Human iPS/ES Cell Technology and Its Application to Toxicology Testing

-Especially focusing on in vitro cardiac function toxicity-

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Outline

Preclinical Testing for Cardiac Electrophysiologic Safety

- ICH S7A and the following S7B Guidance
- hERG: Ability and Limitation
- Beyond hERG: On-chip Quasi-\textit{in vivo} Assay
- Human Cardiomyocytes delivered from hES/hiPS Cells
- Strategy I: Temporal fluctuation measurement
- Strategy II: Spatial tachyarrhythmia/fibrillation measurement

Call for adaptable preclinical strategies to evaluate global cardiac safety
Goals for Preclinical Safety Testing

Primary Goal: Ensuring The Safety of Phase 1 Volunteers

Additional Interests Represent a Mix of Internal and Regulatory Issues
- Guide selection of Discovery candidates
- Provide pharmacodynamic information on metabolites
- Minimize attrition later in Discovery/Development
- Guide conduct of early First in Human studies
- Enable early Concentration-QT(VT/VF) studies
- Regulatory Issues

Call for adaptable preclinical strategies to evaluate global cardiac safety
Preclinical Testing: Regulatory Perspectives

ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals

Objectives:
- To protect clinical trial participants and patients receiving marketed products from potential adverse effects of pharmaceuticals.
- Avoiding unnecessary use of animals and other resources.

“Effects of the test substance on the cardiovascular system should be assessed appropriately. Blood pressure, heart rate, and the electrocardiogram should be evaluated. In vivo, in vitro and/or ex vivo evaluations, including methods for repolarization and conductance abnormalities, should also be considered.”

⇒ S7B represents minimal approach to addressing repolarization safety, not consider global cardiac electrophysiologic effects.
hERG: Ability and Limitations

Exceptions to “link” between hERG block and TdP

- Most drugs that have been removed from the market block hERG current.

- However, not all drugs that block hERG prolong QT or TdP (Verapamil, fluoxetine, tolterodine....)

- IC50 values must be placed in context. (most cmpds block hERG at excessive conc.)

Likely that compounds are unduly discarded in Discovery due to early hERG screening
Necessity to Go “Beyond hERG”

Utility of hERG Assay (in isolation) violates “Pharmacology Rule #2”
– No one assay is 100% Sensitive and Specific

Eliminating hERG liability removes one proarrhythmic risk factor

Ventricular fibrillation might occur without QT prolongation

hERG current assay: A surrogate marker for a surrogate marker

<table>
<thead>
<tr>
<th>Multiple factors</th>
<th>Multiple factors</th>
<th>Torsades de Pointes</th>
</tr>
</thead>
<tbody>
<tr>
<td>hERG ≠ QT</td>
<td>QT ≠ Prolongation</td>
<td>Ventricular Tachycardia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ventricular Fibrillation</td>
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</tbody>
</table>

Predicting VT(TdP), VF
Ensuring “Global” Cardiac Safety
Beyond hERG: Our Solution
On-chip Quasi-in vivo Assay

• Quasi-in vivo assay includes:

1) Using a set of standard human cardiomyocytes prepared from human iPS/ES cells of different races, sexes and also from patients with various diseases to provide an ideal testing panel platform.

2) To predict lethal arrhythmia (TdP/VT/VF) by evaluation of (1) temporal fluctuation of single-cell-level ion channels kinetics, and by evaluation of (2) spatial cell-to-cell conductance fluctuation using the on-chip cell network loop which can choose different conductance pathways of human cardiomyocytes among neighboring circulations.

3) The capacity to quantitatively evaluate the correlation between calcium release and tension generation, and the inhibition on the trafficking pathway of ion-channel proteins by its long-term optical/electrical simultaneous measurement.
Quasi-*in vivo* Assay:
Human Cardiomyocytes delivered from hES/hiPS Cells

**hiPS Cardiomyocyte Cluster**

Prolongation of field potential duration (FPD) caused by E-4031 blocking.

Increasing of the beating rate induced by isoproterenol (ISO)

Quasi-\textit{in vivo} Assay: Strategy I.
Temporal fluctuation of single-cell-level ion channels kinetics

\begin{itemize}
\item Field Potential Recording
\item Currents recorded from a single cell on a microelectrode
\item Long-term measurement during on-chip cultivation
\end{itemize}
Quasi-\textit{in vivo} Assay: Strategy I.
Temporal fluctuation of single-cell-level ion channels kinetics

- Fluctuation measurement of ion channels for TdP prediction

Field Potential Signals  \downarrow  Field Potential Durations (FPDs)

\textbf{X-axis: Mean FPD value (Similar index of APD)}

\textbf{IC}_{50} (7.7 \text{nM})

\textbf{Y-axis: FPD Fluctuation (New index)}

\textbf{E-4031 (n=27)}
Quasi-*in vivo* Assay: Strategy I.
Temporal fluctuation of single-cell-level ion channels kinetics

**on-chip FPD & STV assay using hES-CMCs**

**in vitro assay and clinical**

<table>
<thead>
<tr>
<th>Category</th>
<th><em>in vitro assay</em></th>
<th>Clinical</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>hERG</td>
<td>APD†</td>
</tr>
<tr>
<td>Positive</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>APD false negative</td>
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<td>-</td>
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<tr>
<td>hERG false negative</td>
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<td>hERG false positive</td>
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<td>?</td>
</tr>
<tr>
<td>Negative</td>
<td>-</td>
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</tbody>
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†: Guinea-pig papillary muscle

Drug conc.: 100 ~ 1000-hold $C_{\text{Max}}$ of plasma conc. at clinical use.
Quasi-\textit{in vivo} Assay: Strategy II. Spatial cell-to-cell conductance fluctuation

- Evaluate spatial dispersion of repolarization (a proposed mechanism of proarrhythmia: ion-channel fluctuation + electrical conductivity change)
- Measure changes of QT (ST) intervals, TdP and VT/VF

**A**
- Action potential recordings
- Action potential duration (APD)
- Integration
- Differential

**B**
- Field potential recordings (each single electrode)
- Field potential duration (FPD)
- On-chip cell network assay (Field potential recordings)

**C**
- Quasi ECG (Sum of electrodes)
- Pseudo ST interval

**D**
- In vivo ECG
- QT interval
- QT prolongation

Established \textit{in vitro} assay (Patch clamp assay)

Established \textit{in vivo} assay (ECG)

Single cell information

Cell-to-cell conductance information
Quasi-	extit{in vivo} Assay: Strategy II. Spatial cell-to-cell conductance fluctuation

We can acquire the following three indices simultaneously:

- **APD prolongation (FPD prolongation)**
- **QT interval prolongation** (ST interval ?)
- **Torsades de Pointes** (VT/VF)

**MEA chip**

**2D Sheet Based Network**

**Spin coating of agarose**

**Collagen layer**

**Collagen coating**

**Agarose layer**

**Microfabrication by IR laser**

**ITO electrode with PtB**

**Spin coating of agarose**

**Collagen layer**

**Agarose layer**

**MEA chip**

**Bleu: Nuclear Green: Mitochondria (Cardiomyocyte)**

**50 µm**

**100 µm**

**6 Myocytes & 2 Fibroblasts in yellow box**
Quasi-\textit{in vivo} Assay: Strategy II.

Spatial cell-to-cell conductance fluctuation

Cell network

Ring electrode

Before addition of drug (Astemizole)

10 nM

100 nM

1 \mu M

Informational Content Much Greater than Simply QT(ST) Prolongation
Conclusion:
On-chip quasi-in vivo assay has strong potential to go beyond hERG


1) A set of standard human cardiomyocytes prepared from human iPS/ES cells of different races, sexes and also from patients with various diseases to provide an ideal testing panel platform.

2) Prediction of lethal arrhythmia (TdP/VT/VF) by evaluation of temporal fluctuation of single-cell-level ion channels kinetics, and by evaluation of spatial cell-to-cell conductance fluctuation using the on-chip cell network loop which can choose different conductance pathways of human cardiomyocytes among neighboring circulations.

3) Correlation between calcium release and tension generation, and Inhibition on the trafficking pathway of ion-channel proteins.

Electrical and Mechanical Effects of Novel Drug Candidates Must Both Be Considered in Preclinical Global Cardiac Safety Assessments