In Vitro Systems for the Assessment of Chronic Cardiotoxic Effects:
News from the HESI Stem Cell Working Group

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Abstract

Since long-term exposure of cancer-related therapeutics have been linked to alterations of cardiac function in patients, the Stem Cell Working Group as part of the Health and Environmental Science Institute (HESI) endeavors to gain further insight into chronic cardiotoxicity. The objective of the study was to optimize non-clinical safety assessment strategies of chronic cardiotoxicity by testing prolonged exposure of reference compounds on cell-based assay systems using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). We analyzed the effects of compounds with known cardiotoxic potential and diverse mechanisms of action (MoAs) on contractile and electrophysiological properties of hiPSC-CMs over a period of up to 120 hours. This excerpt of the HESI Stem Cell Working Group study underlines the potential of *in vitro* systems to address contractile function of hiPSC-CMs for chronic safety pharmacological studies of compounds with diverse MoAs.

Study Outline

A panel of 12 compounds was selected to evaluate chronic adverse side effects on hiPSC-CMs, including alterations in energetics and contraction force as well as electrophysiological and structural disturbance.

For the quantification of adverse side effects, a variety of methods was used, covering electrophysiology, mechanobiology, optical methods as well as biochemical assays.

Target Toxicity	Compound	Conc. Range
Energetics/ Mitochondrial Toxicity	**Doxorubcin**	0.1 - 3 µM
	Erlotinib	0.3 - 10 µM
	Sunitib	0.01 - 1 µM
Electrophysiological Disturbance	**Pentamidine**	0.1 - 3 µM
	Arsenic Trioxide	0.1 - 3 µM
Contractility	**BMS-986094**	0.1 - 3 µM
	Milrinone	0.1 - 10 µM
	Nioltinib	0.1 - 10 µM
Structural/myofilament Disturbance	**Endothelin-1**	0.3 - 100 nM
	Vinblastine	1 - 300 nM
	Vincristine	1 - 300 nM
	Vinorelbine	0.1 - 3 µM

green: presented in this poster

Parameter	Method
Electrophysiology	Multielectrode array
	Impedance
Mechanobiology	**Contraction force**
Optical Methods	Optical contraction analysis
	Voltage sensitive dye
	Calcium imaging
Biochemical	Oxygen consumption rate
	Biomarkers

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↑ **Table 2:** Methods used for the quantification of chronic cardiotoxic compound effects

← **Table 1:** Cardiotoxic compounds sorted by target toxicity

Technology

iCell Cardiomyocytes² are highly pure human iPSC-derived cardiomyocytes and allow for robust and reproducible quantification of compound effects as demonstrated in the CiPA study (Blinova *et al.*, 2017).

The **CardioExcyte 96** is an impedance-based system where hiPSC-derived cardiomyocytes are cultured on gold electrodes, enabling label-free and continuous quantification of electrical impedance as a measure of monolayer integrity.

In the **FLEXcyte 96** system, hiPSC-derived cardiomyocytes are cultured on ultra-thin silicone membranes, mimicking the mechanical environment of native human heart tissue. Rhythmic contractions deflect the membranes, quantified by capacitive distance sensing. Parameters analyzed are contractile force (AMP), beat rate (BR), beat rate regularity (BRR), rising time (RT), falling time (FT). (Gossmann *et al.*, 2016 and 2020).

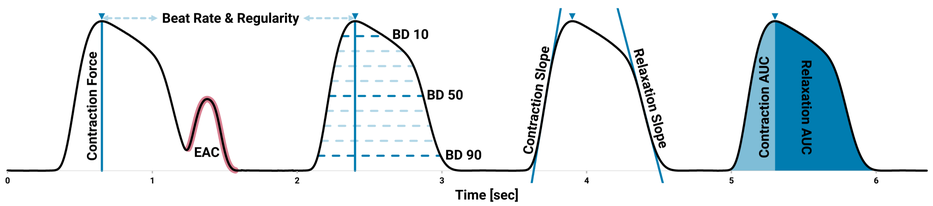

Figure 1: FLEXcyte 96 contraction analysis parameters

Workflow

hiPSC-CMs were seeded from cryopreserved state into the assay format and cultured in maintenance medium for 5 days. 24h prior to compound application, medium was changed to serum-free, protein-containing assay medium. Baseline measurements were performed before compound application. Compounds were added 4x concentrated during a partial media change. The first compound measurement was carried out after 1h. Subsequently, chronic compound responses were recorded in 24h intervals for a total of 120h.

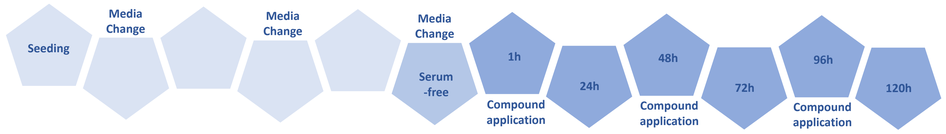

Figure 2: Study workflow

Results

Target Toxicity	Compound	C1	C2	C3	C4
Energetics/ Mitochondrial Toxicity	Doxorubcin	0,75	0,45	0,40	0,36
	Erlotinib	0,95	0,99	1,03	1,00
	Sunitib	0,94	1,10	1,23	1,33
Ephys Disturbance	Pentamidine	0,94	0,94	0,81	0,62
Contractility	BMS-986094	0,93	0,78	0,83	0,77
	Nioltinib	0,96	1,09	1,14	1,00
Structural/myofilament Disturbance	Endothelin-1	0,82	0,83	0,86	0,87
	Vincristine	0,90	0,57	0,43	0,46

Table 3: Effect of the tool compounds on the base impedance as a measure of monolayer integrity

Figure 3: Contraction parameter analysis at 72h post compound application. Parameters: Contraction force (Amplitude), beat rate, contraction slope, relaxation slope, beat duration

- **Doxorubicin** had a strong toxic effect, measured both in contraction force as well as monolayer integrity.
- **Erlotinib** had only mild effects on the contractility of hiPSC-CMs and no effect on monolayer integrity.
- **Sunitinib** had a strong effect on the chronotropy (beat rate, beat duration) and inotropy (amplitude). The monolayer integrity was increased.
- **Pentamidine** had a strong effect on the contraction force and on the monolayer integrity.
- **BMS-986094** had a major impact on all contractile parameters and on the monolayer integrity.
- **Nilotinib** had a strong effect on the contractile parameters, while the monolayer integrity remained unchanged.
- **Endothelin-1** had a positive effect on the contraction force (amplitude), but little or no effect on the monolayer integrity.
- **Vincristine** had a strong effect on both the contractile parameters as well as monolayer integrity.

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