Human iPSC-derived cardiomyocytes: A translational model to predict drug-induced cardiac arrhythmias and long-term toxicity

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BACKGROUND

Human induced pluripotent stem cell (iPSC)-derived cardiomyocytes represent a promising model for *in vitro* prediction of cardiac arrhythmias. Currently, two commercially available hiPSC-derived cardioymocyte products - including Axiogenesis Cor.4U human cardiomyocytes - are validated in an international multi-coresite study inside the Comprehensive in vitro Pro-arrhythmia (CiPA) consortium. This aims to change the paradigm of safety pharmacological assessment from the assessment of drug interaction with the hERG channel to a potentially clinically more relevant assay system using human iPSC-derived cardiomyocytes in MEA-, voltage- and calcium-sensitive dye recordings.

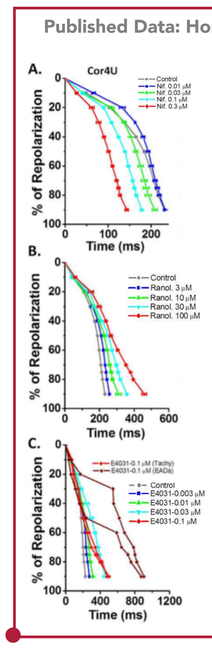
Besides CiPA, a variety of oncological drugs including tyrosine kinase and HDAC inhibitors have been reported to induce long-term cardiac toxicity in the clinic. Recent findings clearly show that Cor.4U cardioymocytes can be applied to a long-term impedance assay and the results from known drugs perfectly translate to the clinical observations.

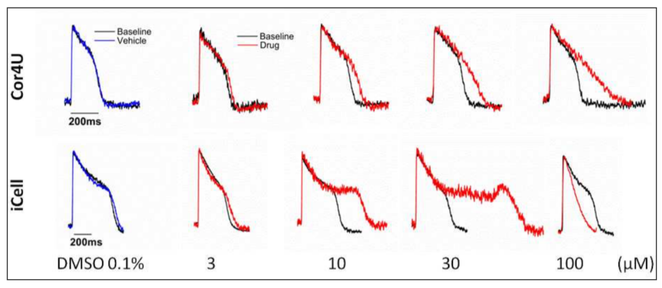
The results shown here imply that iPSC-derived Cor.4U human cardioymocytes are a translational *in vitro* cell model for the prediction of clinically relevant drug-induced cardiac arrhythmias and long-term toxicity.

RESULTS

RECENT PUBLICATIONS: ACUTE ION CHANNEL PHARMACOLOGY AND ARRHYTHMIA ASSESSMENT

Published Data: Hortigon-Vinagre *et al* (2016) *Toxicol Sci.* 154(2):320-331





Left: Repolarization profiles (nifedipine, ranolazine, and E4031). Average rate corrected repolarization profiles for Cor.4U Cardiomyocytes in response to increasing concentrations of (A) nifedipine (B) ranolazine and (C) E4031. (mean±SD, n=4)

Above: Effect of ranolazine on action potentials. Example recordings from Cor.4U or iCell (CDI) cardiomyocytes exposed to ranolazine.

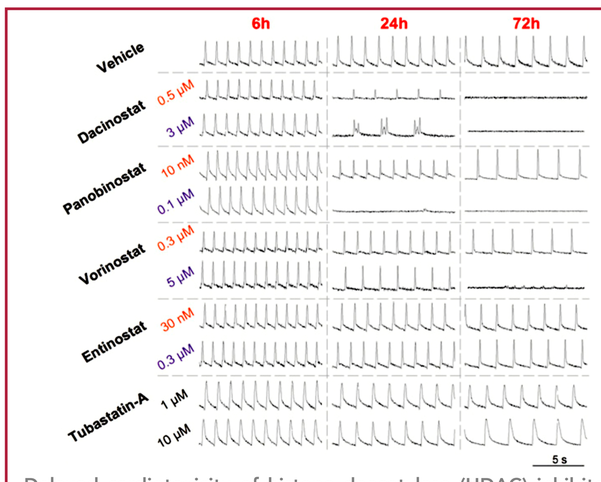
Table (right). Twenty-five drugs studied in acute iPSC-CM experiments are divided into four categories based on the presence of QTc prolongation and TdP reports on FDA-approved labels. Symbols: ↑: drug induced significant (p-value<0.05) change in APDc or FPDc for at least one of the doses. Red shading = drugs induced FPDc or APDc prolongation (↑); green shading = drugs that shortened FPDc or APDc (↓) or had no effect; Cross symbol (×) = drug induced arrhythmias in at least 30% of the replicate wells.

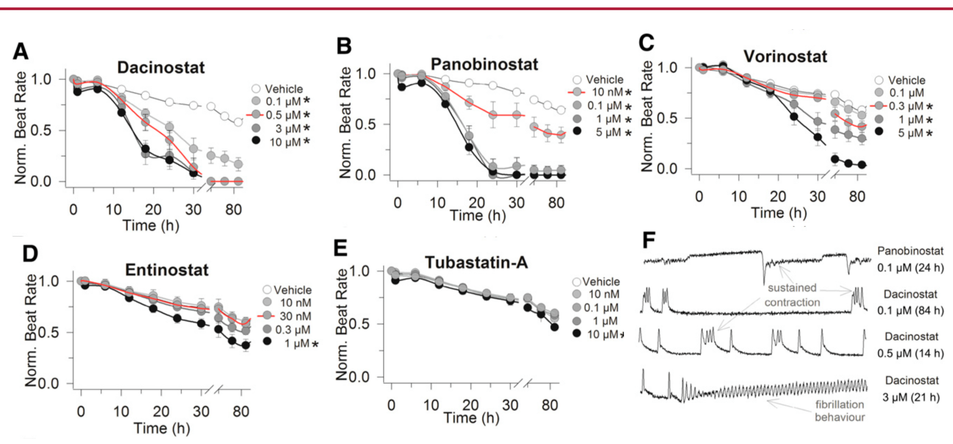
Blinova *et al.* (2016) *Toxicol Sci.* Oct 3 Pre-print

Drug	Repolarization effect in iPS cells: ΔΔFPDc Cor.4U	ΔΔFPDc iCell	ΔΔAPDc Cor.4U	ΔΔAPDc iCell	Arrhythmias induced in iPS: MEA Cor.4 U	MEA iCell	VSD Cor.4U	VSD iCell
QT ↑and TdP on the FDA label								
Quinidine	↑	↑	↑	↑	×	×	-	×
Dofetilide	↑	↑	↑	↑	×	×	×	×
Quinine	↑	↑	↑	↑	-	×	×	×
Propafenone	↑	↑	↑	↑	-	×	-	×
Moxifloxacin	↑	↑	↑	↑	×	×	×	×
Chloroquine	↑	↑	↑	↑	×	×	×	×
Bepridil	↑	-	↑	↑	-	-	-	-
Chlorpromazine	-	-	↑	-	-	-	-	-
Terfenadine	↑	-	↑	↑	-	-	-	-
Cisapride	↑	↑	↑	-	×	-	-	×
Amiodarone	↑	-	-	-	-	-	-	-
Azithromycin	-	↓	-	↓	-	-	-	-
QT ↑, but no TdP on the FDA label								
Ranolazine	↑	↑	↑	↑	-	×	-	-
Ritonavir	↑	-	↑	-	-	-	-	-
Amitriptyline	-	-	-	-	-	-	-	-
Nilotinib	↑	↑	↑	↑	×	×	-	×
Toremifene	-	-	-	↑	-	-	-	-
No QT ↑, nor TdP on the FDA label								
Lidocaine	-	-	-	-	-	-	-	-
Mexiletine	-	-	-	-	-	-	-	-
Mibefradil	-	↓	-	↓	-	-	-	-
Diltiazem	↓	↓	↓	↓	-	-	-	-
Verapamil	↓	↓	↓	↓	-	-	-	-
Licarbazepine	-	-	-	-	-	-	-	-
Not FDA-cleared*								
Cibenzoline	↑	-	↑	↑	-	×	-	×
Sertindole	↑	-	↑	-	-	-	×	-

* cibenzoline (QTc ↑), sertindole (QTc ↑, TdP)(Redfern *et al.* 2003)

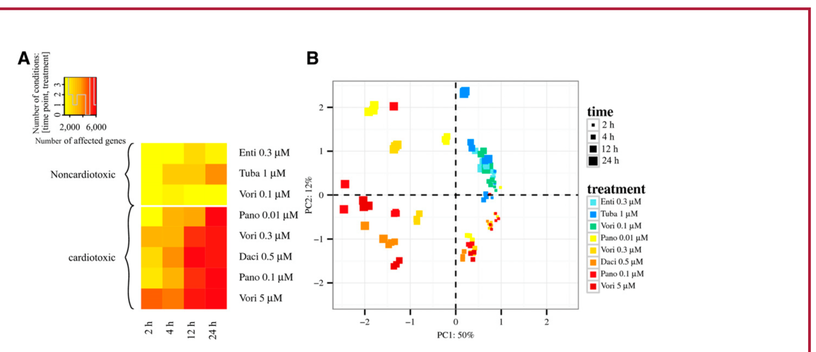
FUNCTIONAL AND TRANSCRIPTIONAL CHARACTERIZATION OF HISTONE DEACETYLASE INHIBITOR-MEDIATED CARDIAC ADVERSE EFFECTS IN COR.4U CARDIOMYOCYTES





Delayed cardiotoxicity of histone deacetylase (HDAC) inhibitors in Cor.4U. Impedance recordings are shown for HDAC inhibitors at 6, 24, and 72 hours after dose compared with vehicles. The different time points represent the same experiment (well) per treatment, where the free peak plasma concentration (C_{eff}) and approximately 10 x Ceff are indicated by red and blue labels, respectively. For tubastatin-a, the C_{eff} is not available because it is a preclinical drug.

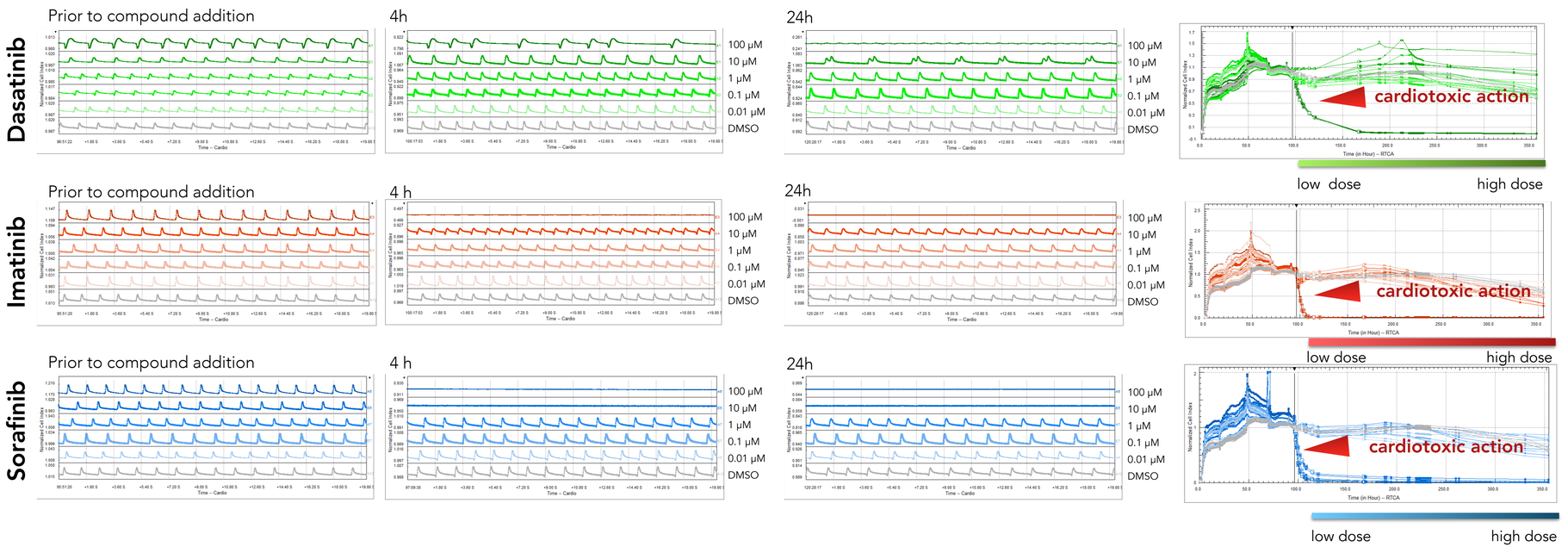
Histone deacetylase (HDAC) inhibitors decrease the beat rate and cause arrhythmic events in Cor.4U (A–E) Concentration-dependent effect of HDAC inhibitors on the normalized beat rate is shown as a function of time. A concentration range based on the effective concentration (C_{eff}) was compared with the vehicle treatment. Note that the red line represents the C_{eff}. Data are presented as mean 6 SEM (n = 7–11). *, p < .05 (differences between treatments and vehicles). **(F)** Examples of arrhythmic-like events representing prolonged/sustained contractions and fibrillation-like pattern on hiPS-CMs. Abbreviation: Norm., normalized.



Overall transcriptional effects of histone deacetylase inhibitors on human induced pluripotent stem cell-derived cardiomyocytes. (A) Heat map representing the number of significantly differentially expressed genes for each selected treatment and time point (limma adjusted p < .05), illustrating the overall expression changes over time. The figure key is shown as an inset. **(B)** Sample similarity based on the log ratios (treatment versus vehicle) for all measured genes illustrating the overall effect of the different treatments and time points. Each treatment is represented by at least three biological replicates. Abbreviations: Daci, dacinostat; Enti, entinostat; Pano, panobinostat; PC, principal component; Tuba, tubastatin-a; Vori, vorinostat.

* Data originally published: Kopljar *et al. Stem Cells Transl Med.* 2016 May;5(5):602-12

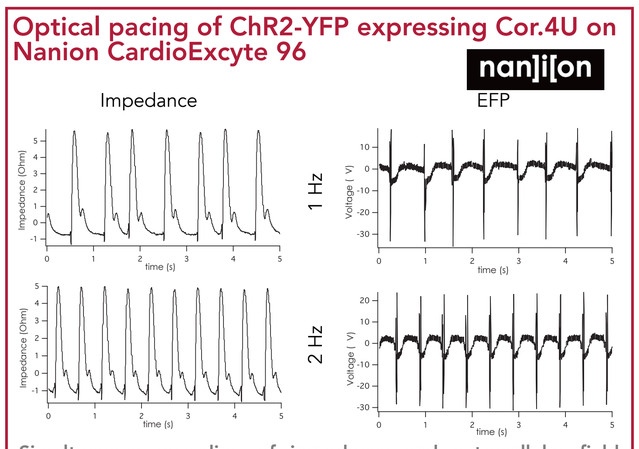
CHRONIC CARDIAC TOXICITY ASSOCIATED WITH TKIs - ASSESSMENT WITH CARDIOMYOCYTE IMPEDANCE ASSAY



Dose-dependent cardiotoxic effect of tyrosine kinase inhibitors on Cor.4U TKI oncological drugs dasatinib, imatinib and sorafinib decrease Cor.4U cell index, a parameter for cardiotoxicity and acute as well as chronic beating irregularities. Cor.4U were plated in E-Plates specialized for impedance measurements using the ACEA xCELLigence RTCA Cardio.

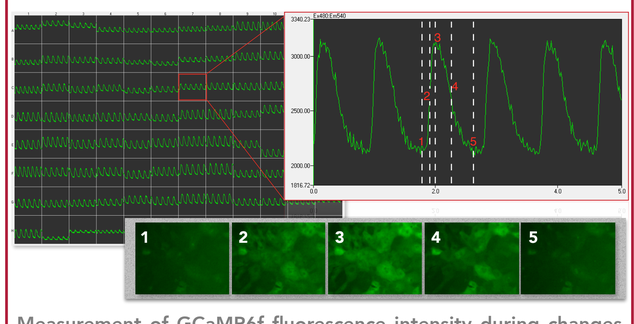
NEW TOOLS TO ACCELERATE DRUG DISCOVERY

Optical pacing of ChR2-YFP expressing Cor.4U on Nanion CardioExcyte 96



Simultaneous recording of impedance and extracellular field potential (EFP) during optical stimulation. Cells were stimulated on CardioExcyte NSP96 plates applying 2 ms blue light pulses. Optical stimulation allows for overlay of the signal giving in-depth mechanistic understanding of the contractility and electrophysiology of iPSC-derived cardiomyocytes.

Recording of calcium transients in GCaMP6f expressing Cor.4U on Hamamatsu FDSS 7000EX



Measurement of GCaMP6f fluorescence intensity during changes in intracellular calcium concentrations in Cor.4U cells. Left: Simultaneous acquisition of fluorescence intensity in all 96 wells. Nearly all wells showed low background fluorescence and high signal to noise. The duration of calcium transients varied between 550 - 600 ms with beat rates comparable to untreated cells (70 bpm). Right: Magnified fluorescence intensity profile. Images 1 - 5 demonstrate high transfection efficiency of GCaMP6f and fluorescence signal during cell contraction.

CONCLUSIONS

- Human iPSC-derived Cor.4U cardiomyocytes can be employed in a number of higher throughput phenotypic screening assays (voltage dyes, microelectrode arrays, impedance) to identify acute ion channel/arrhythmia concerns, chronic cardiotox effects from a number of mechanisms (HDAC, TKI, structural concerns, etc).
- Cor.4U combined with the impedance system allow for long-term as well as label-free cardiotoxicity assays on a medium throughput level.
- HDAC inhibitors can be triaged / prioritized according to their *in vitro* cardiotoxicity profiles as the phenotypic impedance assay/beating characteristics and transcriptional / genetic changes exhibit responses consistent with clinical (cardiotox) observations.
- Overall, human iPSC-derived cardiomyocytes present an ideal tool for acute as well as chronic assessment of drug-induced cardiotoxicity while new optogenetics tools and integrated sensors will usher in a new era of high throughput cardiotoxicity assessment.

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