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

- Multitargeted tyrosine kinase and vascular endothelial growth factor inhibitors
  - Bevacizumab
  - Sunitinib

- Her2-targeted therapies
  - Trastuzumab
  - Pertuzumab

- Proteasome inhibitors
  - Bortezomib

- Anthracyclines
  - Doxorubicin
  - Epirubicin

- Alkylating agents
  - Cyclophosphamide

- Thalidomide

- Microtubule inhibitors
  - Paclitaxel
  - Docetaxel

- Cardiomyocyte damage and heart failure

- Hypertension
- Ischemia
- Vascular effects
- Coronary disease

- Valvular disease

- Pericardial disease

- Thromboembolism

CiPA/JiCSA studies etc

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

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 Asteggianno (Italy), Maurizio Galderisi (Italy), Gilbert Habib (France), Daniel J. Lenihan† (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)
# Cancer therapy associated with Heart failure/Left Ventricular Dysfunction

<table>
<thead>
<tr>
<th>Chemotherapy agents</th>
<th>Incidence (%)</th>
<th>Frequency of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthracyclines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin (Adriamycin®)¹</td>
<td>3-26*#</td>
<td>+++++</td>
</tr>
<tr>
<td>Epirubicin (Ellence®)¹</td>
<td>0.9-3.3#</td>
<td>+</td>
</tr>
<tr>
<td>Idarubicin (Idamycin PFS®)¹</td>
<td>5-18#</td>
<td>++</td>
</tr>
<tr>
<td><strong>Small molecule tyrosine kinase inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dabrafenib (Tafinlar®)²</td>
<td>8-9#</td>
<td>+++++</td>
</tr>
<tr>
<td>Dasatinib (Sprycel®)¹</td>
<td>2-4#</td>
<td>+++++</td>
</tr>
<tr>
<td>Lapatinib (Tykerb®)¹,²</td>
<td>0.9-4.9#</td>
<td>+++++</td>
</tr>
<tr>
<td>Pazopanib (Votrient®)²</td>
<td>0.6-11#</td>
<td>+++++</td>
</tr>
<tr>
<td>Ponatinib (Iclusig®)²</td>
<td>3-15b</td>
<td>+</td>
</tr>
<tr>
<td>Sorafenib (Nexavar®)¹,²</td>
<td>1.9-11</td>
<td>+++++</td>
</tr>
<tr>
<td>Sunitinib (Sutent®)²</td>
<td>1-27#</td>
<td>+++++</td>
</tr>
<tr>
<td>Trametanib (Mekinist®)²</td>
<td>7-11#</td>
<td>+++++</td>
</tr>
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MD Anderson Practices In Onco-Cardiology
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.
Is it possible to predict the effect of drugs on left ventricular function?

### Various methods for functional analyses in iPSC-CMs

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

iPSC-based cardiac contractility using motion vector system

Movie = a series of pictures (frames)

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

(Hayakawa, Kanda et al. JMCC, 2014)
Motion detection from single hiPS-CM

hiPS-CMs (iCell)

Motion profile (5 seconds)

Beating rate: 72/min

Average velocity (μm/s)

Time (s)

0 1 2 3 4 5

0 4 8 12

Single beat profile

Average velocity (μm/s)

0 200 400

0 4 8 12

contraction

relaxation
Effect of isoproterenol on motion vector in hiPS-CMs

- Max contraction velocity
- Max relaxation velocity
- Beating rate

Average velocity (μm/s) vs. Time (s)

- Control
- Isoproterenol

iCell (CDI)
Effect of isoproterenol on motion vector in hiPS-CMs

Isobe et al. JTS 43:493 (2018)
Effect of Doxorubicin on MV system

Before Dox

72h after Dox (3µM)
Effect of Sunitinib on MV system

NIHS

Sunitinib CV

Nippon Shinyaku

Sunitinib RV
Effect of Sunitinib on MV system

We are planning to perform multi-site validation study using this assay system.
hiPSCMs cultured in monolayer sheet show negative force-frequency relationship

- CV (Sheet)
  - Fold change (against 1 Hz)
  - Hz

- DD (Sheet)
  - Fold change (against 1 Hz)
  - Hz

Small rodents = Negative force-frequency relationship

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

- CV (Line)
  - Fold change (against 1 Hz)
  - Hz

- DD (Line)
  - Fold change (against 1 Hz)
  - Hz

Human & large animals = Positive force-frequency relationship

Slide from Dr. Naito (Toho Univ)
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

Sensor

Culture media

Cell sheet

1 mN

1 sec

Contraction force

Ultrasoft electronics devise to monitor cardiomyocytes

Simultaneous measurement of contractility and MEA

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

Non-clinical data

clinical data

Mechanism study

LVEF
Strain rate
PKPB
etc
## Translational research

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

### Table 6  Proposed diagnostic tools for the detection of cardiotoxicity

<table>
<thead>
<tr>
<th>Technique</th>
<th>Currently available diagnostic criteria</th>
<th>Advantages</th>
<th>Major limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echocardiography:</strong></td>
<td>- 3D-based LVEF &lt;br&gt;- 2D Simpson’s LVEF &lt;br&gt;- GLS</td>
<td>- Wide availability. &lt;br&gt;- Lack of radiation. &lt;br&gt;- Assessment of haemodynamics and other cardiac structures.</td>
<td>- Inter-observer variability. &lt;br&gt;- Image quality. &lt;br&gt;- GLS: inter-vendor variability, technical requirements.</td>
</tr>
<tr>
<td><strong>Nuclear cardiac imaging</strong></td>
<td>(MUGA)</td>
<td>- Reproducibility.</td>
<td>- Cumulative radiation exposure. &lt;br&gt;- Limited structural and functional information on other cardiac structures.</td>
</tr>
<tr>
<td><strong>Cardiac magnetic resonance</strong></td>
<td></td>
<td>- Accuracy, reproducibility. &lt;br&gt;- Detection of diffuse myocardial fibrosis using T1/T2 mapping and ECVF evaluation.</td>
<td>- Limited availability. &lt;br&gt;- Patient’s adaptation (claustrophobia, breath hold, long acquisition times).</td>
</tr>
<tr>
<td><strong>Cardiac biomarkers:</strong></td>
<td>- Troponin I &lt;br&gt;- High-sensitivity Troponin I &lt;br&gt;- BNP &lt;br&gt;- NT-proBNP</td>
<td>- Accuracy, reproducibility. &lt;br&gt;- Wide availability. &lt;br&gt;- High-sensitivity.</td>
<td>- Insufficient evidence to establish the significance of subtle rises. &lt;br&gt;- Variations with different assays. &lt;br&gt;- Role for routine surveillance not clearly established.</td>
</tr>
</tbody>
</table>
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.

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

Cardiac toxicity of immune checkpoint inhibitors

Joe-Elie Salem et al. Lancet Oncology, 2018
Modernize non-clinical toxicity to enhance patient safety

- 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.