Development of Allogenic Regenerative Medicine, TEMCELL® HS Inj.

Specifications/Potency:
Specifications and Acceptance Criteria for Cell-based Products

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Group Manager(Associate Director), Cell Therapy, Biopharmaceutical Innovation Research Institute, Research Division
CONTENTS:

» Background: Company Profile
   About TEMCELL® HS inj.

» Case study; TEMCELL® HS inj.
   Establish Specifications and Acceptance Criteria

» Summary
Company Profile
Company profile : JCR Pharmaceuticals

Founded : September 13, 1975
Representative : Shin Ashida (Chairman, President & CEO)
Headquarters : Ashiya, Hyogo Prefecture, JAPAN
Employees : 513 (As of Sep. 30, 2016)
Ownership : Public (listed on 1st Sec. Tokyo Stock Exchange, in 2013)
Securities code : 4552
Mission : To discover, develop, manufacture and market biotherapeutic products for human healthcare
R&D Focus : Rare diseases/orphan drugs,
  Recombinant Protein, Allogeneic Regenerative Medicine
Location and Core Products:

GROWJECT®
(Recombinant human growth hormone)

TEMCELL® HS Inj.

Epoetin Alfa BS inj. JCR
(Recombinant Erythropoietin)

Ashiya : Headquarters
Kobe : Research Institute, 4 Plants

Sendai

Tokyo

Okayama

Nagoya

Takamatsu

Fukuoka
Development History : TEMCELL® HS Inj.

2003 • License agreement with Osiris therapeutics (USA) *

2007 • Phase I/II clinical study

2011 • Phase II/III clinical study

2013 • Orphan drug designation

2014 • Application for new cell-based medicine

2015 • Sep 18, First approval for allogeneic regenerative medicine in Japan

2016 • Feb 24, commercial launch

* The licensor has been changed to Mesoblast Group (Australia) following the assignment of hMSCs-related rights from Osiris to Mesoblast in October 2013
**Background**: TEMCELL® HS Inj.

**Human Bone Marrow-derived Mesenchymal stem cells**

**Components**

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Mesenchymal stem cells</th>
<th>7.2 x 10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive ingredients</td>
<td>DMSO</td>
<td>10% (v/v)</td>
</tr>
<tr>
<td></td>
<td>Human derived Albumin</td>
<td>from donation</td>
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<tr>
<td></td>
<td>Ringer solution</td>
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</tbody>
</table>

**Packaging**: freezing bag packed in paper box

**Storage**: Vapored liquid nitrogen (under -130 degrees C.)

**Expiration period**: 5 years from manufacturing date
**Indication**

Acute GVHD after hematopoietic stem cell transplantation

**Dosage and administration**

administer 2 million cells / kg body weight via intravenous infusion

**Background : TEMCELL® HS Inj.**

- Administer 2 infusions per week for 4 weeks
- Additionally administer one infusion weekly for 4 weeks depending on the degree of symptoms

**total 8 infusions per 4 week-treatment**

(2 infusions per week)
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» Summary
General consideration to establish specifications

Features of Cell Therapy Products;

- Live Cells
- Heterogeneity
- Variation; raw material, process and donor derived
- No Reference Standards
- Mechanism of Action (MoA) is not well elucidated
- Present multiple mode of action

Set multiple candidate of test items and wide range of acceptance criteria

= wide specification
General consideration to establish *Potency assay*;

indicate specific ability or capacity of product

- *ideally measures by quantitative method*
- *mimic biological activity*
- *link in vitro pharmacology test*

assume MoA and its properties
by non clinical study, in vitro tests, clinical study and literature

Need to set “release test” for the final product

Useful for;

✓ Define the shelf life
✓ Establish Comparability study
  → process change, shipping validation, in use stability etc.
  (viability is not enough)
# General consideration to establish specifications

**Reference guideline:** ICH Q6B --- Specification, ICH Q5A --- Safety tests, Technical guidance --- PMDA, 160627

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Test method / items for CTPs</th>
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<tbody>
<tr>
<td><strong>Appearance and description</strong></td>
<td>physical state(solid, liquid), color and clarity</td>
</tr>
<tr>
<td><strong>Identity</strong></td>
<td>highly specific test for the products</td>
</tr>
<tr>
<td><strong>Purity and impurities</strong></td>
<td>purity [viability]</td>
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<tr>
<td></td>
<td>impurity [derived from product, raw material, media or process-related]</td>
</tr>
<tr>
<td><strong>Potency</strong></td>
<td>Secretion of active substance from cells/ stimulate or not</td>
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<td></td>
<td>Specific biological test(link to POC)</td>
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<tr>
<td></td>
<td>cell growth</td>
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<tr>
<td><strong>Quantity</strong></td>
<td>cell number</td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td>karyotyping analysis</td>
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<tr>
<td></td>
<td>Sterility Test, Mycoplasma and Endotoxin</td>
</tr>
<tr>
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<td>Virus</td>
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</tbody>
</table>
Human bone marrow

Donor cell bank (DCB)

Product dose (PD)

Donor screening
  - Acceptance tests
  - Process control tests

Intermediate tests*
  - Especially safety test

Process control tests

Release tests

Set specifications at “intermediate” and “final product”
Established specifications for TEMCELL®HS Inj.

<table>
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<td>physical state(solid, liquid), color and clarity</td>
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<td>purity</td>
<td>viability</td>
</tr>
<tr>
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</tr>
<tr>
<td><strong>Potency</strong></td>
<td>Secretion of active substance from cells/ stimulate or not</td>
<td>ELISA, another quantitative method</td>
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<tr>
<td></td>
<td>Specific biological test(link to POC)</td>
<td>cell based assay</td>
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<tr>
<td></td>
<td>cell growth</td>
<td>cell based assay</td>
</tr>
<tr>
<td><strong>Quantity</strong></td>
<td>cell number</td>
<td>flow cytometry</td>
</tr>
<tr>
<td><strong>Safety</strong></td>
<td>karyotyping analysis</td>
<td>G-band, mFISH, Soft-agar colony formation assay</td>
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<td>Sterility Test, Mycoplasma and Endotoxin</td>
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<td>Virus</td>
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Therapeutic Effects of TEMCELL®HS Inj. on acute GVHD: Possible Mechanisms of Action

1. TEMCELL administered intravenously migrates and accumulates to the inflammatory sites and activated by endogenous inflammatory cytokines including IFN-γ and so forth in the inflammatory sites.

2. Activated TEMCELL suppresses the alloantigen-stimulated donor T-cell functions by a variety of mechanisms including activation of regulatory T-cells, production of anti-inflammatory agents, such as prostaglandin E2 and kynurenine, and so forth.

3. High immunosuppressive potential of the activated TEMCELL as well as its low immunogenic property due to low level expression of HLA class I/II molecules and absence of co-stimulatory factors delays or evades allo-rejection through suppression of patient’s allogeneic immune responses, resulting in prolongation of persistence of TEMCELL in the patients.
Possible mechanism of action for TEMCELL® HS Inj.

1. low immunogenicity
   - possible to administer universally
   - evades allo-rejection through suppression of patient’s allogeneic immune responses

2. immuno-modulation
   - suppress and induce T-lymphocytes
   - secrete anti-inflammatory molecules

3. Homing to the sites of inflammation
   - administer via intravenous infusion
   - need to migrate to the inflammation site to demonstrate therapeutic activity
Test items to measure biological activity for TEMCELL®HS Inj.

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<td>Homing</td>
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Established various testing methods
Assumed mechanism of MSCs at inflammation site:
Based on in vitro test

**inflammatory site**

**Pathogen signal**
- Virus
- dsRNA
- Bacteria
- LPS

**TLR3**
**TLR4**

**IFN-γ**
**TNF-α**

**MSCs** (rest)

**CD4+ T-cell**

**CD4**
**CD25**

**regulatory T-cell**
- FoxP3

**Pathogen clearance**
- IL-6
- IL-8

**IDO1**

**Kynurenines**

**Tryptophan**

**COX2**

**PGE₂**

**Arachidonic acid**

**anti-inflammatory molecules**

**immuno-modulation**
2. immuno-modulation

measured immuno-modulation activity with “Mixed lymphocyte reaction (MLR)”

stimulate PBMC with the CD3/CD28 beads → proliferation

** TEMCELL suppresses T-cell proliferation

** p < 0.01 (Tukey-Kramer)
2. immuno-modulation

evaluate contribution of secreted factor from TEMCELL
Effect of inhibitors for PGE2

co-culture with TEMCELL

PGE2 partially involved T-cell suppression
Assumed migration mechanism of TEMCELL in blood flow: Based on in vitro test

1. **Activation**
   - TNF-α Receptor
   - IGF-1 Receptor
   - PDGF-β Receptor

2. **Rolling**
   - P-Selectin Ligand
   - Chemokine Receptor (CXCR4)
   - Integron (α4) (β1)
   - VCAM-1

3. **Transmigration**
   - MMP2, MMP14
   - TIMP2

**Blood flow**

**Endothelium**

**Basal membrane**

**Inflammatory site**

-migration mechanism of TEMCELL in blood flow:

Based on in vitro test

1. Activation
   - TNF-α Receptor
   - IGF-1 Receptor
   - PDGF-β Receptor

2. Rolling
   - P-Selectin Ligand
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3. Transmigration
   - MMP2, MMP14
   - TIMP2

**Immuno-modulation**
3. Homing to the sites of inflammation

identify molecules involved homing expressed in TEMCELL

- **Expression of Matrix metalloproteinase (PCR)**
  - MMP2, MMP9, MMP14, TIMP1, TIMP2
  - MW: molecular weight marker

- **Expression of Chemokine receptor, CXCR4 (Flow cytometry)**

MW: molecular weight marker

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3. **Homing to the sites of inflammation**

**Analysis for migration mechanism using Boyden chamber method**

- *seed pre cultured cells*
- *migration stain with fluorescent dye*
- *Fluorescence blocking membrane chemo-attractant*
- *microscopic observation and read with bottom-reading microplate reader*

**Quantitative method**

*excitation* *emission*

*positive* *negative*
3. Homing to the sites of inflammation

GM6001: a broad-spectrum matrix metalloproteinase inhibitor

decrease migration activity in a dose dependent manner

MMPs involved homing for TEMCELL
### Test items to measure biological activity for TEMCELL®HS Inj.

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**combine various type of testing methods for each property**

**screening the assay method by collecting the data of the product**

**Select 1 testing method for “immuno-modulation” as potency assay**

**Some of these assays continuously conduct as Verification (evaluate variation)**
Summary;

How to approach to set specifications and potency assay for CTPs?

Set specification refer to current guidelines; Q6B, Q5A and guidance depend on; the cell type for CTPs; somatic stem cells, iPS, ES etc. manufacturing process raw materials expected efficacy of product etc.

Set multiple candidate of test items as much as possible = various spec.
Set “wide” range of acceptance criteria at early stage of product development

Conduct process development and monitoring tests results
→ identify the factor(s) for the variation of the process
→ Re-set acceptance criteria based on the results of process development

To obtain stable data, Correlation with in vivo assay, Suitable method for validation

Confirm “practical” Specifications and acceptance criteria at late stage of development = Tightened set of test items and range of them before Application

Assured quality of product with minimum test items have to consider all of cost(goods, samples, various resources)
Thank you for your attention

– JCR Biotech for a New Tomorrow –
**General consideration to establish specifications**

**Reference guideline**; *ICH Q6B --- Specification, ICH Q5A --- Safety tests, Technical guidance --- PMDA, 160627*

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