

# MicroRNAs in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes (hiPSC-CM) as Predictive Biomarkers of Structural Cardiotoxicity

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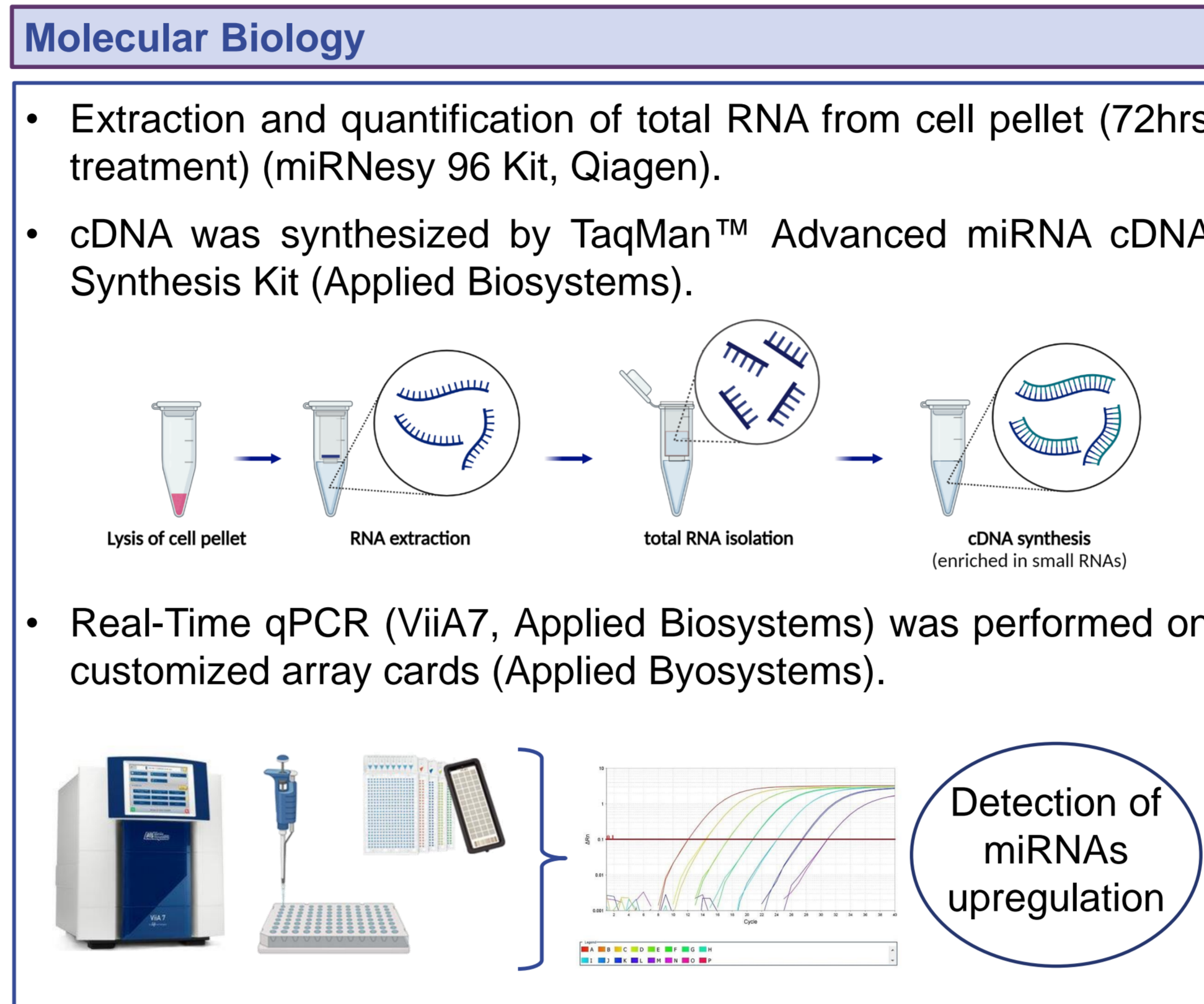
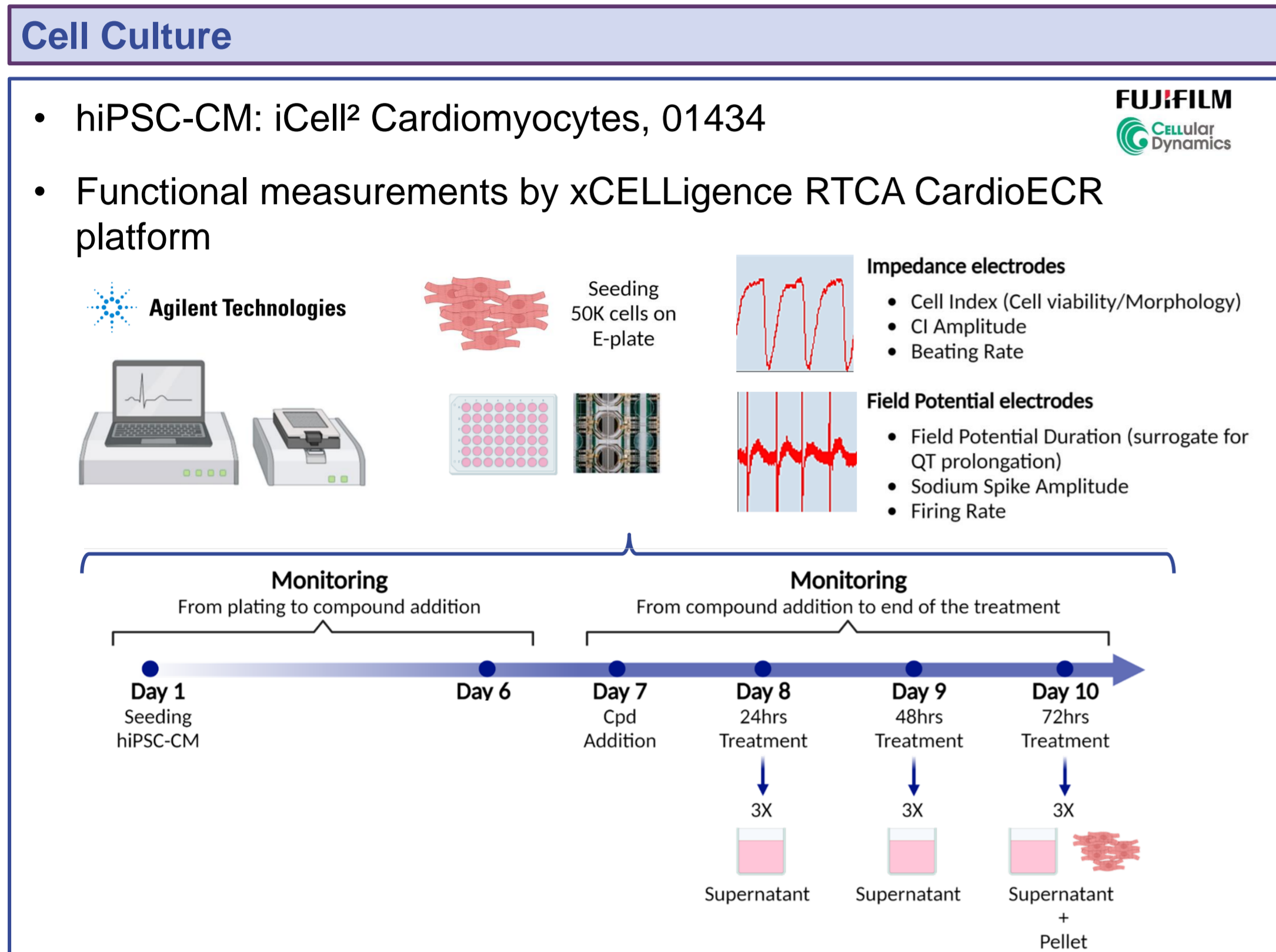
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## BACKGROUND and OBJECTIVES

- Drug-induced cardiotoxicity** remains one of the main causes of drug attrition during preclinical and clinical development<sup>1</sup>, suggesting the need to identify an assay/model that can accurately predict the true effect of a new drug candidate. **Human-induced pluripotent stem cell-derived cardiomyocytes** (hiPSC-CM) based-assays have been broadly employed in drug discovery for proarrhythmic risk assessment, and disease modeling; however, they poorly predict structural changes caused by a drug (i.e., morphological damage or loss of cellular and/or subcellular components)<sup>2,3</sup>. Therefore, identifying novel assays and biomarkers that can identify structural and functional cardiotoxicants, will help to eliminate potential harmful candidates early in drug discovery.
- MicroRNAs** (miRNAs) have been widely investigated in the past decade as alternative **biomarkers** of cardiotoxicity, and several studies have linked their dysregulation to several cardiovascular liabilities<sup>4,5,6</sup>.
- The current study has the principal objective of investigating the **dysregulation** of miRNAs in hiPSC-CM after the treatment with a broad set of drugs known for causing **structural cardiotoxicity**.

## MATERIALS AND METHODS

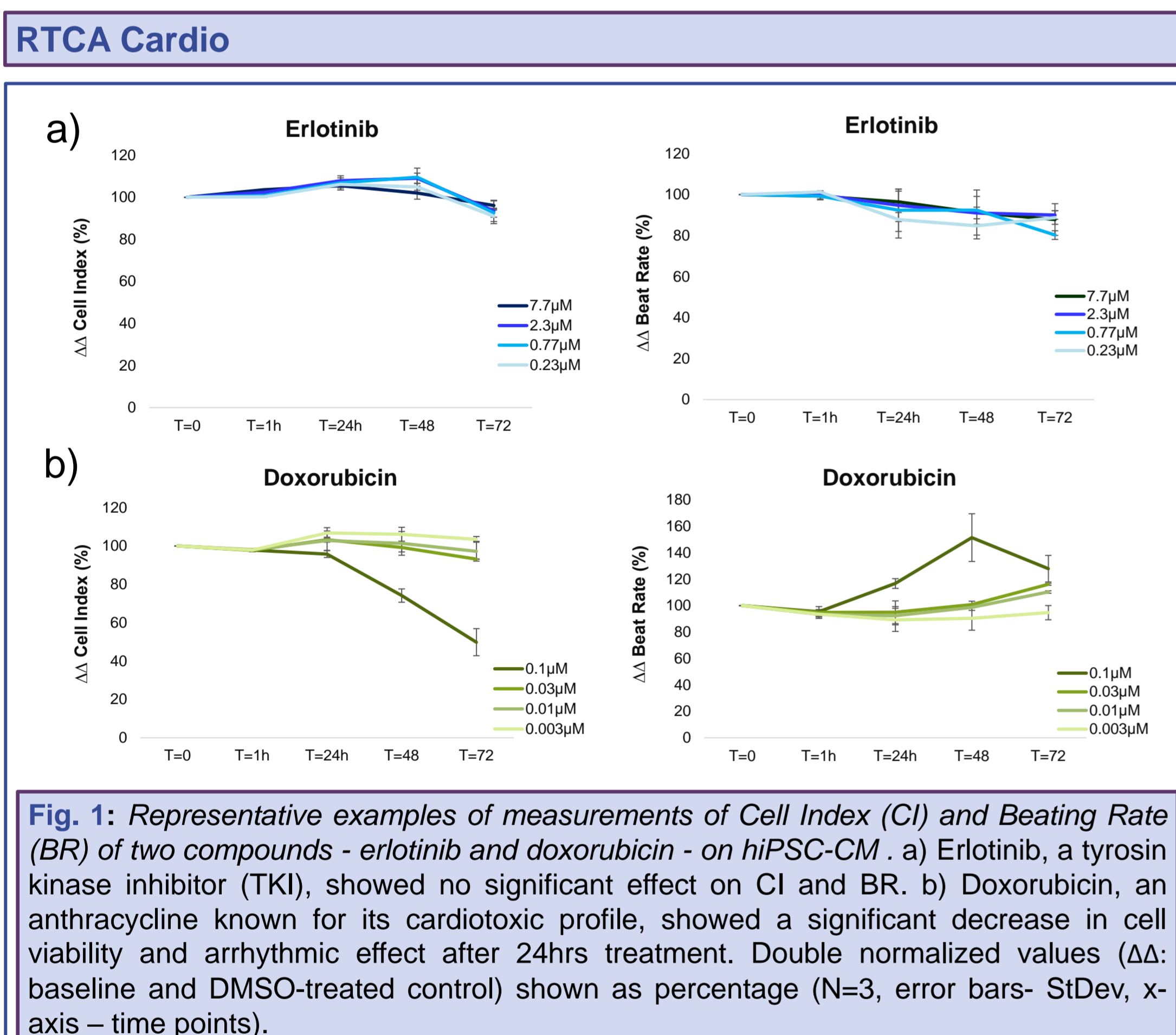


### Table 1: List of structural cardiotoxicants divided by drug class.

\*HESI Stem Cell (SC) working group (wg) blinded compounds in **bold**.

Drug class	Compounds
<b>Anthracyclines</b>	<b>Doxorubicin</b> , Idarubicin, Daunorubicin, Epirubicin
<b>Anthracycline-like mechanism</b>	Mitoxantrone
<b>Tyrosine Kinase (TK) Inhibitors</b>	Imatinib, Lapatinib, <b>Sunitinib</b> , Sorafenib, Crizotinib, Dasatinib, <b>Nilotinib</b> , Erlotinib
<b>Proteasome Inhibitors</b>	Bortezomib, Carfilzomib, Ixazomib
<b>Structure Disruptors (Vinca Alkaloids, Taxanes)</b>	<b>Vincristine</b> , <b>Vinblastine</b> , <b>Vinorelbine</b> , Paclitaxel, Endothelin-1
<b>Effect on DNA/RNA (DNA synthesis, topoisomerase inhibitors, etc.)</b>	5-Fluorouracil, <b>Pentamidine</b> , Etoposide, Cyclophosphamide
<b>Other Mechanism of Action</b>	<b>Milrinone</b> , <b>Arsenic Trioxide</b> , <b>BMS-986094</b> , Tegasero, Dexfenfluramine, Valdecoxib, Rofecoxib

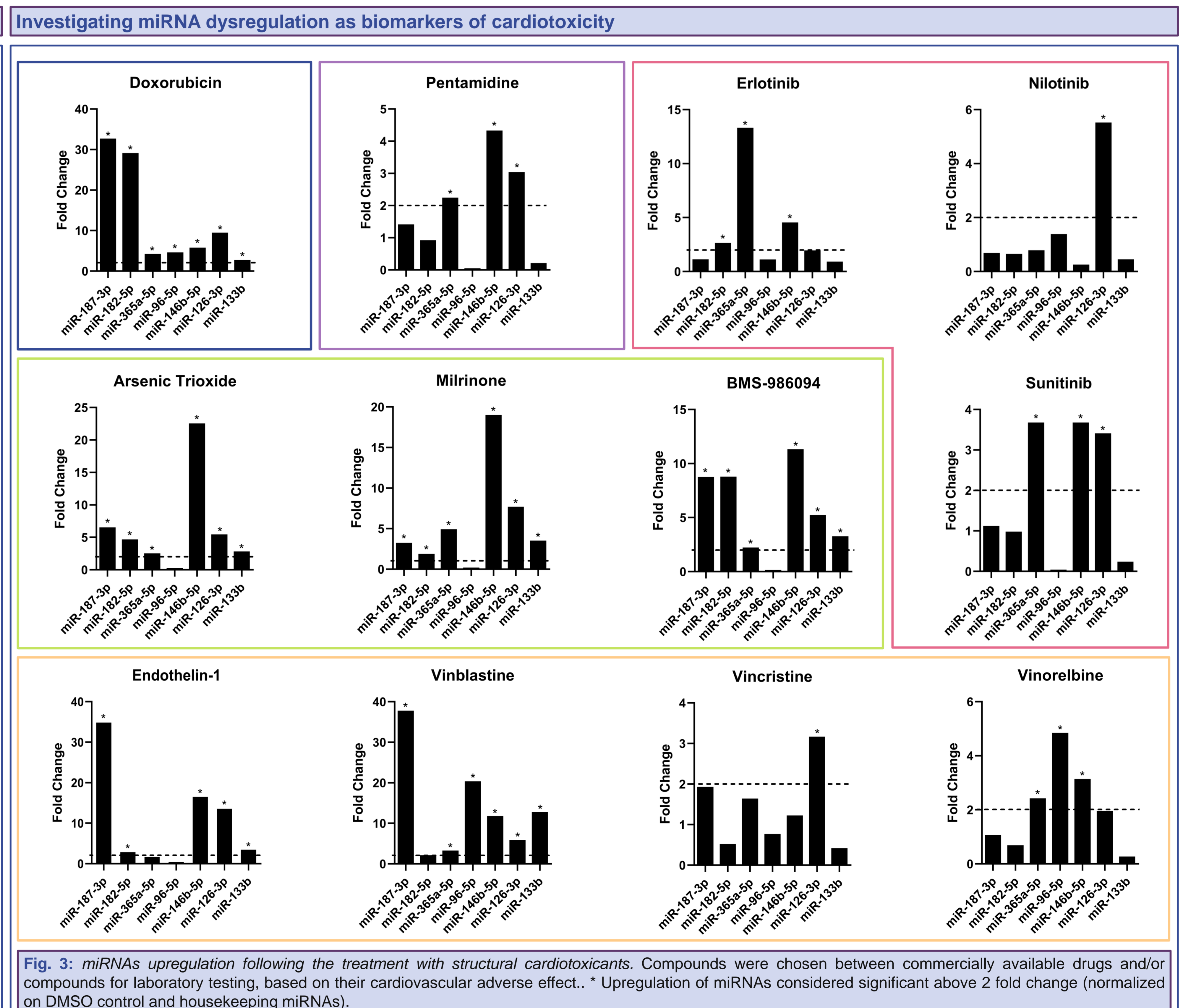
## RESULTS



### Table 2: Structural cardiotoxicants and their effects on hiPSC-CM after 72h treatment among different parameters.

Compound name	Concentration ( $\mu$ M)	Cell Index	Contractility Amplitude	Beating Rate
Doxorubicin	0.1, 0.03, 0.01, 0.003	-50%	-43%	+28%
Endothelin-1	0.1, 0.03, 0.001, 0.0003	+2%	-28%	+15%
Erlotinib	7.7, 2.3, 0.77, 0.23	-9%	+8%	-19%
Arsenic Trioxide	0.48, 0.16, 0.048, 0.016	+15%	+15%	+8%
Pentamidine	3, 1, 0.3, 0.1	-28%	-48%	-20%
Sunitinib	1, 0.3, 0.1, 0.01	-8%	-22%	-32%
BMS-986094	3, 1, 0.3, 0.1	-15%	-8%	+24%
Nilotinib	1, 0.3, 0.1, 0.01	-11%	-37%	-17%
Vincristine	0.3, 0.03, 0.003, 0.0003	-29%	-64%	+18%
Vinblastine	0.3, 0.03, 0.003, 0.0003	-24%	-67%	+35%
Vinorelbine	3, 1, 0.3, 0.1	-64%	-91%	-78%
Milrinone	10, 3, 0.3, 0.1	-9%	-18%	+6%

**Table 2: Structural cardiotoxicants and their effects on hiPSC-CM after 72h treatment among different parameters.** The table captures the maximum percentage change from the baseline (+ increase, - decrease). The effect considered biologically significant at above 20% from the baseline.



## CONCLUSIONS AND FUTURE PERSPECTIVES

- Upregulation of several miRNA candidates in hiPSC-CM was observed following the 72hrs treatment with structural cardiotoxicants, even when Cell Index, measured by RTCA CardioECR system, did not change significantly (endothelin-1, sunitinib, milrinone, etc.).
- The combination of miRNA candidates could be used to screen for cardiotoxic compounds in addition to other functional and structural assays on hiPSC-CM.
- The translatability of the highly upregulated miRNAs will be assessed in patient-derived cardiomyocytes, generated from healthy individuals (control) and patients with cardiovascular liabilities induced by cancer therapy.
- The assay will be further optimized to study miRNAs dysregulation in culture media, as well as in patient's serum, to investigate new potential circulating biomarkers.

### References:

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