



**HESI Cardiac Safety FDA Workshop:
“Leveraging Human-Relevant Cardiomyocytes
in Nonclinical Studies to Provide Mechanistic Insights
into Cardiovascular Safety Liabilities”**

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FDA White Oak Campus, Building 31 Conference Center

Development of in vitro cardiotoxicity assessment for oncology drugs

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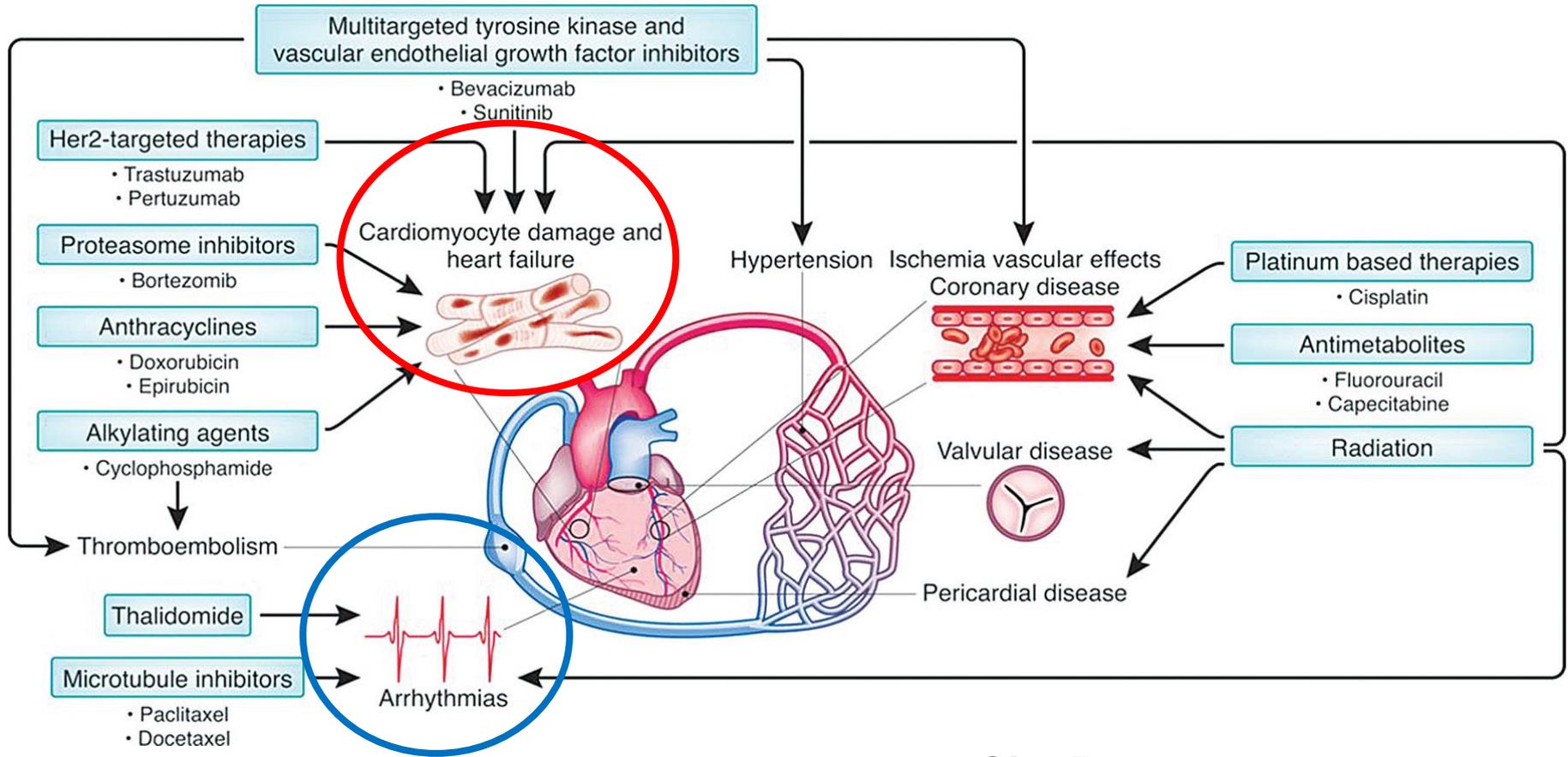
Japan iPS Cardiac Safety Assessment (JiCSA)

☑The opinions in this presentation are our own and do not necessarily reflect the views and policies of Ministry of Health, Labour and Welfare in Japan.

Outline

- Cardiotoxicity: a growing issue in oncology
- Examples with doxorubicin and tyrosine kinase inhibitors using contractility
- Conclusion and Future perspectives

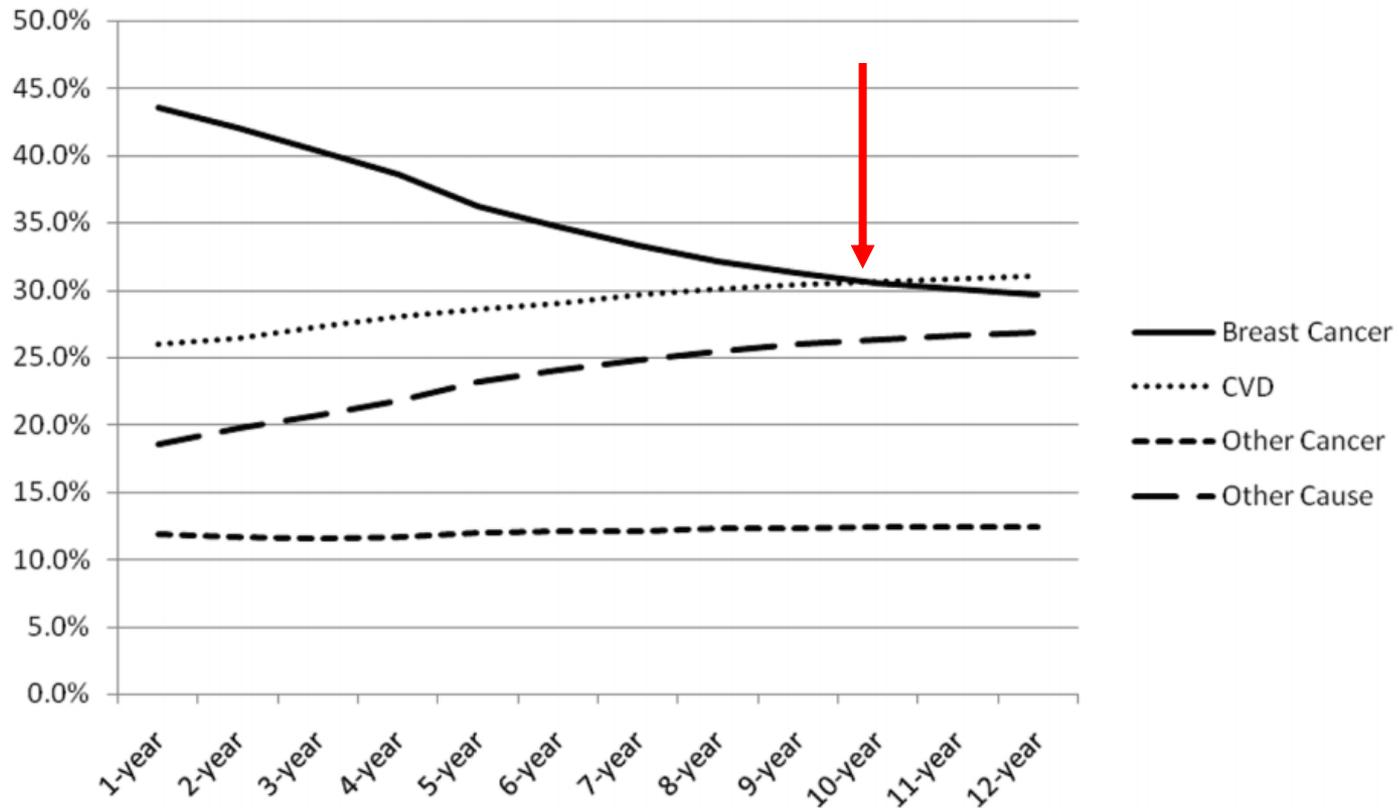
Various Cardiotoxicity



CiPA/JiCSA studies etc

Circ Res 2016;118: 1008-1020.

Cardio-oncology



Patnaik et al. Breast Cancer Research 2011, 13:R64

Attention to reducing the risk of cardiovascular disease should be a priority for the long-term care of women following the diagnosis and treatment of breast cancer.

Cardio-oncology clinical guideline



European Heart Journal (2016) **37**, 2768–2801
doi:10.1093/eurheartj/ehw211

ESC CPG POSITION PAPER

2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines

The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC)

Authors/Task Force Members: Jose Luis Zamorano* (Chairperson) (Spain), Patrizio Lancellotti* (Co-Chairperson) (Belgium), Daniel Rodriguez Muñoz (Spain), Victor Aboyans (France), Riccardo Asteggiano (Italy), Maurizio Galderisi (Italy), Gilbert Habib (France), Daniel J. Lenihan¹ (USA), Gregory Y. H. Lip (UK), Alexander R. Lyon (UK), Teresa Lopez Fernandez (Spain), Dania Mohty (France), Massimo F. Piepoli (Italy), Juan Tamargo (Spain), Adam Torbicki (Poland), and Thomas M. Suter (Switzerland)

European Heart Journal (2016) 37:2768–2801

Cancer therapy associated with Heart failure/Left Ventricular Dysfunction

Chemotherapy agents	Incidence (%)	Frequency of Use
Anthracyclines		
Doxorubicin (Adriamycin®) ¹	3-26* [#]	++++
Epirubicin (Ellence®) ¹	0.9-3.3 [#]	+
Idarubicin (Idamycin PFS®) ¹	5-18 [#]	++
Small molecule tyrosine kinase inhibitors		
Dabrafenib (Tafinlar®) ²	8-9 [#]	++++
Dasatinib (Sprycel®) ¹	2-4 [#]	++++
Lapatinib (Tykerb®) ^{1,2}	0.9-4.9 [#]	++++
Pazopanib (Votrient®) ²	0.6-11 [#]	++++
Ponatinib (Iclusig®) ²	3-15 ^b	+
Sorafenib (Nexavar®) ^{1,2}	1.9-11	++++
Sunitinib (Sutent®) ²	1-27 [#]	++++
Trametanib (Mekinist®) ²	7-11 [#]	++++

CV safety issue of oncology drugs

- **Development of better in vitro models may improve screening of drug candidates for potential CV toxicity and mechanistic characterization**
- **Are there any additional studies to predict potential CV risk?**
- **How can we mitigate CV toxicity in patients?**

Our regulatory challenge is to minimize and predict potential cardiotoxicity by oncology drugs at the early non-clinical testing process.

Various methods for functional analyses in iPSC-CMs

A

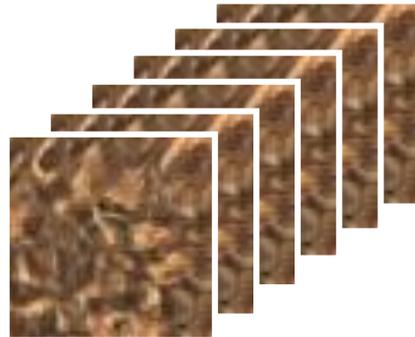
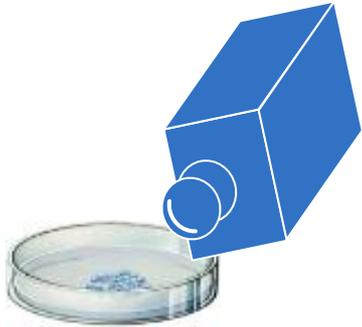


METHOD	MAIN TARGET	MAIN CHARACTERISTICS
1. Patch clamp	Single cells	Precise data on action potentials, requires advanced skills and equipment, invasive and terminal
2. Multielectrode array	Clusters, sheets	Non-invasive method for obtaining electrophysiological data (field potentials), non-terminal, does not record single cells
3. Impedance assays	Cell sheets	Non-invasive, non-terminal, based on measuring electric impedance of an electrode with a cell on it
4. Fluorescent imaging	Single cells, clusters, sheets	Specific fluorescent dye based measurement of local membrane voltage or ion concentrations, toxic and potentially terminal.
5. Atomic force microscopy	Single cells, clusters, sheets	Measures beating force directly from the cell, not invasive but contacts with the cell, requires advanced equipment
6. Traction force microscopy	Single cells, small clusters	Measures movements of fluorescent beads and determines the beating forces indirectly, non-invasive, non-terminal
7. Video microscopy	Single cells, clusters, sheets	Non-invasive, non-toxic, non-terminal, only basic equipment needed, potential for automatization

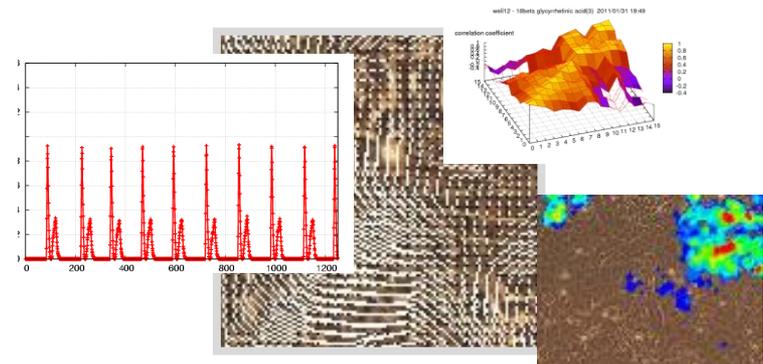
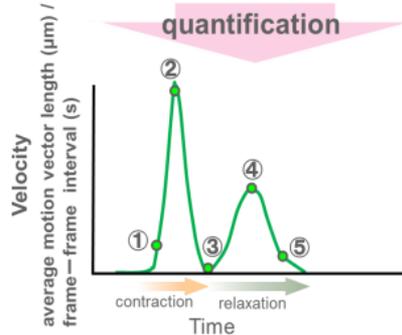
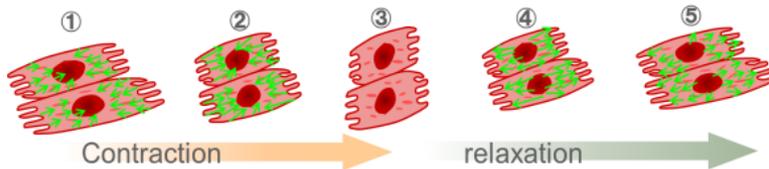
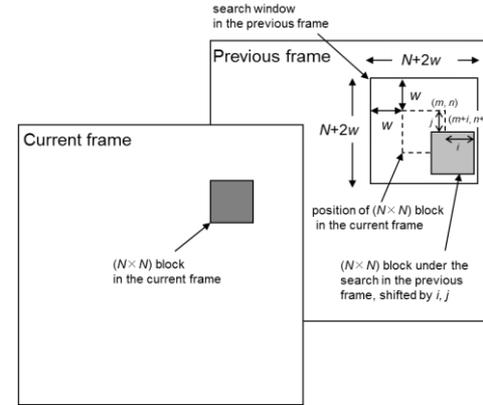
BBA 1863:1864–72 (2016)

Is it possible to predict the effect of drugs on left ventricular function?

iPSC-based cardiac contractility using motion vector system



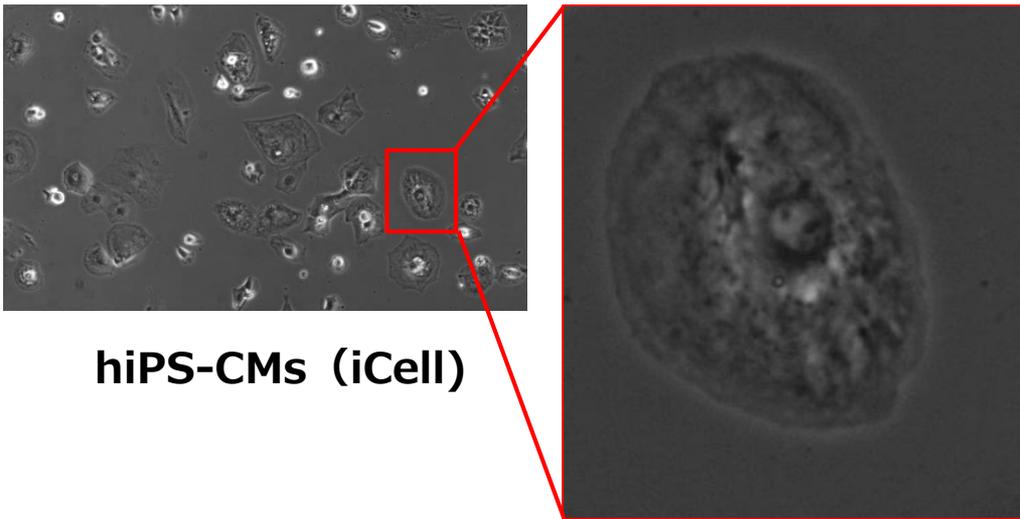
Movie= a series of pictures (frames)



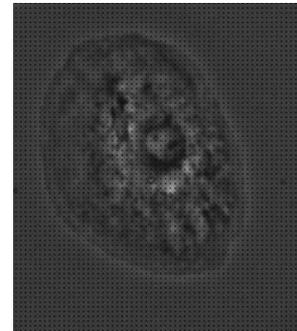
(Hayakawa, Kanda et al. *JMCC*, 2014)

Video microscopy provides a non-invasive method for cardiomyocyte beating analysis and can be scaled up toward high throughput.

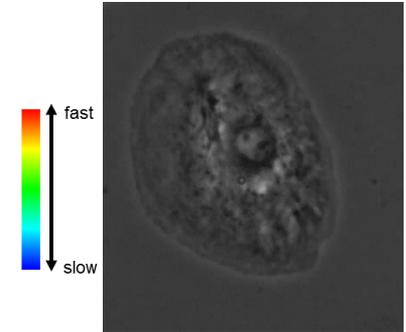
Motion detection from single hiPS-CM



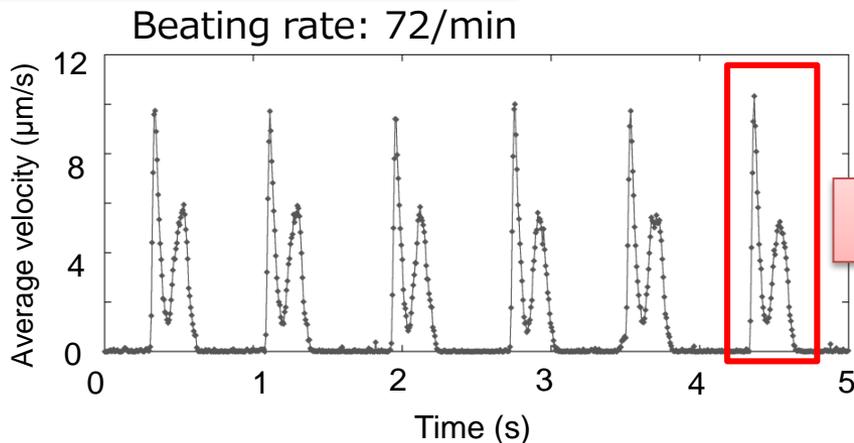
● Velocity field



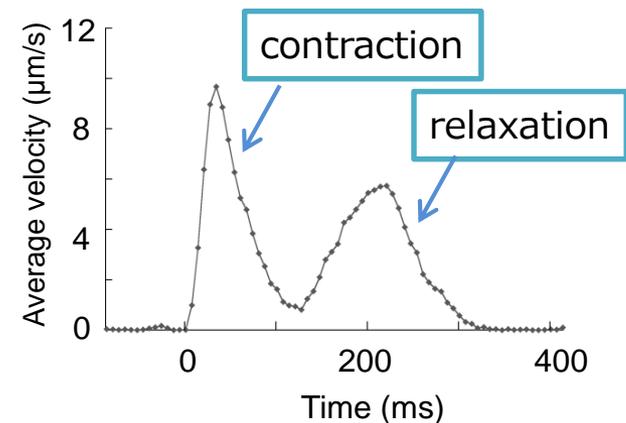
● Velocity amplitude



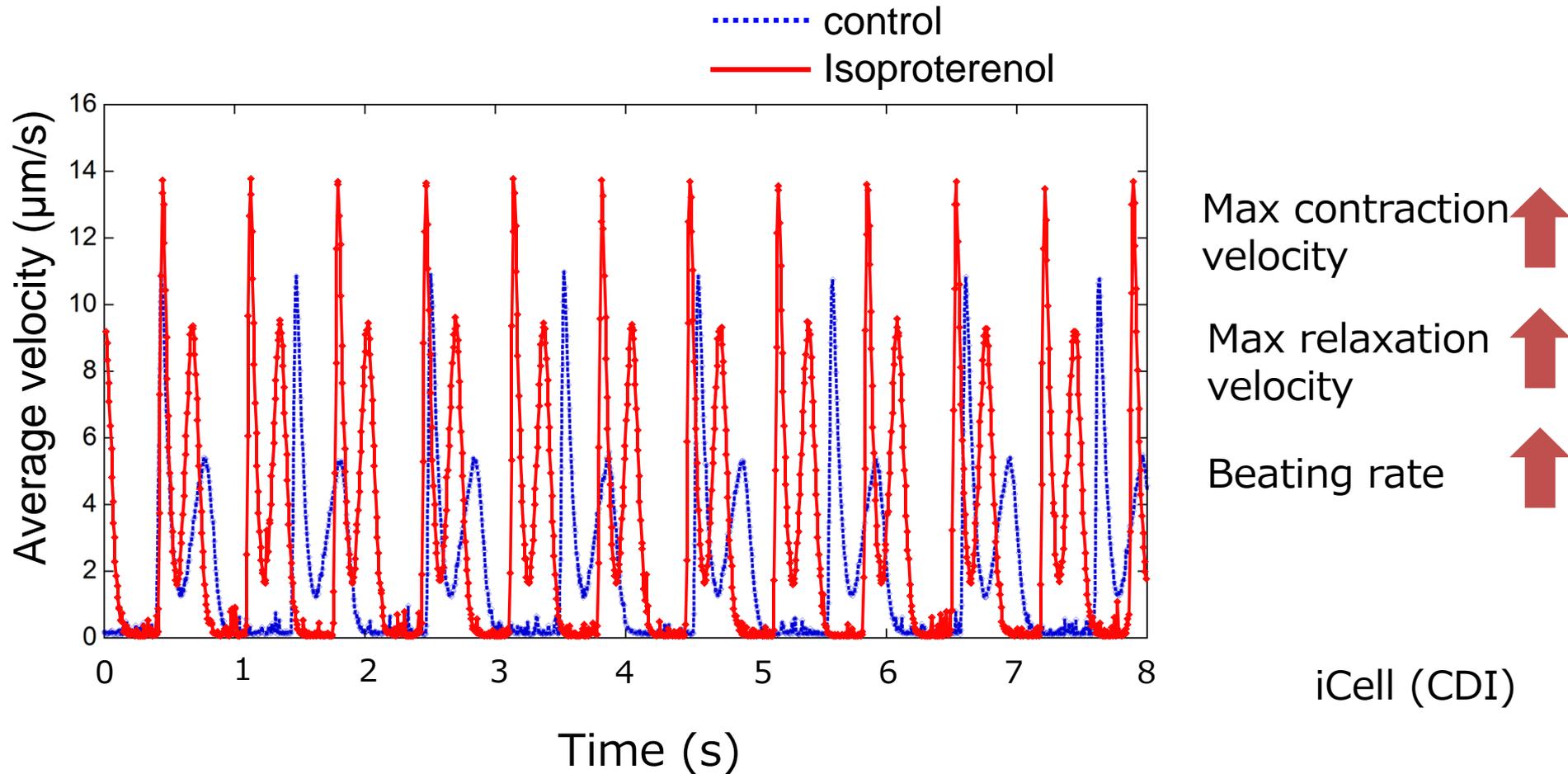
Motion profile (5 seconds)



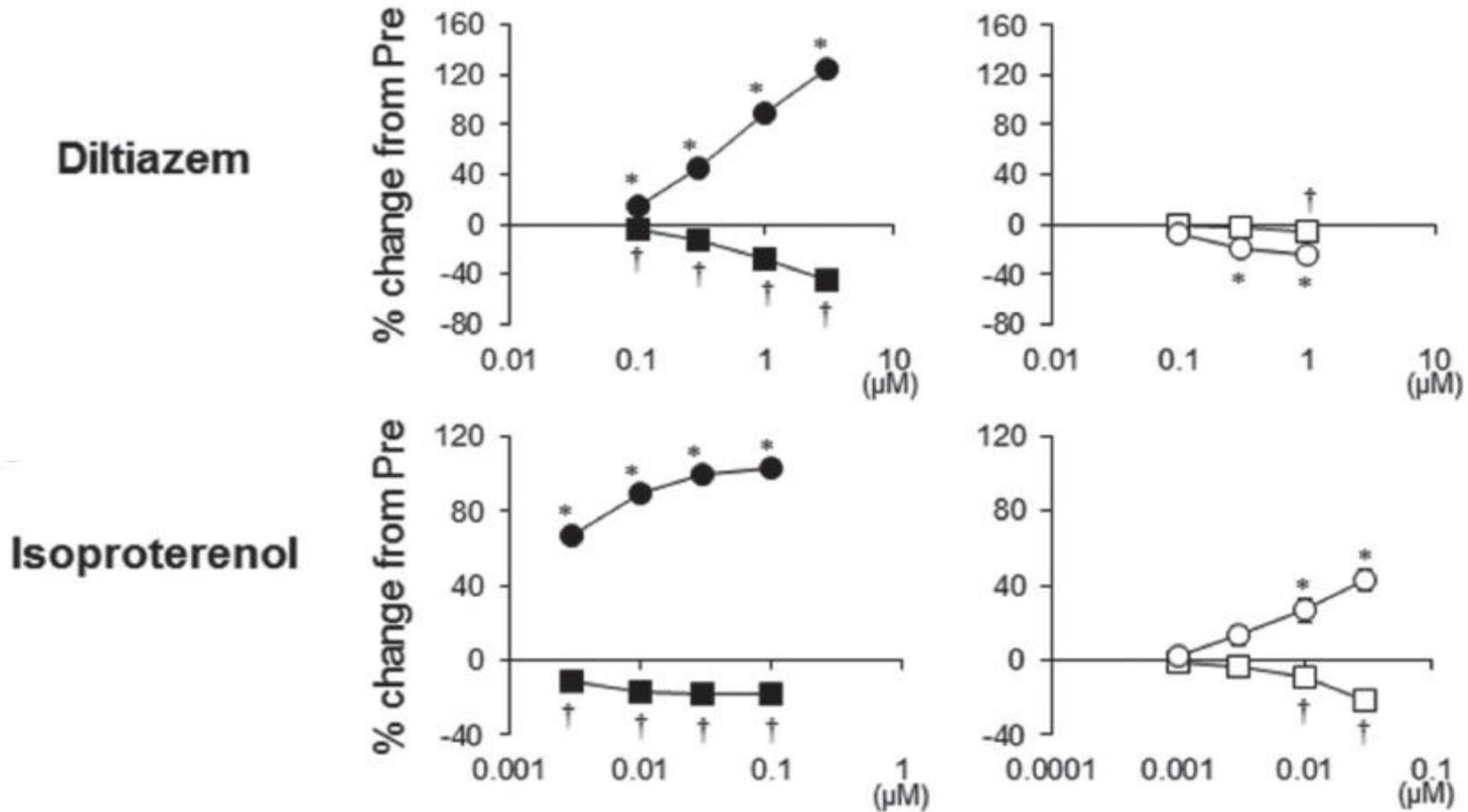
Single beat profile



Effect of isoproterenol on motion vector in hiPS-CMs

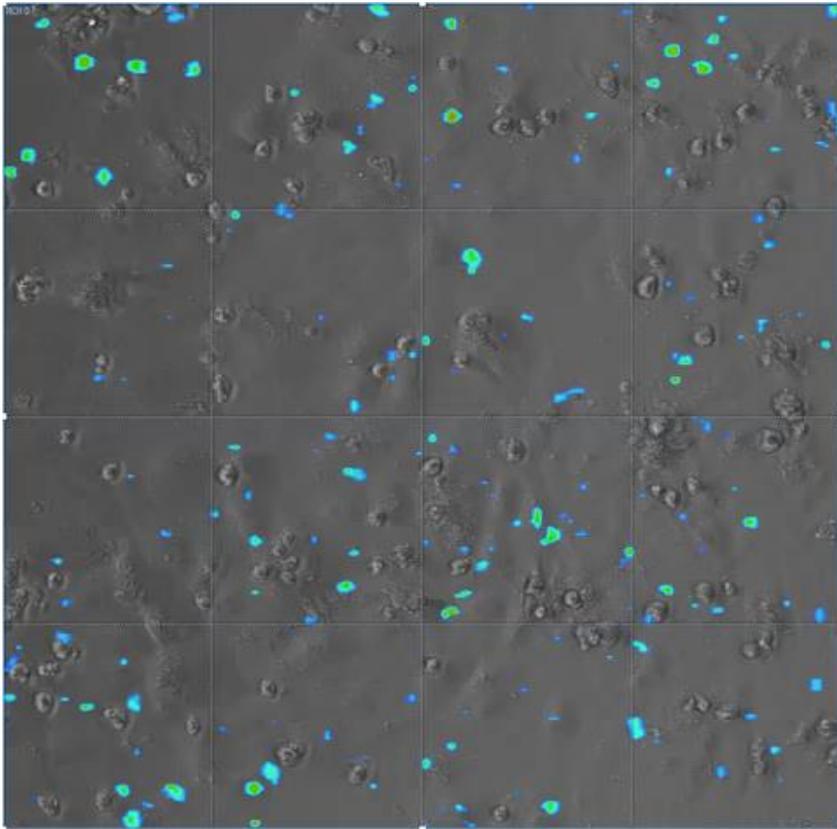


Effect of isoproterenol on motion vector in hiPS-CMs

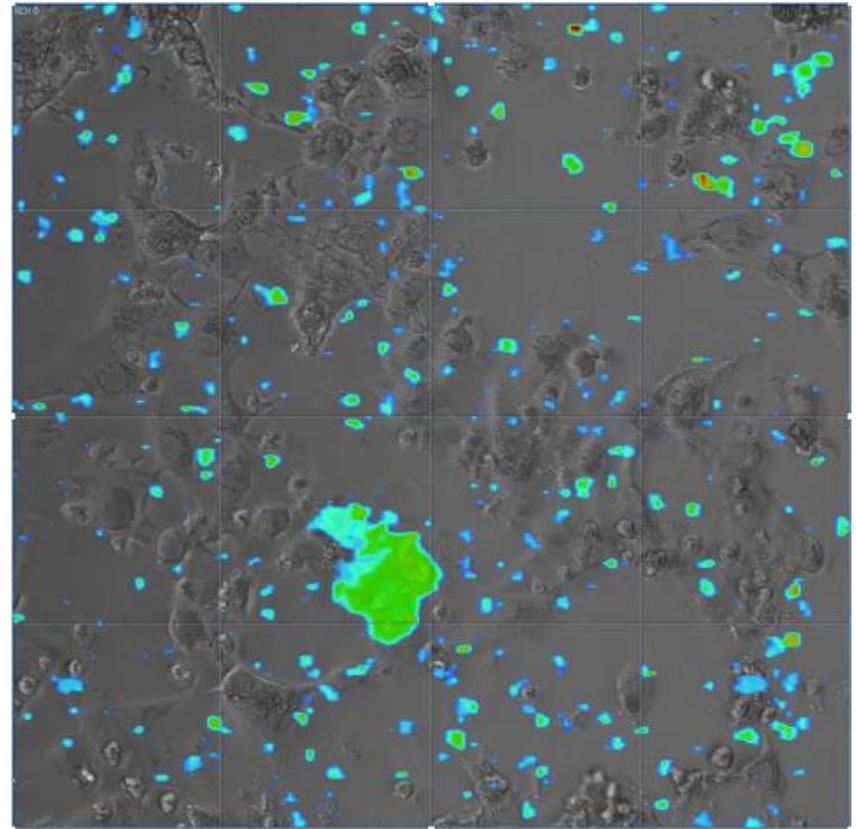


Effect of Doxorubicin on MV system

Before Dox



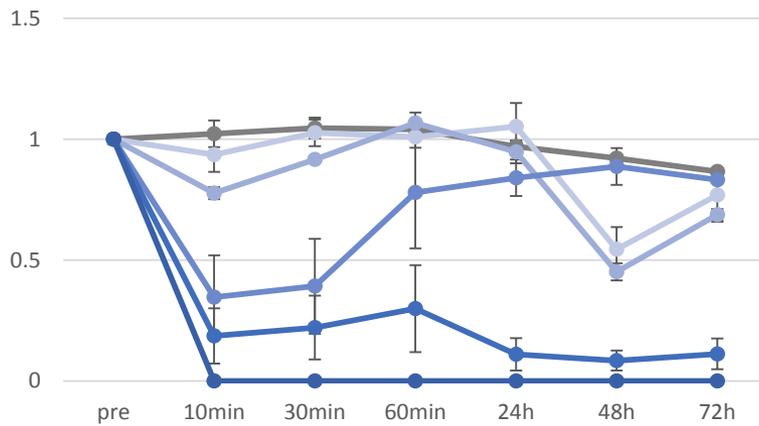
72h after Dox (3 μ M)



Effect of Sunitinib on MV system

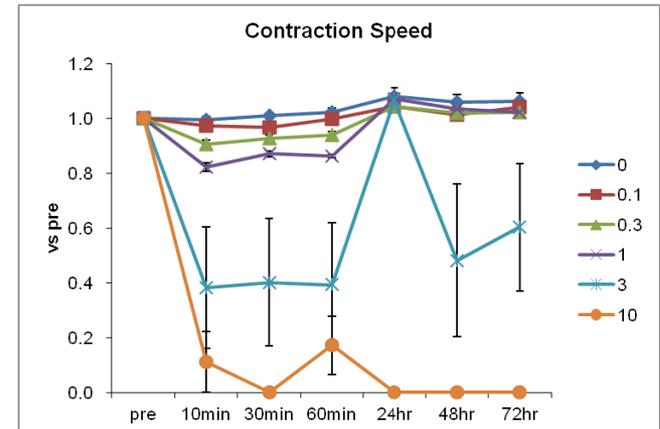
NIHS

Sunitinib CV

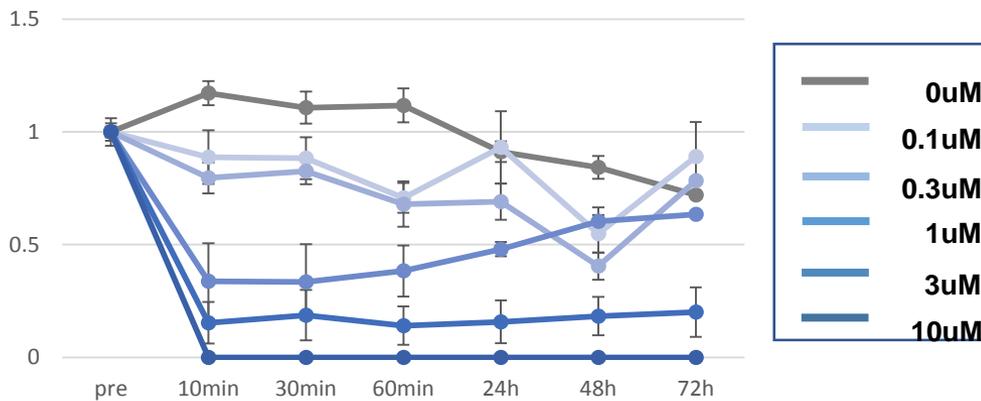


Nippon Shinyaku

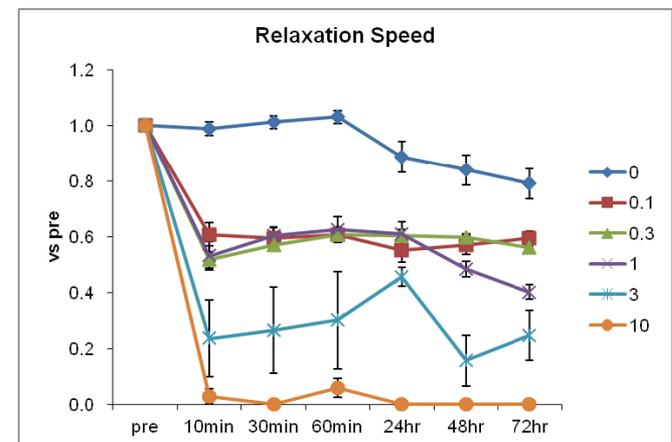
Contraction Speed



Sunitinib RV



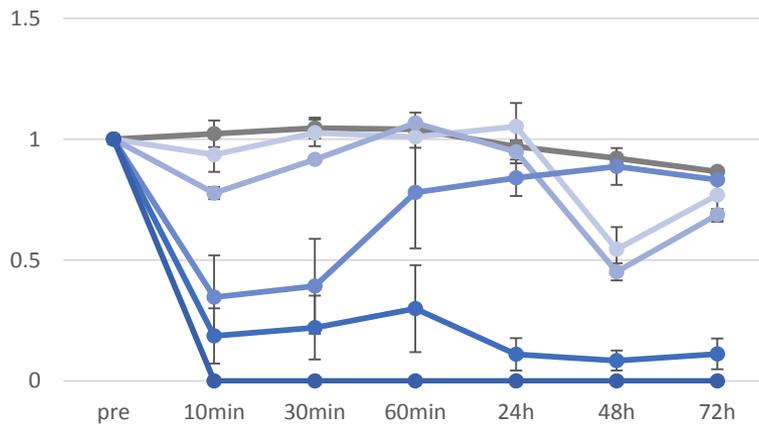
Relaxation Speed



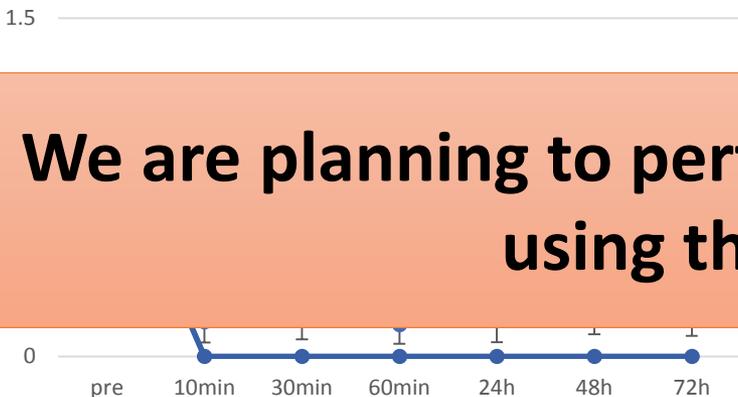
Effect of Sunitinib on MV system

NIHS

Sunitinib CV

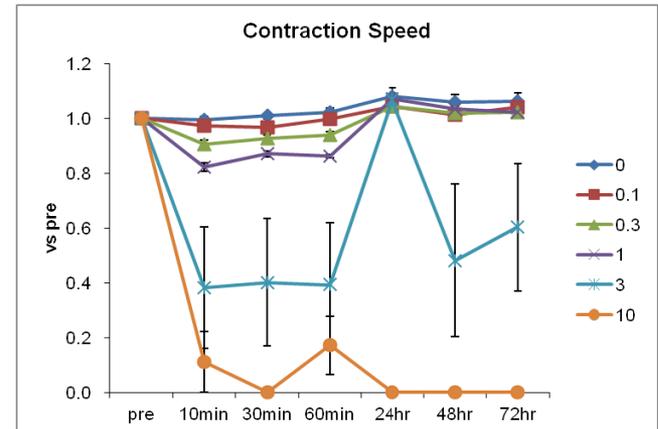


Sunitinib RV

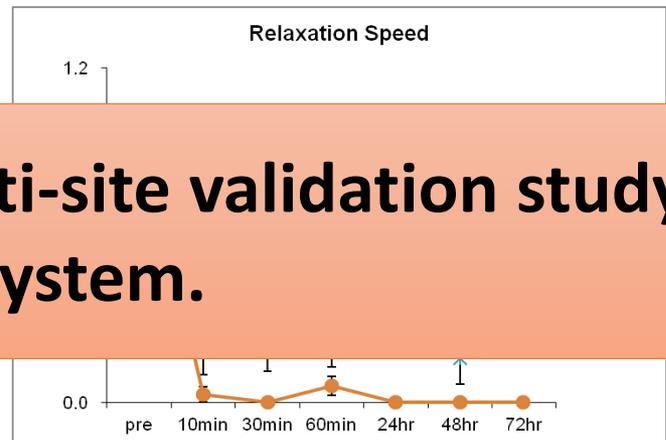


Nippon Shinyaku

Contraction Speed



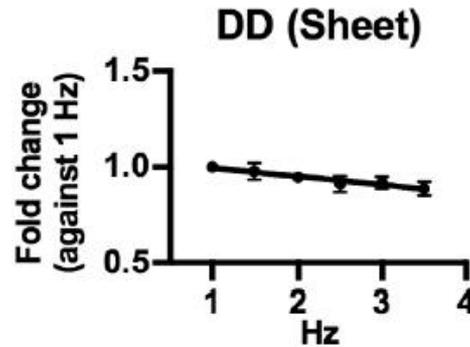
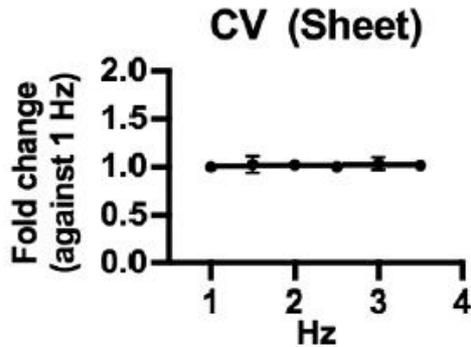
Relaxation Speed



We are planning to perform multi-site validation study using this assay system.

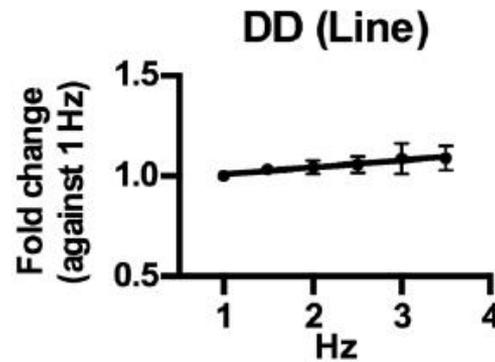
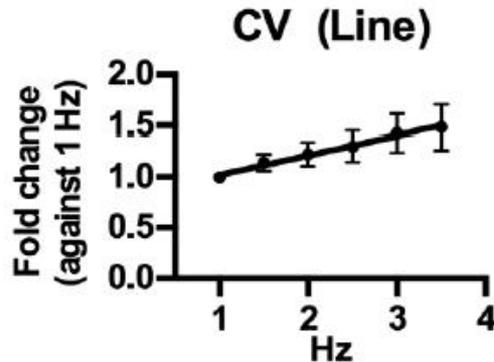
Force-frequency relationship in iPS-CMs

hiPSCMs cultured in monolayer sheet show negative force-frequency relationship



Small rodents
= **Negative** force-frequency relationship

hiPSCMs cultured in line-pattern with specific width show positive force-frequency relationship

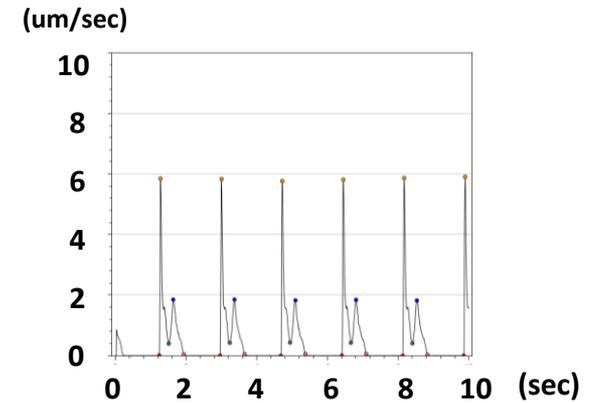
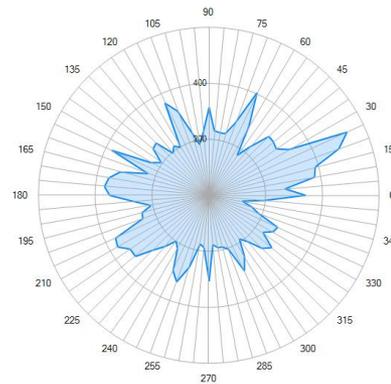
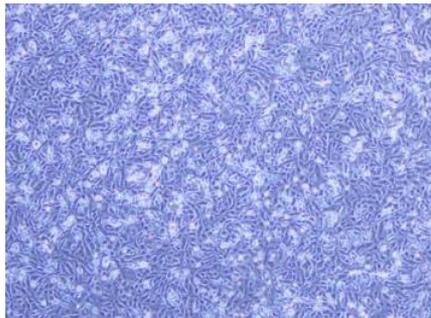


Human & large animals
= **Positive** force-frequency relationship

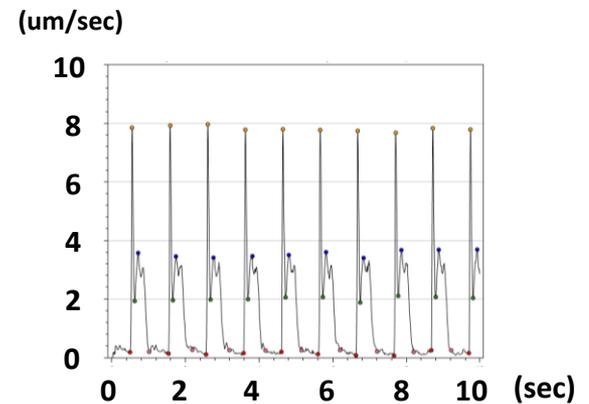
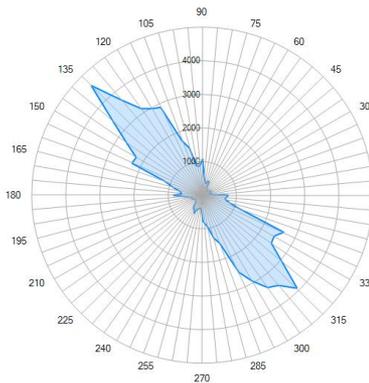
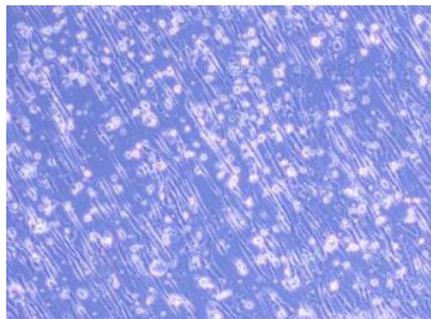


Effect of line-patterned plate on contraction in 2D monolayer

Conventional plate

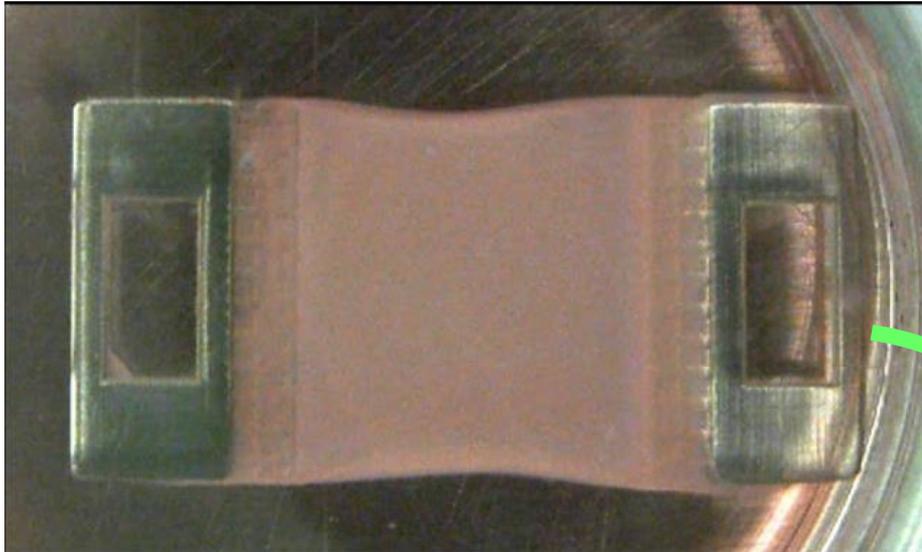


Line-patterned plate

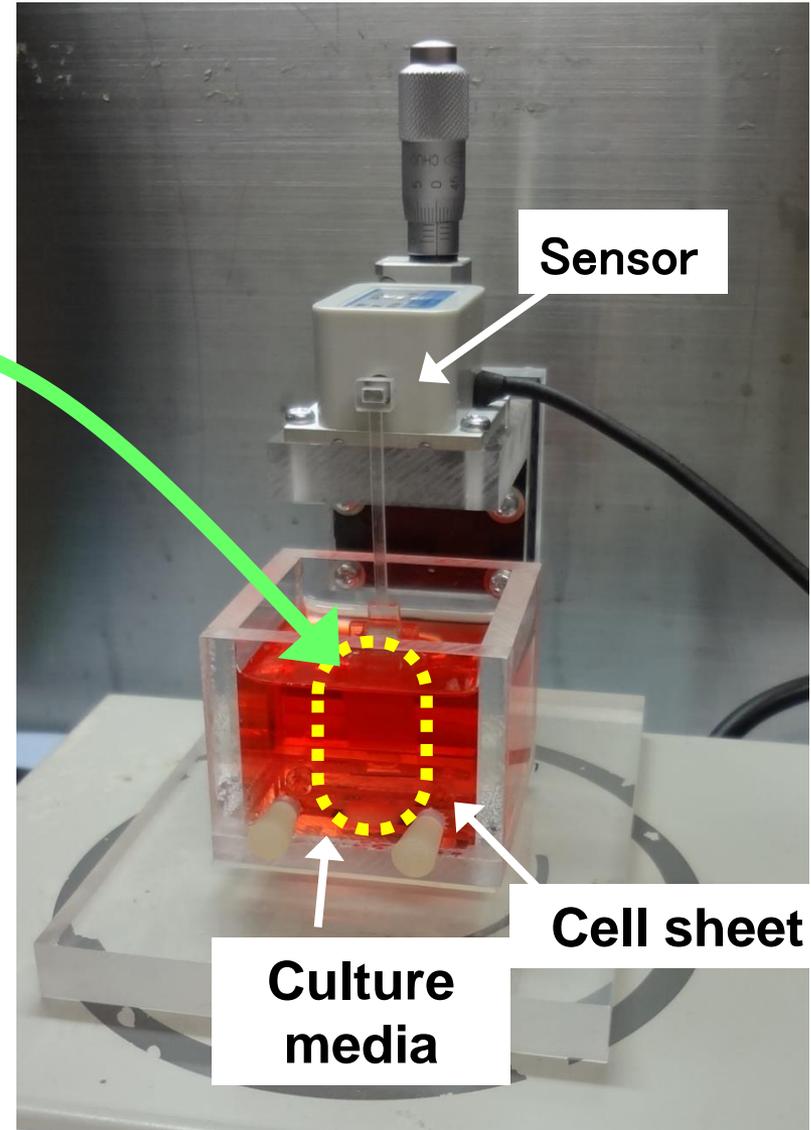
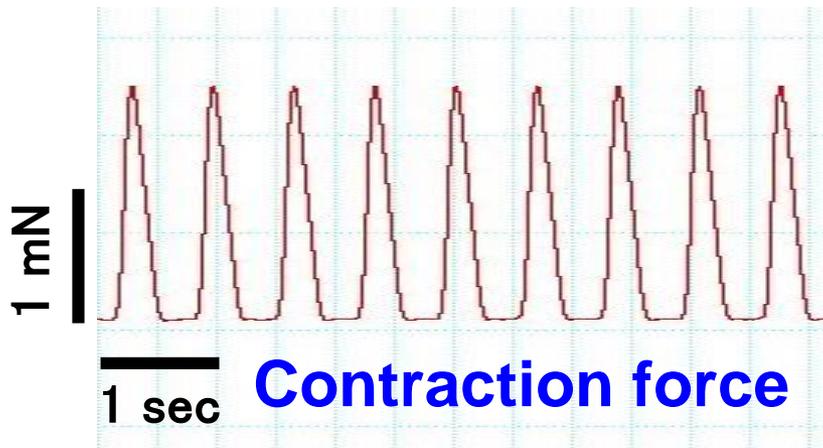


iPS-CMs with alignment improve the drug response?

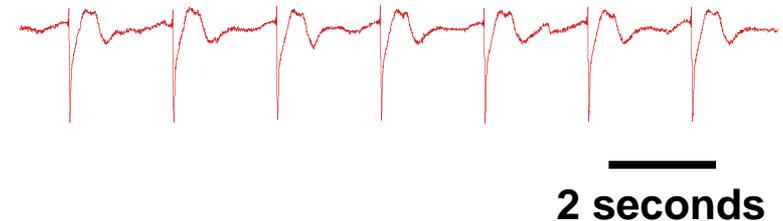
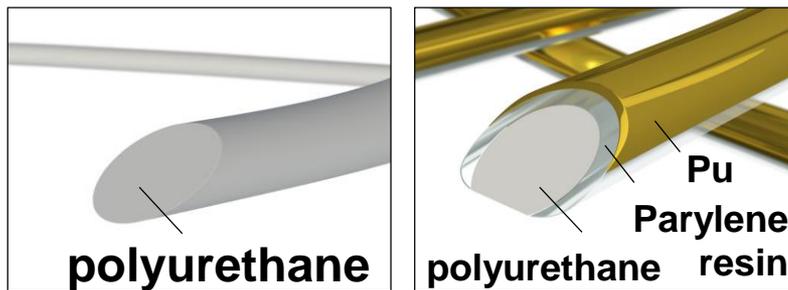
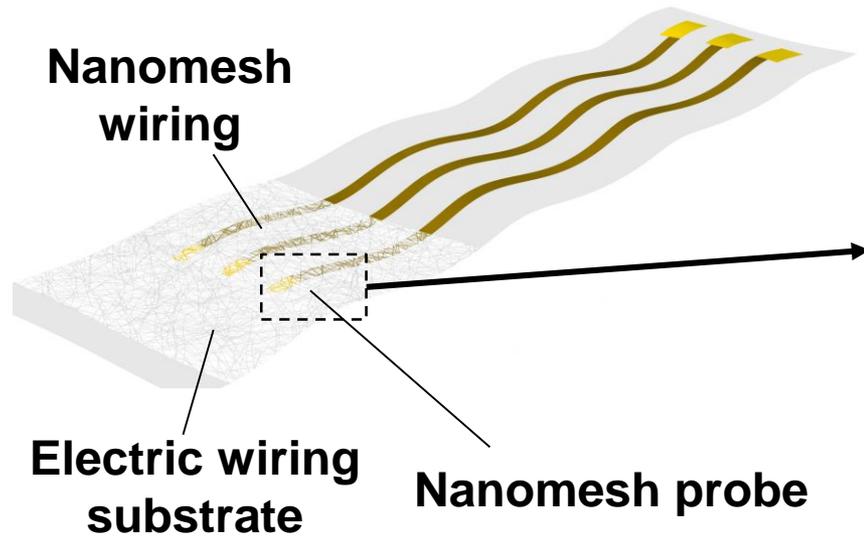
Direct Contraction Force Measurement using Human iPS Cardiac Cell Sheet



iPSC-derived cardiac cell sheet



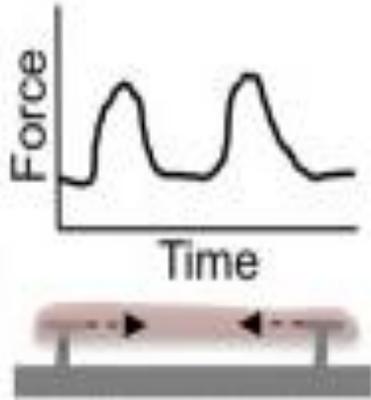
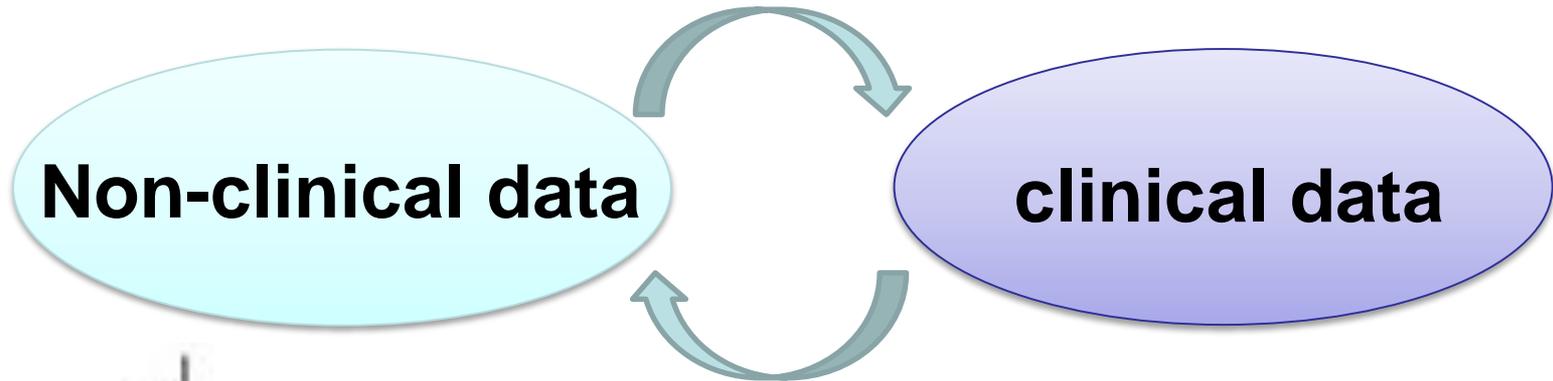
Ultrasoft electronics devise to monitor cardiomyocytes



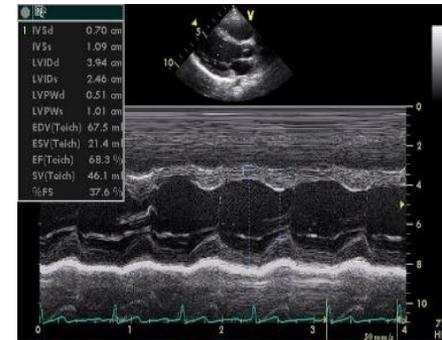
Lee et al. Nat Nanotechnol. 14:156-160, 2019

Simultaneous measurement of contractility and MEA

Translational research



Mechanism study



LVEF
Strain rate
PKPB **etc**

Translational research

Table 6 Proposed diagnostic tools for the detection of cardiotoxicity

Technique	Currently available diagnostic criteria	Advantages	Major limitations
Echocardiography: - 3D-based LVEF - 2D Simpson's LVEF - GLS	<ul style="list-style-type: none"> LVEF: >10 percentage points decrease to a value below the LLN suggests cardiotoxicity. GLS: >15% relative percentage reduction from baseline may suggest risk of cardiotoxicity. 	<ul style="list-style-type: none"> Wide availability. Lack of radiation. Assessment of haemodynamics and other cardiac structures. 	<ul style="list-style-type: none"> Inter-observer variability. Image quality. GLS: inter-vendor variability, technical requirements.
Nuclear cardiac imaging (MUGA)	<ul style="list-style-type: none"> >10 percentage points decrease in LVEF with a value <50% identifies patients with cardiotoxicity. 	<ul style="list-style-type: none"> Reproducibility. 	<ul style="list-style-type: none"> Cumulative radiation exposure. Limited structural and functional information on other cardiac structures.
Cardiac magnetic resonance	<ul style="list-style-type: none"> Typically used if other techniques are non-diagnostic or to confirm the presence of LV dysfunction if LVEF is borderlines. 	<ul style="list-style-type: none"> Accuracy, reproducibility. Detection of diffuse myocardial fibrosis using T1/T2 mapping and ECVF evaluation. 	<ul style="list-style-type: none"> Limited availability. Patient's adaptation (claustrophobia, breath hold, long acquisition times).
Cardiac biomarkers: - Troponin I - High-sensitivity Troponin I - BNP - NT-proBNP	<ul style="list-style-type: none"> A rise identifies patients receiving anthracyclines who may benefit from ACE-Is. Routine role of BNP and NT-proBNP in surveillance of high-risk patient needs further investigation. 	<ul style="list-style-type: none"> Accuracy, reproducibility. Wide availability. High-sensitivity. 	<ul style="list-style-type: none"> Insufficient evidence to establish the significance of subtle rises. Variations with different assays. Role for routine surveillance not clearly established.

European Heart Journal (2016) 37:2768–2801

How can we compare in vitro data with in vivo/human data?

Biomarkers and Omics

✓ Biomarkers

- BNP/ Nt-proBNP
 - Troponin (T and I)
 - miRNAs (miR-1, -499, -208 ...)
 - Fatty acid binding protein (FABP-3)
- etc

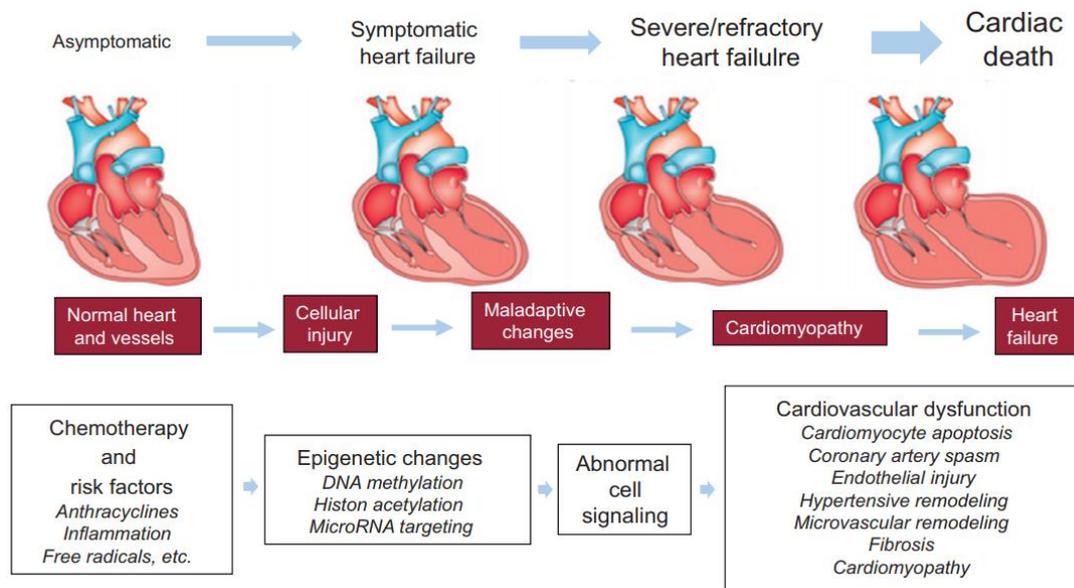
✓ Omics

- SNPs
- Epigenetic modifications.

Circulation. 2005;112:3754–3762.

Oncologist. 2013;18:446–453.

Rev Esp Cardiol. 2017;70(7):576–582

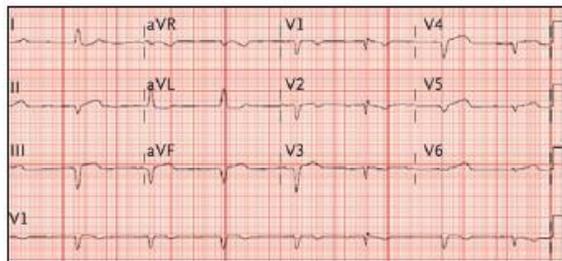


We are currently trying to find suitable biomarkers to bridge non-clinical and clinical settings.

Cardiac toxicity of immune checkpoint inhibitors

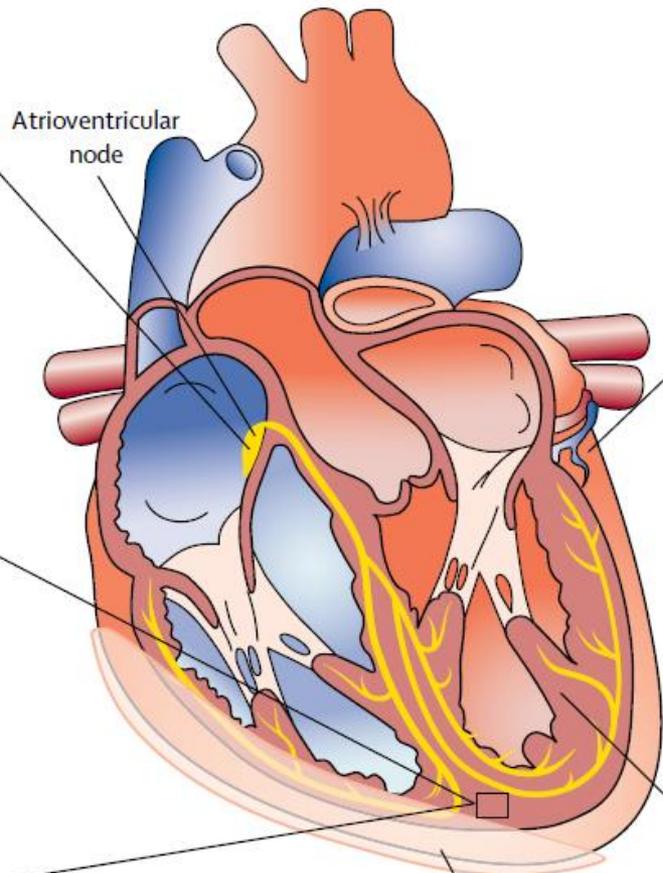
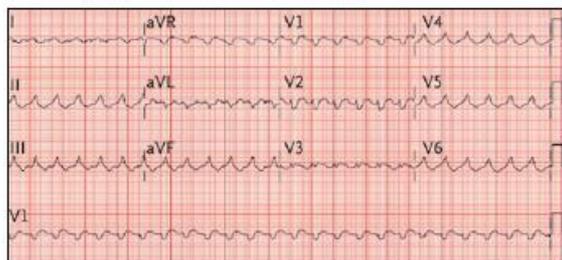
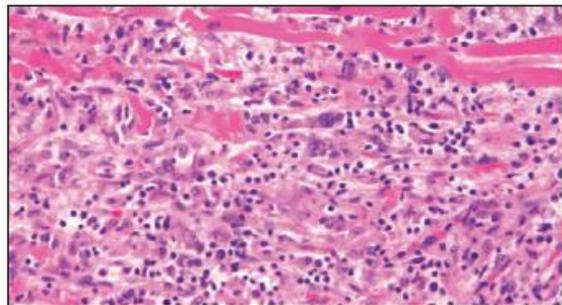
Conduction disease

- Atrioventricular block



Myocarditis

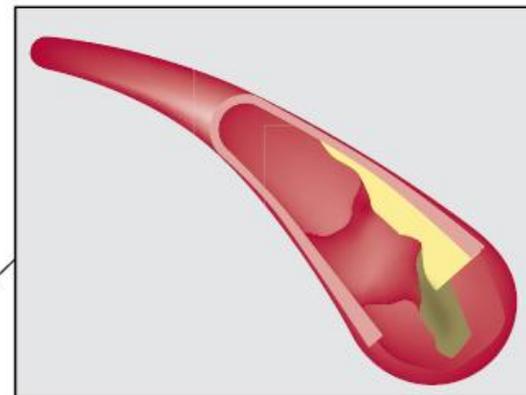
- Heart failure
- Ventricular arrhythmias



- Pericarditis
- Effusion
 - Tamponade

Coronary artery disease

- Atherosclerotic plaque rupture
- Acute myocardial infarction
- Coronary vasculitis

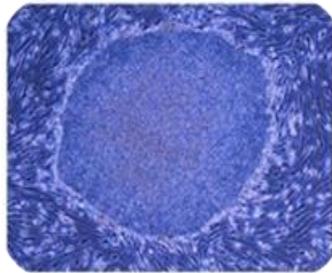


Non-inflammatory left ventricular dysfunction

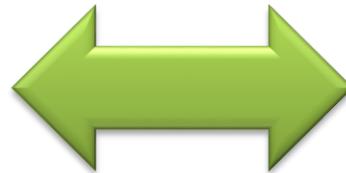
- Heart failure
- Takotsubo syndrome



Modernize non-clinical toxicity to enhance patient safety



Human iPS cells



Safety assessment

- CV toxicity evaluation and mitigation is importance particularly in longer term survivors.
- LV dysfunction can be obtained by imaging methods, such as Echo and GLS.
- A bridge between non-clinical and clinical efforts are needed for patients' safety. Emerging modalities include use of iPS-CMs/motion vector system, biomarkers and omics technologies in the clinic.