline idarubicin (C). Graphs show dose and time-dependent effects on hiPSC-CMs over the five days incubation span. Graphs represent mean ± SEM. Asterisks represent statistical significance with $p < 0.05$ (*) or $p < 0.01$ (**) (Wilcoxon-Mann-Whitney test, n = 4).

## 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, sirolimus and temsirolimus 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., 2017) (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 QT-prolonging properties (Lee 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 TKIs assessed with the FLEXcyte technology, indicate the suitability of this technology for (sub)chronic 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.