Could secreted microRNAs in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes (hiPSC-CM) Be Predictive Biomarkers of Structural Cardiotoxicity?

Martina Cherubin[1,2], Annie Delaunois[1], Jean-Pierre Valentin[1], Maaike Alaerts[2], Pieter-Jan Guns[3], Vitalina Gryshkova[1]

[1] UCB Biopharma SRL, Braine-l'Alleud, Belgium
[2] Center of Medical Genetics, University of Antwerp and Antwerp University Hospital, Antwerp, Belgium
[3] Physio-pharmacology, University of Antwerp, Antwerp, Belgium

SP 117

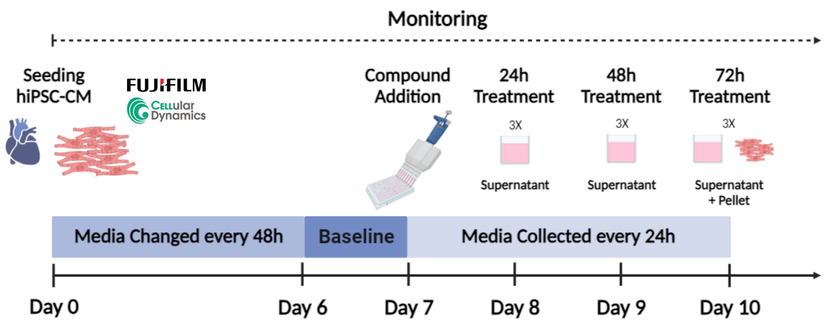
BACKGROUND and OBJECTIVES

- In pharmaceutical industries, the identification of novel biomarkers to predict **structural cardiotoxicity** is pivotal to avoid late-stage drug attrition. Indeed, almost half of safety-related attrition is caused by cardiovascular toxicity, of which 48% is due to drug-induced structural changes (i.e., morphological damage or loss of cellular and/or subcellular components)[1,2].
- **MicroRNAs** (miRNAs) have been widely investigated in the past decade as alternative **biomarkers**, and several studies have linked their dysregulation to cardiovascular liabilities [3,4,5].
- miRNAs secreted in **biofluids** have been investigated as **translatable** biomarkers to clinic. However, *in vitro* detection methods present several challenges. A broader application of miRNA-based assays to detect cardiotoxicity is limited by their low abundance in the supernatant of cell cultures, as well as the lack of high-throughput platforms and established methods for RNA extraction[6].
- This study aims to investigate the **upregulation** of circulating miRNAs in hiPSC-CM **supernatant** after the treatment with known structural cardiotoxicants.

MATERIALS AND METHODS

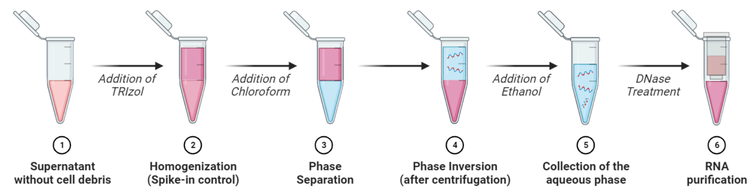
Cardiomyocytes Cell Culture

- hiPSC-CM: iCell² Cardiomyocytes, 01434
- Functional measurements by xCELLigence RTCA CardioECR platform (data previously presented at SPS 2022)
- Collection of supernatant every 24h and cell pellet at 72h



miRNAs Detection

- Analysis of miRNAs in hiPSC-CM pellet and supernatant by RT-qPCR
- The panel of miRNAs analyzed included 4 normalizers and 12 target miRNAs



Issue with Supernatant RNA	Solution
Low abundance of miRNAs in supernatant	Experiment run in 24-well plate: 275'000 cells/mL
Lack of established housekeeping miRNAs	Spike-in control (cel-miR-39-3p)

Table 1: List of 32 structural cardiotoxicants divided by drug class. *HESI Stem Cell (SC) working group (wg) blinded compounds in **bold.**

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

RESULTS

miRNAs upregulated in Cell Pellet

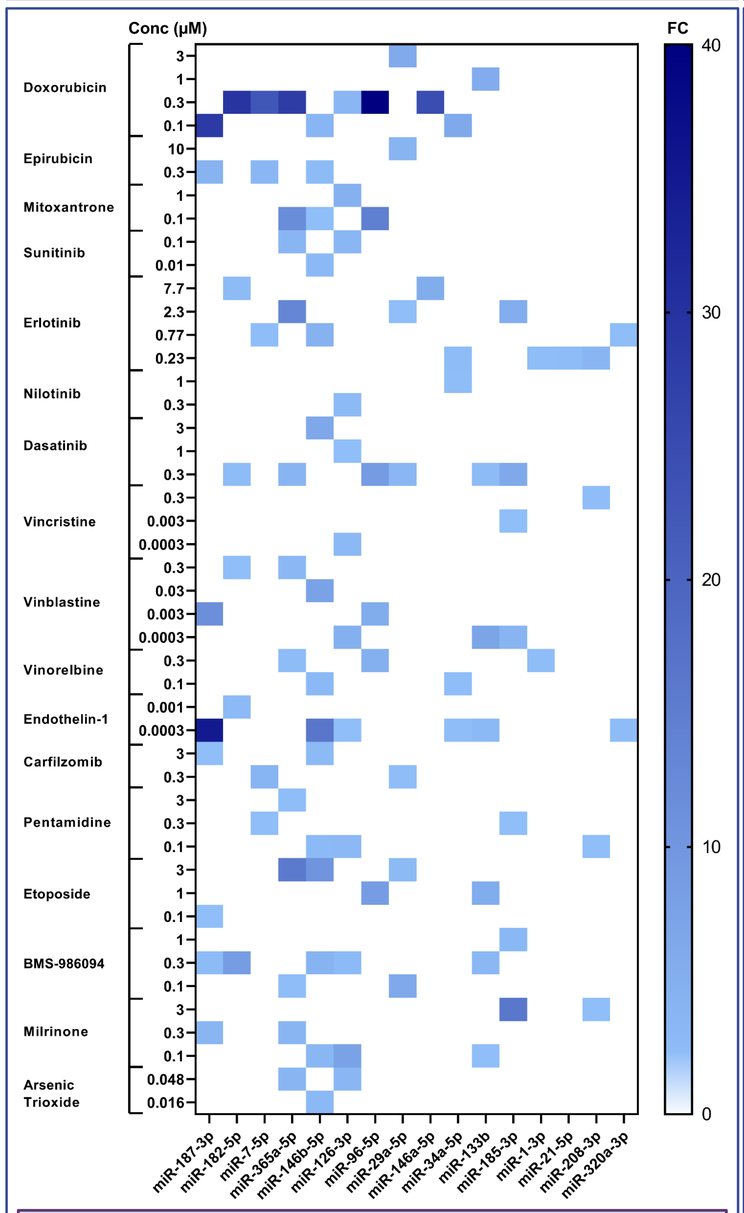


Fig. 1: *Heatmap of miRNAs upregulation in hiPSC-CM pellet after 72h treatment with structural cardiotoxicants.* Upregulation of miRNAs considered significant above 2 fold change (FC).
Data normalized on DMSO control and housekeeping miRNAs.

miRNAs upregulated in Supernatant

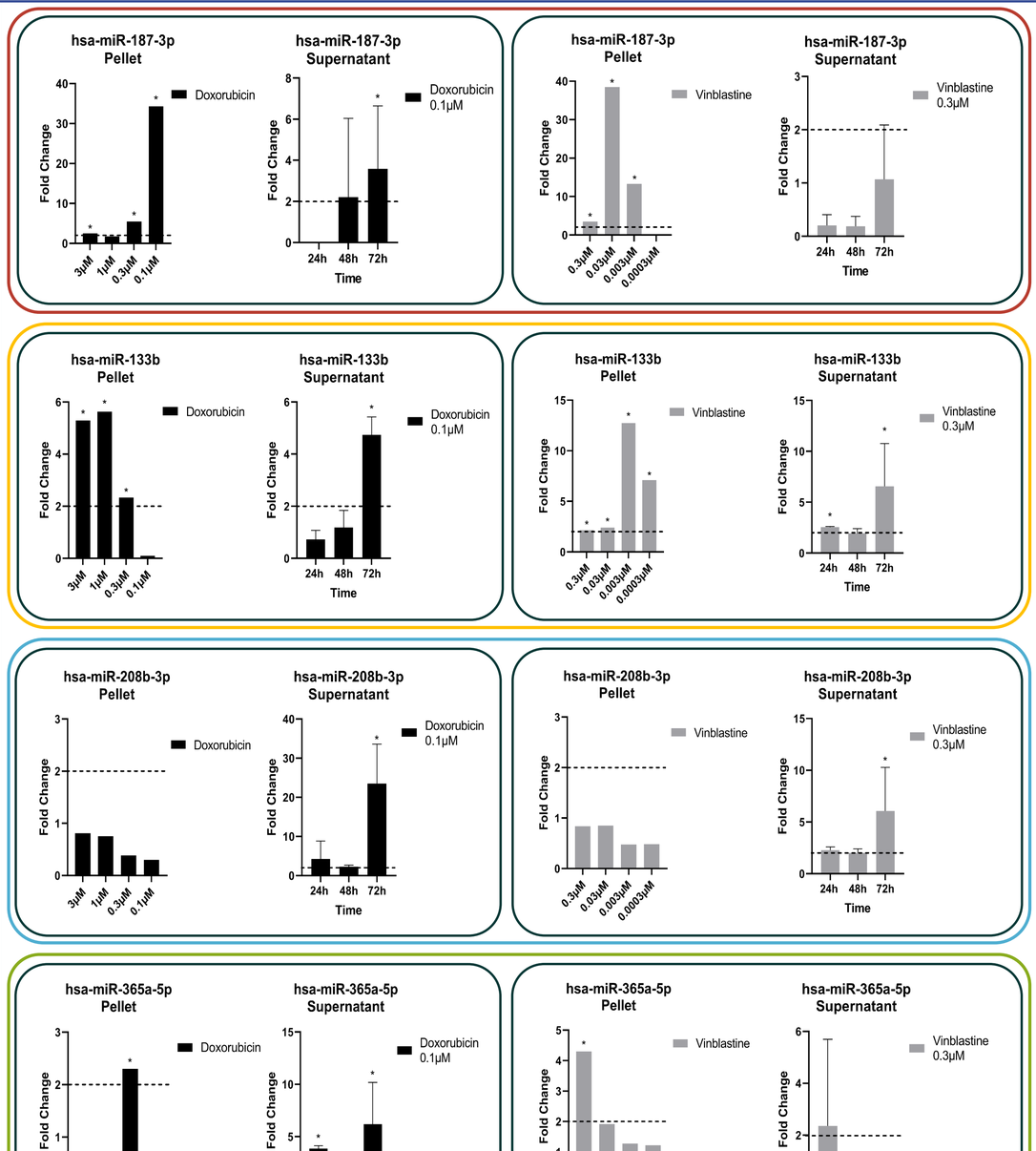


Fig. 2: *Time-dependent miRNAs upregulation in supernatant of hiPSC-CM.* Out of 12 miRNAs selected, only 4 miRNAs were significantly upregulated in the supernatant at 72h incubation with the drug. From literature, hsa-miR-133b found upregulated in heart failure patient; hsa-miR-208b-5p *in vivo* and in patients with myocardial infarction. Threshold was set at Ct = 32 to calculate FC. Upregulation of miRNAs considered significant above 2 FC (normalized on DMSO control and housekeeping miRNAs).

Treatment		hsa-miR-182-5p	hsa-miR-126-3p	hsa-miR-29a-5p	hsa-miR-146b-5p	hsa-miR-96b-5p
Doxorubicin 0.1µM	24h	ND	33.386	33.932	ND	ND
	48h	ND	32.707	ND	ND	ND
	72h	ND	32.080	27.400	ND	ND
Idarubicin 0.1µM	24h	ND	33.057	32.739	ND	ND
	48h	ND	33.321	31.775	ND	ND
	72h	ND	32.183	26.935	ND	34.398
Mitoxantrone 1µM	24h	ND	33.056	28.198	ND	ND
	48h	ND	36.574	30.659	ND	ND
	72h	ND	34.247	26.251	ND	ND
Vinblastine 0.3µM	24h	ND	34.524	31.444	ND	ND
	48h	35.923	34.960	29.858	ND	ND
	72h	ND	32.172	29.507	38.82	38.239

Table 2: *RT-qPCR raw data for other miRNAs tested on hiPSC-CM supernatant over 72h.* Treatments' concentration selected based on upregulation observed in cell pellet after 72h. Out of 12 miRNAs targets, only 4 were significantly upregulated in response to structural cardiotoxicants. For the other miRNAs selected, DMSO values were not detected (ND). Normalization to FC was not possible. Data are presented as Ct values.

CONCLUSIONS AND FUTURE PERSPECTIVES

- Upregulation of several miRNA candidates in hiPSC-CM **pellet** was observed following the 72hrs treatment with structural cardiotoxicants, with the largest upregulation induced by anthracyclines and drugs affecting the microtubular structure (Vinca Alkaloids, Taxanes, etc.). Highest FC were observed for hsa-miR-187-3p, hsa-miR-146b-5p, hsa-miR-133b, hsa-miR-126-3p, hsa-miR-96-5p and hsa-miR-365a-5p.
- The optimized assay to study miRNAs dysregulation in the **supernatant** revealed the upregulation of several miRNAs in a time-dependent manner. Significant upregulation was found for hsa-miR-187-3p, hsa-miR-133b, hsa-miR-208b-3p and hsa-miR-365a-5p after 72h incubation with structural cardiotoxicants.
- 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, both in cell pellet and supernatant. The upregulation of circulating miRNAs in the supernatant will be compared to **serum/circulating miRNAs** from patients treated with structural cardiotoxicants.

References:

1. Weaver et Valentin, *Toxicol Sci* (2019)
2. Gryshkova et al., *Arch. Toxicol.* (2022)
3. Vegter et al., *Eur J Heart Fail 18* (2016)
4. Kim et al., *Yonsei Med J* (2018)
5. Mirna et al., *Cells* (2019)
6. Felekkis et Papaneophytou, *Int. J. Mol. Sci* (2020)

Inspired by patients. Driven by science.

INSPIRE receives funding from the EU Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 858070

The images are created with BioRender.com