In vivo tumorigenicity testing for pluripotent stem cell-derived products

FIRM-CoNCEPT-MEASURE
STEP2 In Vivo Tumorigenicity Subgroup
Acting Leader

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Focusing on the Pluripotent Stem Cell derived Cell Therapy Products (CTPs)

• There are **two kinds of risks of tumorigenicity** of CTPs derived from the pluripotent stem cells, **the one is teratoma derived from undifferentiated iPS cells, the other is transformation of cells.**
• Somatic/somatic stem cell derived CTPs have less concern for tumorigenicity compared to iPS/ES cell derived CTPs.
Types of Tumorigenicity Tests/Assays

1. Residual undifferentiated iPS cells
2. Contamination of transformed cells
3. Tumor formation in micro environment after transplantation

- *In vitro* assays currently reported are for detection of residual undifferentiated cells and contamination of transformed cells.
- *In vivo* assay can be detected all types of tumorigenicity using iPS cells and final product (CTPs).
## In vivo Tumorigenicity Tests

<table>
<thead>
<tr>
<th>Tumorigenicity in micro environment</th>
<th>Transformed cells</th>
<th>Residual undifferentiated iPS cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal 1 species is enough. Immuno-deficient mouse or nude rat</td>
<td>1 species is enough. Immuno-deficient mouse or nude rat</td>
<td>Residual undifferentiated iPS cells</td>
</tr>
<tr>
<td>Number of animals</td>
<td>10/group (at the final evaluation)</td>
<td>10/group (at the final evaluation)</td>
</tr>
<tr>
<td>Dose (Number of cells)</td>
<td>MTD or MFD should be dosed. Negative control is needed (positive control is not mandatory)</td>
<td>MTD or MFD should be dosed. Negative control is needed (positive control is not mandatory)</td>
</tr>
<tr>
<td>Regimen</td>
<td>Single Dose</td>
<td>Single Dose</td>
</tr>
<tr>
<td>Administration route</td>
<td>Basically, Clinical intended route</td>
<td>Clinical intended route or Subcutaneous</td>
</tr>
<tr>
<td>Observation period</td>
<td>Basically, Life Span</td>
<td>On the case by case basis, 4 to 16 weeks could be applicable</td>
</tr>
</tbody>
</table>
**FIRM-CoNCEPT-MEASURE**

*In vivo* Tumorigenicity Subgroup

**Purpose:** development of a standard method for detecting residual undifferentiated iPS cells in CTPs in *in vivo* tumorigenicity test.

**Participant 5 companies**

<table>
<thead>
<tr>
<th>Timeline</th>
<th>2017</th>
<th>2018</th>
<th>2019</th>
<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>S</td>
<td>T</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>F</td>
<td>S</td>
<td>T</td>
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<tr>
<td></td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>S</td>
</tr>
</tbody>
</table>

- Preliminary study
- Validate the method by main multisite study
- Summarize & documentation

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Preliminary Multisite Study for Detection of Residual Undifferentiated iPS Cells

Preliminary Protocol

• **Purpose**: Confirm test procedures in 6 studies at 5 sites (CROs)
  – Transplantation method, dosing-cell preparation, observation, mortality, histopathological examination and so on

• **Animal**: NOD/Shi-scid, IL-2RγKO Jic (NOG) mouse
  – Highly immunocompromised animal, no activity of T cell, B cell and NK cell, and easy to purchase as a commercial animal

• **Dose**: Transplantation of $10^2, 10^3, 10^4$ or $10^5$ iPS cells (Cellartis® human iPS cell line (ChiPSC18)) with Corning® Matrigel®, ROCK inhibitor, and $10^6$ Normal Human Dermal Fibroblasts (NHDF) (treated with mitomycin-C)
  – Matrigel®: to advance induction of teratomas by iPS cells
  – ROCK inhibitor: supporting survival of iPS cells
  – NHDF: useful feeder cells to *in vivo* tumorigenicity studies
Preliminary Multisite Study for Detection of Residual Undifferentiated iPS Cells

Preliminary Protocol (continued)

• **Group size:** 6 male mice/group

• **Dosing:** single subcutaneous injection, abdominal region

• **Study duration:** 20 weeks after dosing

• **Parameters:** clinical observations, body weights, food consumption, measurement of subcutaneous nodule size in the injection site, gross pathology, histopathology, and the 50% Tumor Producing Dose (TPD50 value) calculated

⇒ Compare the results from each studies and sites, and decide the method to be validated by the main multisite study

Preliminary Multisite Study for Detection of Residual Undifferentiated iPS Cells

Results from preliminary study

• **Measurement of subcutaneous nodule size in the injection site**
  - Formation of subcutaneous nodule is observed in all groups of all studies, not all animals.
  - Increase of nodule size is observed from Week 2 at the highest dose group.
  - Animals are sacrificed from Week 6 at the highest dose group by humane endpoint based on excessive growth of subcutaneous nodule.

• **Clinical observation, Body weight, Food Consumption**
  - No abnormal findings are observed in these parameters in all groups of all studies except for subcutaneous nodule.
### Preliminary Multisite Study for Detection of Residual Undifferentiated iPS Cells

**Results from preliminary study (continued)**

**“Teratoma” occurrence in the injection site and TPD50**

<table>
<thead>
<tr>
<th>Dose of iPS cells</th>
<th>$10^2$</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
<th>TPD50</th>
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</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Study 1</td>
<td>6#</td>
<td>6</td>
<td>6</td>
<td>6</td>
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<td>Study 2</td>
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<td>6</td>
<td>6</td>
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<tr>
<td>Study 5</td>
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<td>6</td>
<td>6</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
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<td>4</td>
<td>5</td>
<td>6</td>
<td>680</td>
</tr>
</tbody>
</table>

#: No. of animals bearing “Teratoma” which is diagnosed histopathologically

*: Actual injection doses of this study are $0.75 \times 10^2$, $0.75 \times 10^3$, $0.75 \times 10^4$ and $0.75 \times 10^5$. 
Preliminary Multisite Study for Detection of Residual Undifferentiated iPS Cells

Results from preliminary study (continued)

Histopathological findings of “Teratoma”

- Ectoderm: melanocyte
- Endoderm: intestine
- Mesoderm: cartilage
Main Multisite Study for Detection of Residual Undifferentiated iPS Cells

Protocol for the main multisite study (now conducting)

- **Purpose**: Confirm test procedures in [4 studies at 4 sites](#) (CROs)
- **Animal**: NOG mouse
- **Dose**: Transplantation of $10, 10^2, 10^3$ or $10^4$ iPS cells (ChiPSC18) with Matrigel®, ROCK inhibitor and $10^6$ NHDF
- **Group size**: 6 male and 6 female mice/group
- **Dosing**: Single subcutaneous injection, abdominal region
- **Study duration**: 20 weeks after dosing
- **Parameters**: clinical observations, body weights, food consumption, measurement of subcutaneous nodule size, gross pathology, histopathology, and TPD50 value

*if necessary, immunochemical stain using human antibody*

Results will be obtained by the end of October 2018.
Thank you for your attention!!
Back Up
Reference

Standardization of the teratoma assay for analysis of pluripotency of human ES cells and biosafety of their differentiated progeny.


iPS 4x10^5/ml
300µl
+ NHDF 4x10^7/ml
300µl

iPS medium 270µl
+ NHDF 4x10^7/ml
270µl

iPS medium 270µl
+ NHDF 4x10^7/ml
270µl

iPS medium 270µl
+ NHDF 4x10^7/ml
270µl

discard

60µl

600
↓
540µl

iPS 1x10^4
/100µl

iPS 1x10^3
/100µl

iPS 1x10^2
/100µl

iPS 1x10
/100µl

+MG 540µl
↓
1080µl

+MG 540µl
↓
1080µl

+MG 540µl
↓
1080µl

+MG 540µl
↓
1080µl