Qualification of *in-vitro* Cardiac Cell Models for Preclinical Assessment of Oncology Drug-induced Cardiotoxicity

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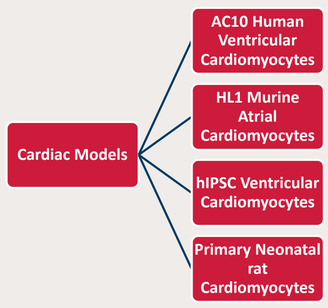
INTRODUCTION

Cardiotoxicity is a major complication of many anticancer therapies that impacts the quality of life and overall survival of patients. Preclinical models with improved ability to predict structural and functional cardiac liabilities are required to identify toxicological mechanisms, reduce clinical cardiotoxicity potential and identify therapeutic strategies to mitigate these life-threatening effects.

STUDY AIMS:

- Using impedance based real-time cell analysis (xCELLigence RTCA), evaluate the ability of different cardiac cell models to detect structural and functional drug-induced cardiotoxicity

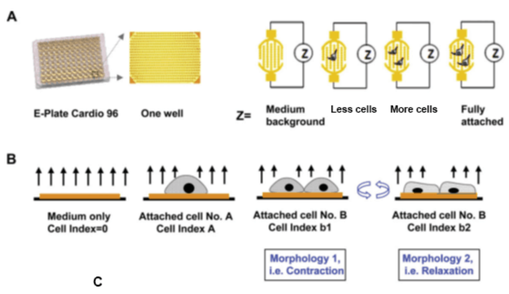


Detection of cardiotoxicity in response to Histone deacetylase inhibitor class of drugs

- Several HDAC inhibitors have been introduced to the clinic or are currently in trial
- HDAC inhibitors demonstrate clinical cardiotoxicity
- **What is the involvement of HDAC sub-classes in HDACi-induced cardiotoxicity?**

METHODOLOGY

Fig 1. xCELLigence Real-Time Cell analysis and Contractility:



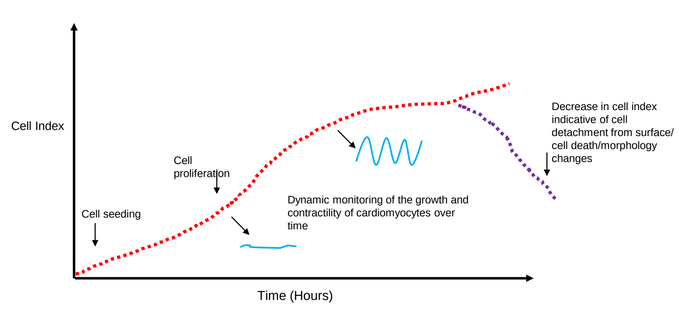


Fig.1 ***Impedance-based Real Time Cell Analysis – How does it work?*** Cells are seeded on an electrode coated plate. When a mild current is applied the attachment of the cells will impede the current flow. ***What does it measure?*** Changes in impedance due to cellular growth and morphology are recorded in real time. These can change in response to drugs or toxic insults.

Fig 2. Qualification of xCELLigence system to detect functional changes in cardiomyocyte contractility

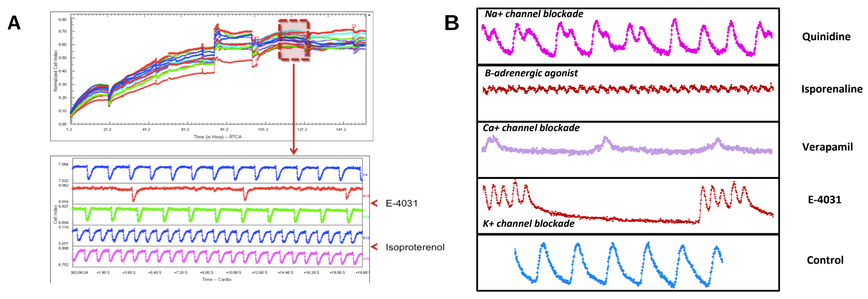


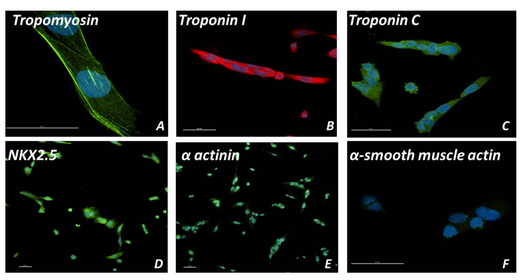
Fig 2. Changes in contractility in hiPSC-CM (A) and primary neonatal CMs (B) induced by cardioactive agents

RESULTS

HDAC inhibitor at clinically relevant concentrations induces structural changes in AC10 ventricular cardiomyocytes

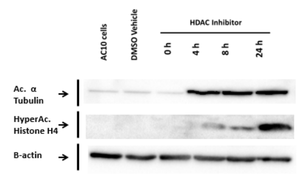
AC10 ventricular human cell line

Fig 3. Cardiac phenotype of AC10 cells



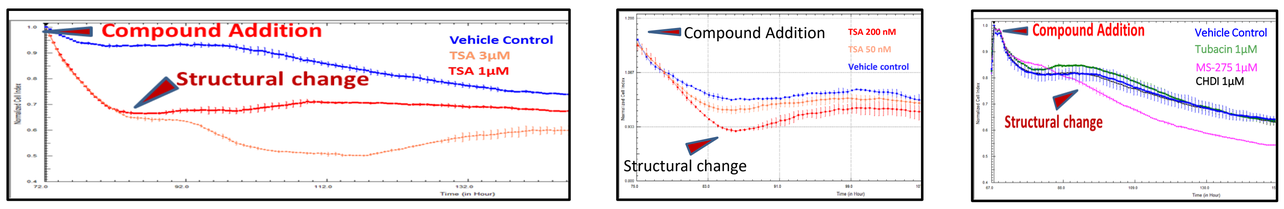
AC10 cells are positive for A. Tropomyosin, B. Troponin I, C. Troponin C, D. NKX2.5 and E. alpha-actin. Negative for F. alpha smooth muscle actin

Fig 4. TSA-mediated HDACi induces Time-dependent Histone and Tubulin acetylation in human AC10 cardiomyocyte cell line



AC10 cells at confluency were exposed to the pan-HDACi TSA (1 µM). Ex pression of acetylated histone H4 and acetylated α-tubulin protein was determined by western blot analysis

Fig 5. Inhibition of HDACs at sub-toxic concentrations induces structural change in AC10 cells



Sub-toxic concentration of TSA induced a decrease in cell index but not a decrease in cell number, suggesting cytotoxicity may depend on cell hypotrophy secondary to HDACi downstream effects

Selective inhibition of class I HDACs induces structural changes in AC10 cell line. Selective inhibition of class II HDACs does not induce significant cell index alterations.

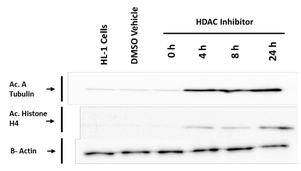
HDAC inhibitor at clinically relevant concentrations induces structural and partial functional changes in HL1 atrial cardiomyocytes

HL1 atrial murine cell line

Fig 6. Electrophysiology of HL1 cells

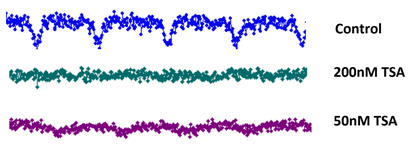
Compound	*Mechanism*	*Detectable effect in HL-1 cell line using xCELLigence Cardio*
Quinidine	Sodium channel blocker class Ia	☑
Disopyramide	Sodium channel blocker class Ia	X
Lidocaine	Sodium channel blocker class Ib	☑
Propafenone	Sodium channel blocker class Ic	X
Metoprolol	Selective B-adrenergic blocker (short acting)	X
Carvedilol	Non-selective α/B-adrenergic blocker (long acting)	X
Amiodarone	Potassium channel blocker	X
Verapamil	Calcium channel blocker	☑
E-4031	Experimental hERG blocker	☑
Isoprenaline	B-adrenoceptor agonist	☑

Fig 7. TSA-mediated HDACi induces Time-dependent Histone and Tubulin acetylation in HL-1 cell line



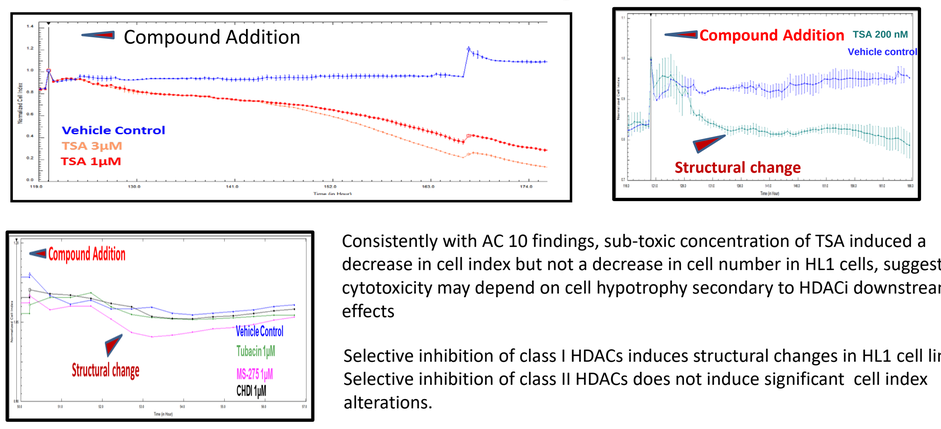
HL-1 cells at confluency were exposed to the pan-HDACi TSA (1 µM). Expression of acetylated histone H4 and acetylated α-tubulin protein was determined by western blot analysis

Fig 8. TSA-mediated HDACi induces functional changes in HL1 contractility



HL1 show functional changes in response to HDACi treatment. Loss of contractility is observed within 24h post drug exposure at 200nM.

Fig 9. Inhibition of HDACs at sub-toxic concentrations induces structural change in HL1 cells



Consistently with AC 10 findings, sub-toxic concentration of TSA induced a decrease in cell index but not a decrease in cell number in HL1 cells, suggesting cytotoxicity may depend on cell hypotrophy secondary to HDACi downstream effects

Selective inhibition of class I HDACs induces structural changes in HL1 cell line. Selective inhibition of class II HDACs does not induce significant cell index alterations.

hIPSC- CMs detect functional changes but not structural changes in response to HDAC inhibitor

Human iPS-derived cardiomyocytes

HDAC inhibitor at clinically relevant concentrations induces functional and structural changes in primary rat cardiomyocytes

Primary rat-derived cardiomyocytes

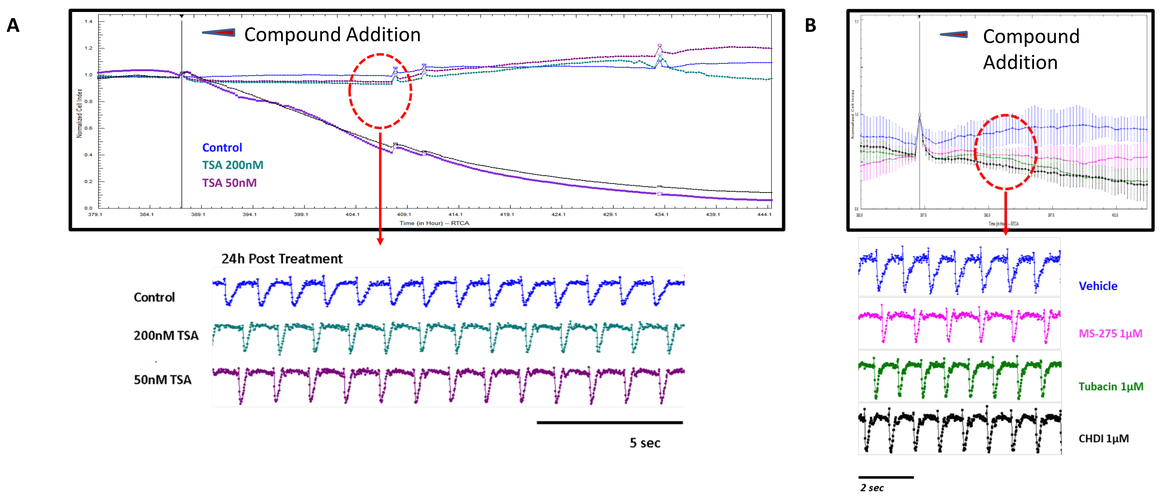


Fig 10. Trichostatin-A (TSA; pan-HDAC inhibitor) is associated with proarrhythmic and acute cytotoxic events detected using impedance assays in Cor.4U cells (A).
Selective Class II HDACis (Class IIa-Tubacin and Class IIb-CHDI) induce moderate decreases in cell index but no beating irregularities (A and B) ; changes in contractility frequency are
observable upon treatment with class I HDACi (MS-275) (B).

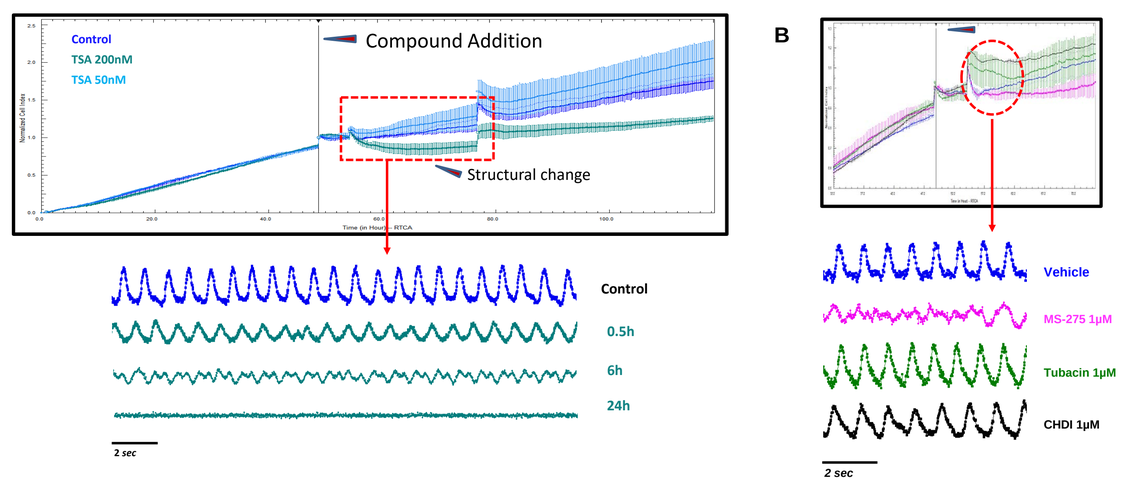


Fig 11. Trichostatin-A (TSA; pan-HDAC inhibitor) displayed a decrease in cell index at sub-therapeutic dose of 200nM in primary rat cells. Complete loss of contractility was achieved within 24hours from initial exposure (A).
Selective Class II HDACis (Class IIa-Tubacin and Class IIb-CHDI) did not induce changes in contractility, changes in contractility pattern are observable upon treatment with class I HDACi (MS-275) (B).

CONCLUSIONS

The combination of non-contractile primary cardiomyocytes and contractile cardiomyocytes offers a comprehensive model system for the detection of drug-induced structural and functional cardiotoxicity

Cardiac Model	Structural Screening	Functional screening
AC10 Human Ventricular Cell Line	YES	NO
HL-1 Murine Atrial Cell Line	YES	LIMITED
Human hiPS-CMs	LIMITED	YES
Primary Neonatal Rat CMs	YES	YES

The integration of different in-vitro models allowed to gain insights into HDACi-mediated cardiotoxocity

HDACI- induced Cardiotoxicity
HDAC inhibition causes both structural and functional aberrations to cardiac cells at sub-clinical drug concentrations
Class I HDACi induced detectable toxicity in the form of structural and functional perturbations
Class IIa and IIb HDACi did not cause detectable toxicity

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MRC Medical Research Council
ittp Integrative toxicology training partnership