Multiparametric assessment of the effects of tyrosine kinase inhibitors (TKIs) on the (electro)physiology of human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes

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Abstract

Introduction

Treatment with TKIs has improved the management of various types of cancer. However, TKI therapy is often associated with (severe) cardiotoxicity. To test a broader spectrum of potentially cardiotoxic effects of TKIs, we developed a multiparametric assay platform in which various mechanisms of drug-induced cardiotoxicity can be assessed in hiPSC-derived cardiomyocytes (Pluricyte® Cardiomyocytes).

Method

Using this platform, the effects of a set of TKIs (e.g. lapatinib, imatinib, nilotinib) on electrophysiology, contractility and viability of Pluricyte® Cardiomyocytes (day 9 post-thaw) were analyzed using multielectrode array (MEA), impedance, calcium transient and ATP assays. In addition, a PamChip® assay was performed to profile kinase activities in Pluricyte® Cardiomyocytes in presence and absence of TKIs.

Results

Multiparametric analyses revealed different types of pharmacological responses of Pluricyte® Cardiomyocytes and enabled the assessment of the multiple TKI-induced mechanisms that could cause cardiotoxicity.

Conclusion

We conclude that our approach, combining Pluricyte® Cardiomyocytes with various (electro)physiology-based assays, is very useful to generate unique cardiotoxicity profiles of TKIs and other cardiotoxic compounds at an early stage of drug development. Furthermore, application of this platform may greatly reduce the use of animal experiments in preclinical development.

Effects of TKIs on viability of Pluricyte® Cardiomyocytes

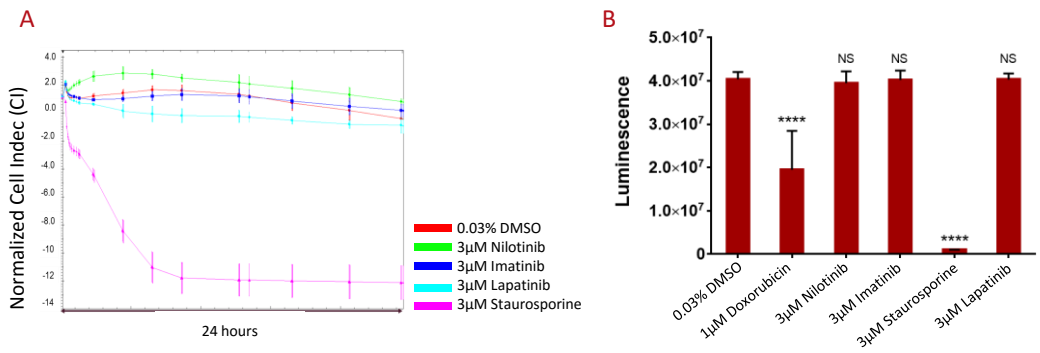

Figure 1.
A: Cell Index (i.e. impedance) measurements can be used as a surrogate to identify compound effects on the viability of Pluricyte® Cardiomyocytes. Staurosporine is identified as a toxic compound, as expected. Other TKIs did not affect cell viability when compared to 0.03% DMSO. Data were generated using the xCELLigence® RTCA CardioECR system, N=4 individual wells per compound. **B:** Effect of TKIs on viability of Pluricyte® Cardiomyocytes was confirmed by a CellTiter-Glo® ATP cell viability assay (Promega). Doxorubicin was used as positive control for cytotoxicity. Incubation time: 24 hr. N=4 individual wells per compound. P value **** ≤ 0.0001.

Inhibition of different kinases by TKIs in Pluricyte® Cardiomyocyte cell lysates

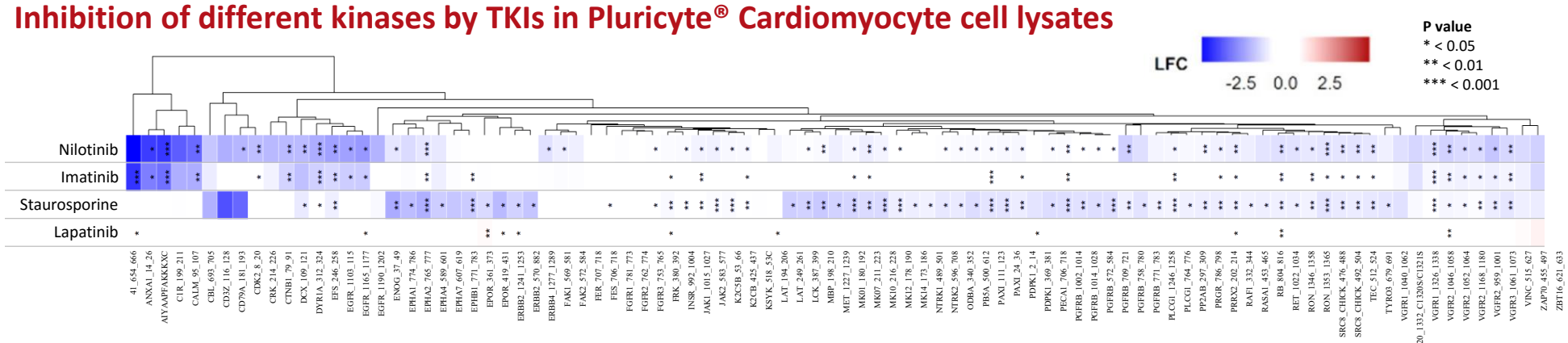

Figure 2. Heatmap of tyrosine kinase inhibition in Pluricyte® Cardiomyocyte lysate. Pluricyte® Cardiomyocytes were cultured for 8 days and subsequently lysed. The lysate was incubated with 3µM nilotinib, imatinib, staurosporine or lapatinib. Kinase activity was profiled using the **PamChip® assay**, which is based on measuring peptide phosphorylation by protein kinases using fluorescently labelled anti-phospho antibodies. Blue color represents reduced kinase activity compared to control. Nilotinib and imatinib generated similar inhibition profiles as expected from literature [Mellor, H.R. *et al*, Toxicological Sciences 2011]. Lapatinib appeared less potent and more specific compared to the other TKIs. Staurosporine, a multi-targeted TKI inhibits a broad range of phosphorylation activities (known to be non-selective). This profiling of kinases provides information about kinase activity in Pluricyte® Cardiomyocytes as well as the specificity and potency of kinase inhibition in cardiomyocytes by TKIs.

Effect of TKIs on calcium transients in Pluricyte® Cardiomyocytes

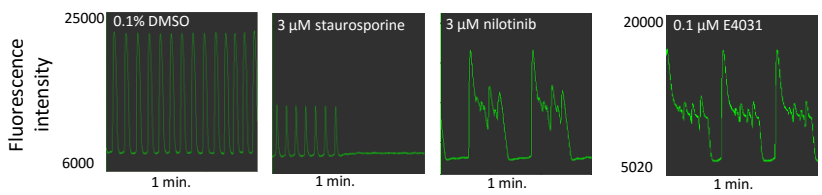

Figure 3. Fluorescent calcium transients in Pluricyte® Cardiomyocytes upon TKI treatment assessed using the FDSS®/µCELL system. **3µM staurosporine** induced acute toxicity, leading to very acute diminishing of the calcium transients. Although **3µM nilotinib** did not affect cell viability (shown in **Fig. 1**), it induced arrhythmic events in calcium transients in Pluricyte® Cardiomyocytes similar to the well-known hERG-channel blocker, E4031. It is known that Nilotinib blocks the hERG-channel with an IC50 of 0.7µM [Xu, Z. *et al*, Hematology Reviews 2009], which might explain the arrhythmic events. **3µM imatinib** (hERG IC50 of 20µM, Guo, L. *et al*, Toxicological Sciences 2013) and **3µM lapatinib** did not affect calcium transients within 30 minutes after compound addition compared to DMSO control (data not shown). Measurements were performed directly after compound addition.

Long-term effect of lapatinib on the impedance peak width of Pluricyte® Cardiomyocytes

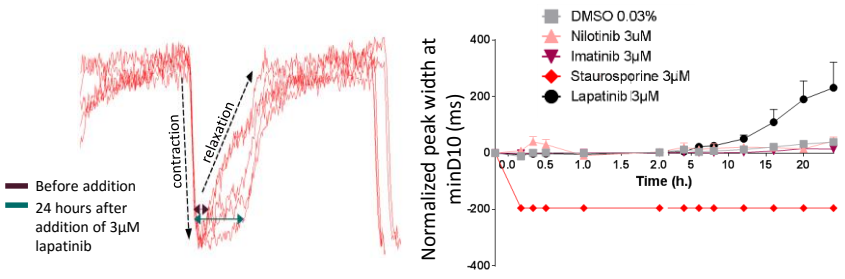

Figure 5. Overlay of Cell Index (i.e. impedance, surrogate for cell contraction) waveforms showing an increase in peak width in Pluricyte® Cardiomyocytes after long-term incubation (12-24h) with 3µM lapatinib. The contraction-relaxation cycle of Pluricyte® Cardiomyocytes gives rise to a distinct, rhythmic fluctuation in impedance. Lapatinib-induced increase in peak width suggests an effect on the relaxation time of the cardiomyocytes. No other effects of lapatinib on the impedance of Pluricyte® Cardiomyocytes were observed (see also **Fig. 4**).

Acknowledgments

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Effect of TKIs on electrophysiology of Pluricyte® Cardiomyocytes

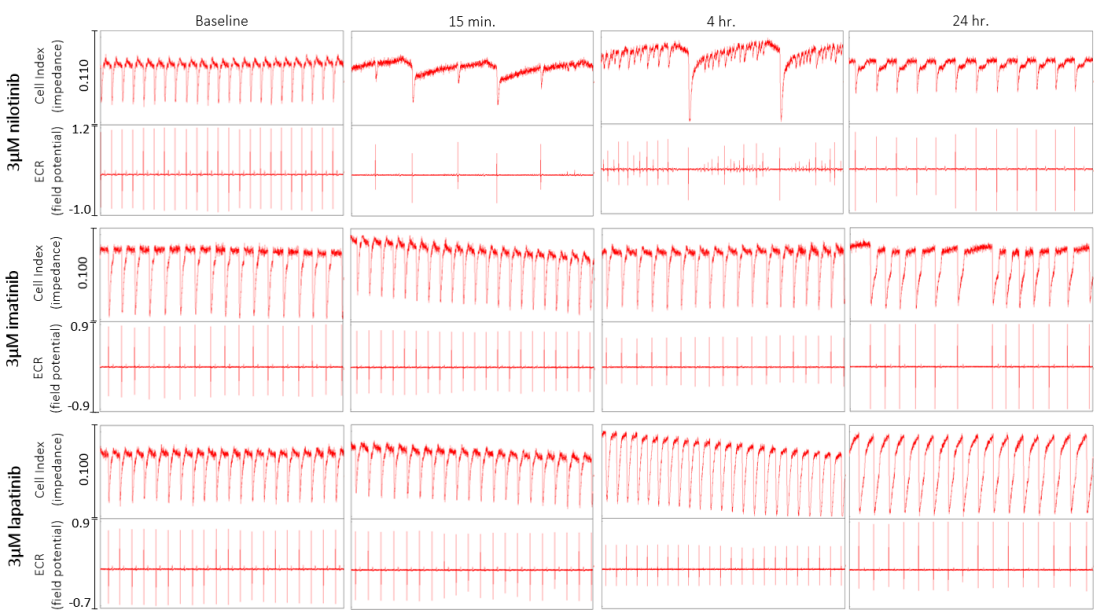

Figure 4. Simultaneous measurement of ECR (i.e. field potential) and Cell Index (i.e. impedance, surrogate for cell contraction) of Pluricyte® Cardiomyocytes using the xCELLigence® RTCA CardioECR system. Baseline recordings show Pluricyte® Cardiomyocyte field potentials with robust and well-pronounced de- and repolarization peaks. Addition of **3µM nilotinib** caused acute and long-term (until t=16 hr., not shown) arrhythmias in Pluricyte® Cardiomyocytes (similar effects can be observed in **Fig. 3**). The cells appeared to recover after 24 hours. **3µM imatinib** did not induce arrhythmic-like events. However, it did induce irregular beating patterns after 24 hours of incubation. 24 hours of incubation with **3µM lapatinib** induced an increase in impedance amplitude and an increase in impedance peak width, suggesting an effect on the relaxation time of the cardiomyocytes, as depicted in **Fig. 5** in more detail. **3µM staurosporine** dramatically affected cell viability already very early after compound addition, which can be clearly observed from **Fig. 1**. No traces of the field potential nor the impedance could be detected after 15 minutes of incubation (data not shown).

Concluding Remarks

- **Nilotinib** and **imatinib** generated similar kinase inhibition profiles, as expected. They both did not affect cell viability, which was confirmed by both impedance as well as ATP viability assays. However, in contrast to imatinib, nilotinib induced arrhythmias, as detected in MEA and calcium transient assays. This is likely due to additional hERG-channel inhibition.
- **Lapatinib** appeared less potent and more specific in inhibiting kinases compared to the other TKIs. Furthermore, it affected the impedance peak width after long-term incubation, suggesting an effect on the relaxation time of the cardiomyocytes, which could be related to *in vivo* decrease in left ventricular ejection fraction [Mellor, H.R. *et al*, Toxicological Sciences 2011].
- **Staurosporine**, showed a non-specific kinase inhibition profile and induced acute toxicity in Pluricyte® Cardiomyocytes, as expected.
- In conclusion, Pluricyte® Cardiomyocytes provide a highly relevant *in vitro* model to study cardiac safety and toxicity at various stages of drug development. The integration of a multiparameter approach, as described here for the assessment of cardiotoxicity, could provide a more complete and detailed cardiotoxicity profile of novel (anti-cancer) compounds.