Application Note

FLEXcyte 96

Chronic cardiotoxic effects of tyrosine kinase inhibitors and anthracyclines analyzed with the FLEXcyte 96 on human iPSC-derived cardiomyocytes

Summary

In cancer research, the intense development of targeted therapeutics such as tyrosine kinase inhibitors (TKIs) has brought tremendous improvement to the survival rate of cancer patients over the last two decades. The goal to reduce diverse toxic side effects of cancer treatment with targeted therapy has been widely achieved in comparison to traditional anti-cancer treatments like anthracyclines. Nevertheless, both therapeutics, TKIs and anthracyclines, still lead to adverse cardiotoxic side effects such as left ventricular dysfunction and heart failure. [1] [2]
The severity of these side effects depends on dosage and time span of treatment which brings chronic assessment of cardiotoxicity into focus.
However, acute testing (min to hours) of cell models with low predictive value remains the primary application so far, due to the inability of common cell-based assays to analyze cellular behavior reliably over prolonged periods of time.

Figure 1. FLEXcyte 96 device with FLEXcyte 96 plate

To ensure a safer transition of new drug candidates into clinical stages, a modern preclinical approach is of vital importance to evaluate chronic cardiotoxic side effects.

To meet this need, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are employed for acute as well as chronic evaluation of preclinical cardiotoxic risk using the FLEXcyte 96 system. This new technology analyzes cardiac contractility with special flexible membranes as substrate for the cells on a 96 well plate instead of commonly used stiff glass or plastic surfaces. This physiological *in vitro* environment has a proven pro-maturation effect on the cells leading to adult-like cardiac responses to known compounds. [3] [4] [5]

Here, we present the applicability of hiPSC-CM contractility measurements for chronic toxicological assessment using the high-throughput FLEXcyte 96 system. 15 kinase inhibitors and 3 anthracyclines with well-known cardiotoxic profiles were selected to evaluate the reproducibility of clinical data. The results showed the expected cardiotoxic effects, including negative inotropy and induction of proarrhythmic events and ceased beating in a dosage and timespan dependend manner and proving the advantage of the FLEXcyte system over commonly used cell-based assays for preclinical cardiac risk assessment.

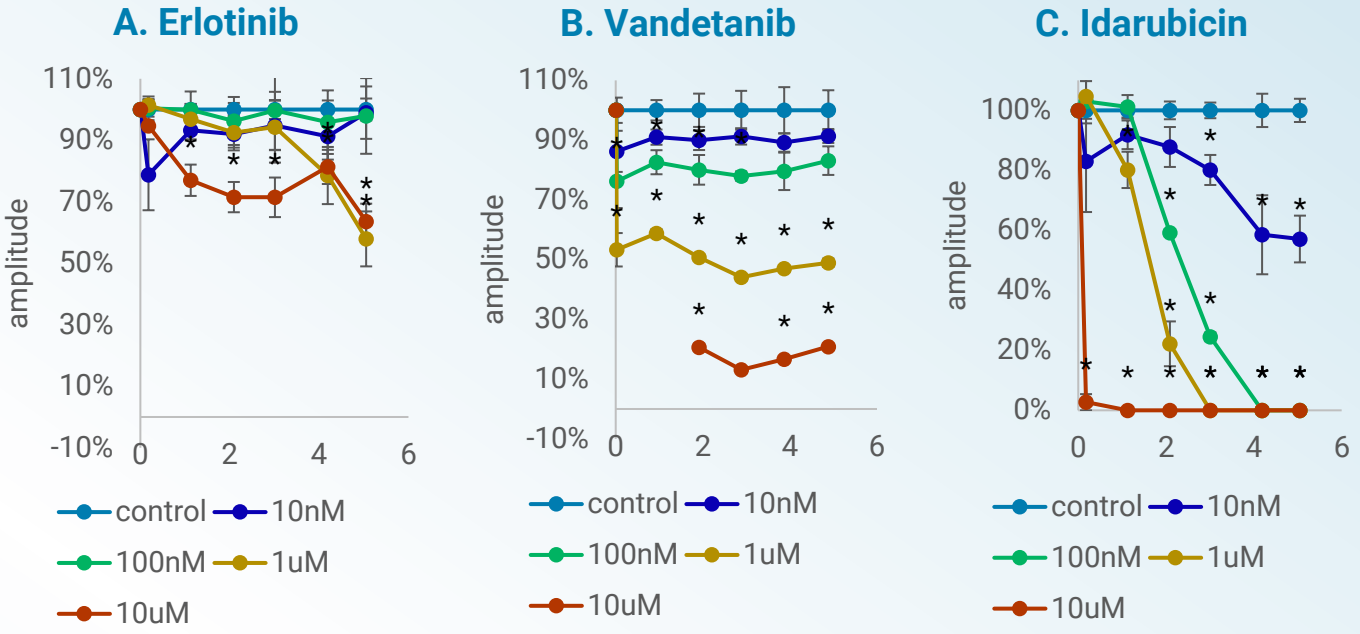

Figure 2. Chronic cardiotoxic effects of kinase inhibitors and anthracyclines. Amplitude of hiPSC-CM (iCell® CM², FCDI) cultured on FLEXcyte 96 well plates after treatment with kinase inhibitors erlotinib (A), vandetanib (B) and anthracycline idarubicin (C). Graphs show dose and time-dependent effects 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

Figure 2 shows the time and dose-dependent effects of kinase inhibitors erlotinib, vandetanib as well as athracycline idarubicin treatment on hiPSC-CMs cultured on FLEXcyte 96 well plates.

Cardiotoxic effects were evaluated in a timeframe of 5 days of incubation. For this purpose, hiPSC-CMs were incubated with concentrations ranging from 10 nM to 10 µM and beat parameters were monitored daily. Erlotinib, an EGFR (epidermal growth factor receptor) and tyrosine kinase inhibitor generally regarded as non-cardiotoxic, had a minor dose and time-dependent effect on hiPSC-CMs only at concentrations in the micromolar range (Fig. 2A). At 1 µM, a decrease in beat amplitude was observed from day 4 on, reaching 60 ± 9% of control on day 5 with a tendency to further decrease. At the highest concentration of 10 µM, a statistically significant decrease in amplitude to 70 ± 6% of control was observed from day 2 on. On day 5, the amplitude reached a value of 60 ± 6% of control, comparable to results at 1 µM (Fig. 2A).

Vandetanib, a VEGFR (vascular epidermal growth factor receptor), EGFR and RET (receptor tyrosine kinase) inhibitor had a dose-dependent but not time-dependent effect on hiPSC-CMs (Fig. 2B). At the highest concentration of 10 µM, cells ceased beating transiently between initial compound addition and day 2. Subsequently, the beat amplitude was constant at 20 ± 1% of control. At 1 µM, the amplitude was relatively constant at 50 ± 1% of control during the timeframe of observation. The two lowest doses of 100 nM and 10 nM resulted in a decrease of amplitude to 80 ± 5% and 90 ± 3% of control, respectively (Fig. 2B). The effect of idarubicin, an anthracycline chemotherapy agent, was both time and dose dependent (Fig. 2C). At the highest concentration of 10 µM, the cells ceased beating already after 2 h of incubation. At 1 µM and 100 nM, the beat amplitude decreased to arrest over a time period of 3 and 4 days, respectively. At the lowest concentration of 10 nM, the amplitude decreased to 60 ± 8% of control over the period of 5 days (Fig. 2C).

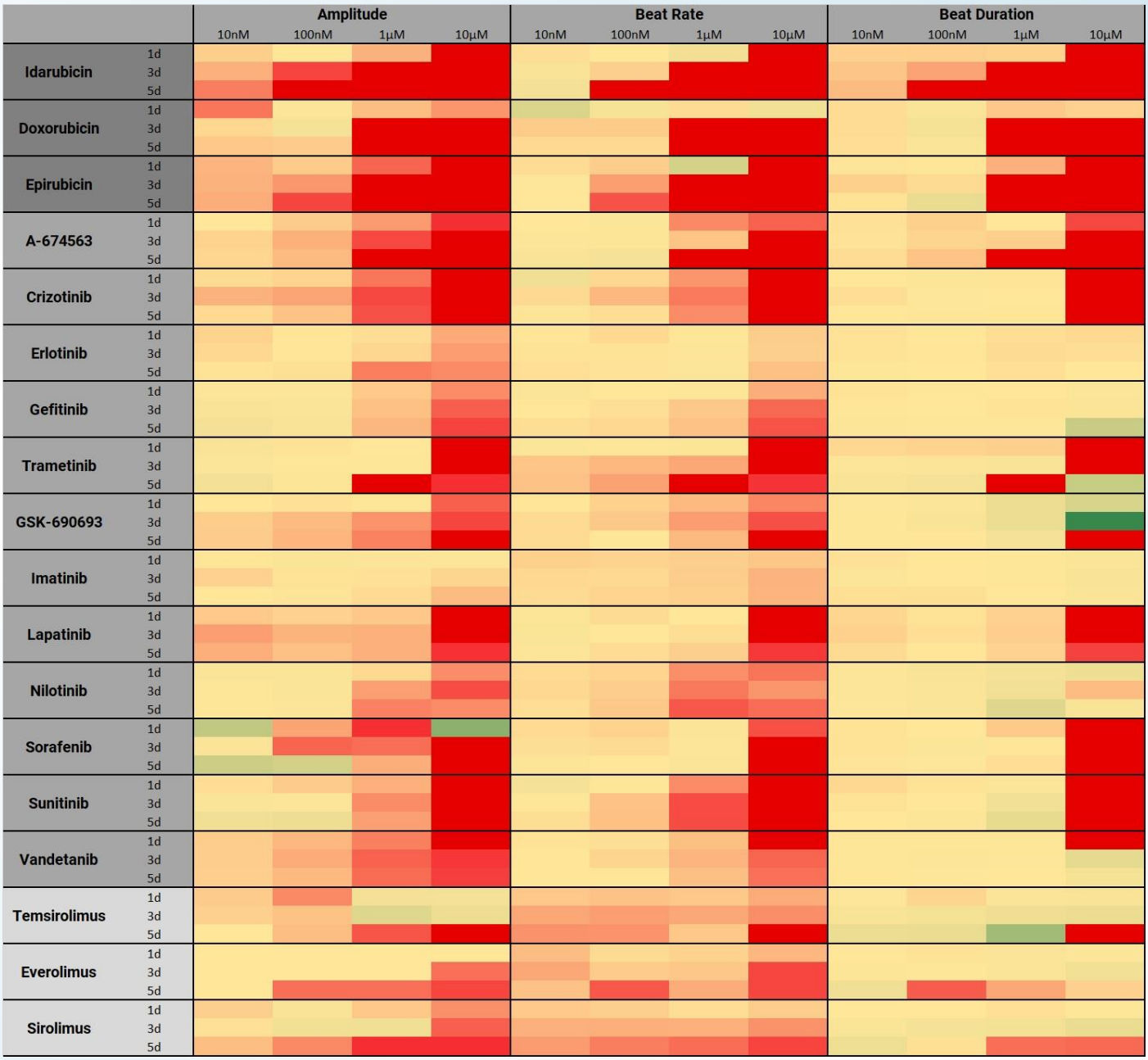

Figure 3. Heatmap of chronic cardiotoxic effects of kinase inhibitors and anthracyclines.hiPSC-CM analyzed after 1 d, 3 d and 5 d of compound treatment on the FLEXcyte 96. Shown parameters are amplitude, beat rate and beat duration. The heat map colours indicate increasing effects (green) of hiPSC-CMs, 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.

In total, 15 kinase inhibitors and 3 anthracyclines were analyzed upon cardiotoxic side effects using hiPSC-CMs on the FLEXcyte 96. Figure 3 shows a heatmap summarizing the results regarding effects on amplitude, beat rate and beat duration. Chosen time points are 1 d, 3 d and 5 d after administration of compounds ranging between 1 nM – 10 µM to reveal time and dose-depended effects. Known cardiotoxic anthracyclines such as Doxorubicine and Epirubicine show expected toxic effects even at lower concentrations after longer incubation times (deep red). Negative controls with known low cardiotoxic impact such as erlotinib, imatinib, everolimus, sirolimus and temsirolimus only show toxic side effects at high non-physiological concentrations in a time-dependend manner.

The FLEXcyte 96 device employing hiPSC-CMs results in a pre-clinical cardiotoxicity approach that unites cost effective high-throughput for acute and particular chronic analysis of cardiotoxic side effects.

Methods

Human iPSC-CMs were purchased from Fujifilm Cellular Dynamics International (Madison, WA, USA).

The cells were cultured on FLEXcyte 96 well plates according to manufacturers' guidelines in 200 µL maintenance medium per well. Cells were seeded 6 days before compound treatment at 100k (iCell® CM², FCDI) per well 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.

The CardioExcyte/ FLEXcyte Control software enables direct analysis of contractility parameters. An adaptive signal detection algorithm extracts the positions and values of beating events. Beat intervals, amplitudes, rising and falling time, pulse witdhs are detected as well as integrals and arrhythmia.

Acknowledgments

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References

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Notes

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