FLEXCYTE 96 APPLICATION NOTE

# Beating the Risk

## Human Cell-Based Profiling of TKIs and Anthracyclines for Cardiotoxicity Screenings

# Introduction – Cardiotoxicity Profiling Reinvented

Targeted cancer therapies like tyrosine kinase inhibitors (TKIs) have significantly improved survival rates over the past two decades. While they generally exhibit fewer side effects than traditional treatments such as anthracyclines, both drug classes can still cause serious cardiotoxic outcomes, including left ventricular dysfunction and heart failure. [1][2]

These adverse effects are often dose- and time-dependent, emphasizing the need for chronic cardiotoxicity assessment. However, most conventional human-based assays support only acute testing and lack the ability to monitor cellular function label-free over extended periods.

To address this, human iPSC-derived cardiomyocytes (hiPSC-CMs) are combined with the FLEXcyte 96 system, which analyzes contractility on mechanically compliant membranes in a 96-well format to mimic physiological conditions more closely than traditional rigid substrates. This environment supports maturation and elicits adult-like drug responses. [3][4][5]

Here, we tested a panel of 12 TKIs, 3 mTOR inhibitors and 3 anthracyclines with known cardiotoxic profiles confirming expected effects, including negative inotropy, proarrhythmic events, and loss of contractility, all in a dose- and time-dependent manner, highlighting this assay's value for predictive, long-term cardiac safety assessment.

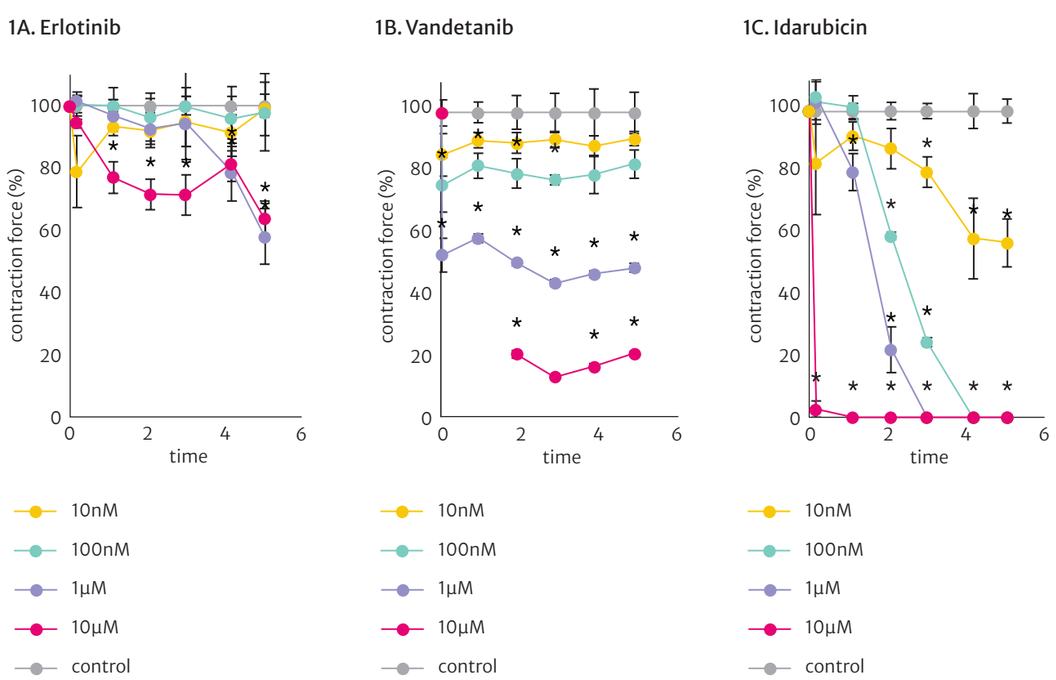


Figure 1. Contraction force of hiPSC-CMs (iCell® CM², FCDI) cultured on FLEXcyte 96 plates following treatment with erlotinib (A), vandetanib (B), and idarubicin (C). Graphs display dose- and time-dependent effects over a 5-day incubation. Data are shown as mean ± SEM; asterisks indicate statistical significance (*p < 0.05, **p < 0.01; Wilcoxon-Mann-Whitney test, n = 4).

# Methods

- iCell® CM², FCDI, were cultured on FLEXcyte 96 plates following manufacturers' protocols using 200 µL maintenance medium per well.
- Seeding was performed ~6 days prior to treatment at 60k cells per well to ensure monolayer and network formation. A final medium change was performed 4–6 hours before compound application.
- For compound testing, 50 µL of medium was removed and replaced with 50 µL of 4× concentrated compound solution to achieve the desired final concentration.
- Contractility was recorded using the CardioExcyte Control software. Parameters such as contraction force (mN/mm²), beat rate, beat duration and arrhythmic events were quantified.

# Acknowledgements

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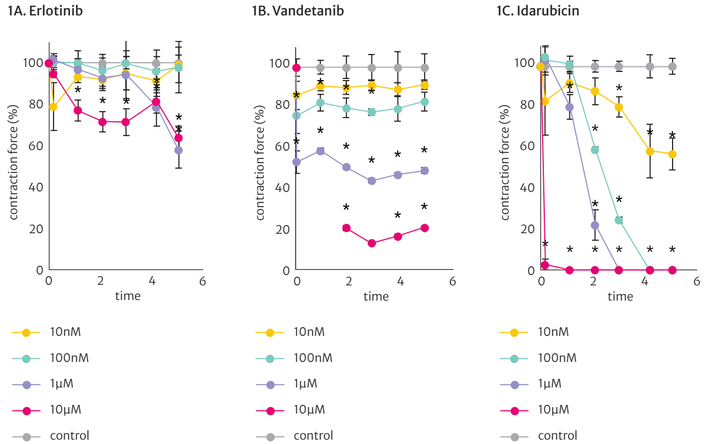


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# Results – Reflecting Clinical Human Response

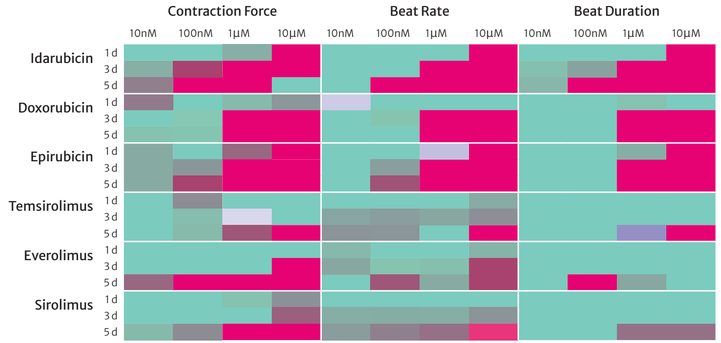


Figure 2. Heatmap of chronic cardiotoxic effects of anthracyclines and mTOR inhibitors. Parameters include contraction force, beat rate, and beat duration. Color coding indicates increasing effects (purple), stable responses (green), and decreasing to ceasing activity (magenta to deep magenta).

Time- and dose-dependent cardiotoxic effects of kinase inhibitors erlotinib, vandetanib, and the anthracycline idarubicin on hiPSC-CMs cultured on FLEXcyte 96 well plates are shown in figure 1. Cells were treated with concentrations ranging from 10 nM to 10 µM and monitored over 5 days.

**Erlotinib**
Typically considered non-cardiotoxic, showed only minor effects at micromolar doses. At 1 µM, contraction force declined from day 4 onward, reaching 60 ± 9% of control by day 5. At 10 µM, a significant decrease to 70 ± 6% was seen from day 2, dropping to 60 ± 6% by day 5 (Fig. 1A).

Vandetanib
Targeting VEGFR and EGFR, showed a clear dose-dependent response without strong time dependency. At 10 µM, cells stopped beating temporarily before stabilizing at 20 ± 1% of control. At 1 µM, contraction force remained around 50 ± 1%, with lower doses producing milder effects (Fig. 1B).

Idarubicin
Induced both dose- and time-dependent toxicity. At 10 µM, beating ceased within 2 hours. At 1 µM and 100 nM, contraction force progressively declined to arrest over 3–4 days. Even at 10 nM, contraction force dropped to 60 ± 8% over 5 days (Fig. 1C).

Figure 2 and Figure 3 illustrate a heatmap of anthracyclines, mTOR and kinase inhibitors evaluated for chronic functional cardiotoxic effects using hiPSC-CMs on the FLEXcyte 96. Changes in contraction force, beat rate, and beat duration at 1, 3, and 5 days after compound exposure (1 nM–10 µM), highlight time- and dose-dependent responses.

+ Known cardiotoxins like doxorubicin and epirubicin showed pronounced toxic effects even at low concentrations over time.
+ Negative controls of the mTOR and kinase inhibitor families such as erlotinib, imatinib, everolimus, sirolimus, and temsirolimus exhibited minimal effects, limited to high, non-physiological doses.

The FLEXcyte 96 platform offers a scalable, human-relevant solution for preclinical cardiotoxicity testing, enabling label-free monitoring of contractile function in hiPSC-derived cardiomyocytes. In light of the FDA's regulatory shift away from mandatory animal studies, this technology presents a future-ready approach that combines physiological relevance with industrial scalability, positioning it as a valuable tool for next-generation cardiac risk assessment.


Figure 3. Heatmap of chronic cardiotoxic effects of kinase inhibitors. Parameters include contraction force, beat rate, and beat duration. Color coding indicates increasing effects (purple), stable responses (green), and decreasing to ceasing activity (magenta to deep magenta).

# Contact us

**innoVitro GmbH**
Artilleriestraße 2
Jülich, Germany

+49 (0)2461 317 05 61
info@innovitro.de

**innovitro.de**

JOHNS HOPKINS UNIVERSITY
Center for Alternatives to Animal Testing

FUJIFILM Cellular Dynamics