Report on the Deliberation Results

September 2, 2015

Medical Device and Regenerative Medicine Product Evaluation Division,
Pharmaceutical and Food Safety Bureau,
Ministry of Health, Labour and Welfare

[Classification]	Human cellular/tissue-based products, 2 Human somatic stem cell-processed products
[Non-proprietary name]	Human (allogeneic) bone marrow-derived mesenchymal stem cells
[Brand name]	Temcell HS Inj.
[Applicant]	JCR Pharmaceuticals Co., Ltd.
[Date of application]	September 26, 2014

[Results of deliberation]
In the meeting held on September 2, 2015, the Committee on Regenerative Medicine Products and Biotechnology made the following decision and concluded that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affiars and Food Sanitation Council.

The product may be approved. A conditional and time-limited approval is not applicable to this product. The re-examination period should be 10 years.

The following conditions for approval should be imposed.

[Conditions for approval]
1. The applicant is required to ensure that the product is used by or under the supervision of a physician with adequate knowledge of and experience with hematopoietic stem cell transplantation at a medical institution with adequate facilities for the treatment of emergencies and in a setting where appropriate measures are taken, such as laboratory monitoring.
2. The applicant is required to ensure that a use-results survey that covers all patients treated with the product is conducted during the re-examination period and that appropriate actions are taken as needed.

Review Report

August 11, 2015
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following regenerative medical product submitted for registration are as follows.

[Brand name]	Temcell HS Inj.
[Classification]	Human cellular/tissue-based products, 2 Human somatic stem cell-processed products
[Non-proprietary name]	Human (allogeneic) bone marrow-derived mesenchymal stem cells
[Applicant]	JCR Pharmaceuticals Co., Ltd.
[Date of application]	September 26, 2014
[Shape, structure, active ingredient, quantities, or definition]	Each bag (10.8 mL) contains 72×10^6 human mesenchymal stem cells.
[Application classification]	(1-1) New regenerative medical product
[Items warranting special mention]	Orphan regenerative medical product (Designation No. 326 of 2013 [*25 yaku*], Notification No. 1212-1 from the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated December 12, 2013)
[Reviewing office]	Office of Cellular and Tissue-based Products

[Results of review]
As shown in the Attachment, the Pharmaceuticals and Medical Devices Agency (PMDA) has concluded that a certain level of efficacy of the product in the treatment of acute graft versus host disease following a hematopoietic stem cell transplant has been demonstrated by the submitted data and its safety is acceptable in view of its observed benefits.

As a result of its review, PMDA has concluded that the product may be approved for the indication or performance and dosage and administration or method of use as shown below, with the following conditions.

[Indication or performance]
Acute graft versus host disease following hematopoietic stem cell transplantation

[Dosage and administration or method of use]

The usual dosage of Temcell is 2×10^6 human mesenchymal stem cells/kg body weight administered as a slow intravenous infusion at a controlled rate of 4 mL/min. One bag of Temcell should be diluted with 18 mL of physiological saline. Temcell should be administered twice weekly for 4 weeks with infusions at least 3 days apart. The dose of 2×10^6 human mesenchymal stem cells/kg can futher be given once weekly for additional 4 weeks, depending on the degree of symptoms.

[Conditions for approval]

1. The applicant is required to ensure that the product is used by or under the supervision of a physician with adequate knowledge of and experience with hematopoietic stem cell transplantation at a medical institution with adequate facilities for the treatment of emergencies and in a setting where appropriate measures are taken, such as laboratory monitoring.
2. The applicant is required to ensure that a use-results survey that covers all patients treated with the product is conducted during the re-examination period and that appropriate actions are taken as needed.

Attachment

Review Report (1)

May 22, 2015

1. Product submitted for registration

[Brand name] Temcell HS Inj.

[Classification] Human cell-based products, 2 Human somatic stem cell-based products

[Non-proprietary name] Human (allogeneic) bone marrow-derived cell suspension

[Applicant] JCR Pharmaceuticals Co., Ltd.

[Date of application] September 26, 2014

[Shape, structure, active ingredient, quantities, or definition]

Each bag (10.8 mL) contains 72×10^6 human mesenchymal stem cells.

[Proposed indication or performance]

Acute graft versus host disease following hematopoietic stem cell transplantation

[Proposed dosage and administration or method of use]

1. Dosage and administration

The usual dosage of Temcell is 2×10^6 human mesenchymal stem cells/kg body weight administered as a slow intravenous infusion. Temcell should be diluted with physiological saline prior to infusion. As a rule, Temcell should be administered twice weekly for 4 weeks for a total of 8 infusions (at least 3 days apart). Continued therapy with 4 once-weekly infusions is allowed for additional 4 weeks, depending on the degree of symptoms.

2. Method of use

1) In order to maintain cell viability, the cells in a bag should be rapidly thawed in a water bath (37 ± 1°C) before use and immediately diluted with 18 mL of physiological saline. Once thawed, Temcell must be kept at room temperature and must be infused within 3 hours.
2) In order to prevent allergic reactions, patients should be premedicated intravenously with either hydrocortisone sodium succinate (or phosphate) (the adult dose, 100-200 mg) or chlorpheniramine maleate (the adult dose, 5-10 mg), or both, 30 minutes to 1 hour prior to the administration of Temcell.
3) A dose of Temcell prepared for administration should be given as a slow intravenous infusion at a controlled rate of 4 mL/min (≤6 mL/min) and should not be co-infused with other agents. For patients weighing ≤50 kg, Temcell should be infused slowly over ≥10 minutes.
4) During infusion, the infusion bag should occasionally be massaged gently to mix the suspension since sinking of cells may result in a non-uniform suspension of cells within the bag.

5) The patient's respiratory status, vital signs, and arterial oxygen saturation should be monitored constantly during and after infusion of Temcell.
6) Any unused portion must be discarded.

The data submitted in the application and the review thereof by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined in the sections below.

[Table of Contents]

Review Report (1) 4
1. Product submitted for registration 4
2. Origin or History of Discovery, Use in Foreign Countries, and Other Information 9
2.1. Outline of product submitted for registration 9
2.2. Development history, etc. 9
3. Manufacturing Process and Specifications 10
3.1. Cells as the main constituent of the product 10
3.2. Product 18
3.3. Reference materials 19
3.4. Outline of the review by PMDA 20
4. Stability 27
4.1. Product stability 27
4.2. Stability of infusion solutions 27
4.3. Outline of the review by PMDA 28
5. Indication or Performance 28
5.1. Studies to support the indication or performance 28
5.2. Outline of the review by PMDA 30
6. Biodistribution of the Product 30
6.1. Single-dose biodistribution study (CTD 4.2.2.3.1) 30
6.2. Outline of the review by PMDA 31
7. Nonclinical Safety Data 33
7.1. Mouse 4-week repeated intravenous dose toxicity study (CTD 4.2.3.2.3) 33
7.2. Reference data 33
7.3. Outline of the review by PMDA 34
8. Clinical Data 35
8.1. Japanese phase I/II study (CTD 5.3.5.2.1, Study JR-031-201 [[redacted] to [redacted]]) 35
8.2. Japanese phase I/II extension study (CTD 5.3.5.2.2, Study JR-031-202 [[redacted] to [redacted]]) 37
8.3. Japanese phase II/III study (CTD 5.3.5.2.3, Study JR-031-301 [[redacted] to [redacted]]) 39
8.4. Reference data (Foreign studies with Prochymal) 40
8.5. Outline of the review by PMDA 43
9. Risk Analysis 66
10. Results of Compliance Assessment Concerning the Data Submitted in the New Regenerative Medical Product Application and Conclusion by PMDA 67
10.1. PMDA's conclusion on the results of document-based GLP/GCP inspection and data integrity assessment ..67
10.2. PMDA's conclusion on the results of GCP on-site inspection 67
11. Overall evaluation at the time of preparing Review Report (1) 67
Review Report (2) 68

[List of abbreviations]

Abbreviation	Definition
ATG	Anti-thymocyte globulin
BSA	Bovine serum albumin
B19	Human parvovirus B19
$CD4^+CD25^+$T cells	CD4- and CD25-positive cells
$CD4^+$T cells	CD4-positive T cells
CI	Confidence interval
CMV	Cytomegalovirus
CR	Complete response
^{51}Cr-JR-031	^{51}Cr-labeled JR-031
CSP	Cyclosporin
CXCR4	C-X-C chemokine receptor type 4
DCB	Donor cell bank
DMSO	Dimethyl sulfoxide
EBV	Epstein-barr virus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
FISH	Fluorescence *in situ* hybridization
GRO	Growth-related oncogene
γ-GTP	γ-glutamyl transpeptidase
GVHD	Graft versus host disease
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HHV	Human herpes virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papilloma virus
HTLV	Human T-cell leukemia virus
IBMTR	International bone marrow transplant registry
IDO	Indoleamine 2,3-dioxygenase
IGF-1	Insulin-like growth factor 1
IFN-γ	Interferon-gamma
IL	Interleukin
ITGB1	Integrin β1
Studies JR-031-201/202	Study JR-031-201 and Study JR-031-202
JSHCT	The Japan Society for Hematopoietic Cell Transplantation
LPS	Lipopolysaccharide
MCP	Monocyte chemoattractant protein
MHC	Major histocompatibility complex
MMF	Mycophenolate mofetil
MMP	Matrix metalloproteinase
MR	Mixed response
hMSC	Human mesenchymal stem cell
rMSC	Rat mesenchymal stem cell
OR	Overall response
Osiris	Osiris Therapeutics Inc.
PCR	Polymerase chain reaction
PD	Product dose
PDGF	Platelet-derived growth factor
PGE_2	Prostaglandin E_2

PR	Partial response
TAC	Tacrolimus
TIMP	Tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNF	Tumor necrosis factor
PMDA	Pharmaceuticals and Medical Devices Agency
Ex-vivo cultured hMSCs	Cells as the active ingredient of the product
Commercial manufacturing process	Process D
JSHCT guideline	Hematopoietic cell transplantation guideline—GVHD
Manufacturing process for Japanese phase I/II clinical study	Process A
Adverse reactions	Adverse events other than those assessed by the investigator as causally unrelated to JR-031 or adverse events whose causality to JR-031 cannot be ruled out by the sponsor
Temcell	Temcell HS Inj.
Study 280 III/IV subpopulation	Subjects with JSHCT grade III or IV acute GVHD in the mITT population of Study 280

2. Origin or History of Discovery, Use in Foreign Countries, and Other Information

2.1. Outline of product submitted for registration

Temcell HS Inj. (Temcell) consists of human mesenchymal stem cells (hMSCs) obtained from culture-expanded nucleated cells isolated from the bone marrow of healthy adult donors, which is a regenerative medical product administered as an intravenous infusion in expectation of its therapeutic effect arising from pharmacological action, in the same way as drug products.

Temcell was developed to treat corticosteroid-refractory acute graft versus host disease (GVHD) following hematopoietic stem cell transplantation (corticosteroid therapy is considered to be the first-line treatment for the disease). hMSCs have been reported to reduce T-cell activation and thus suppress immune responses by mechanisms such as inhibition of T-cell secretion of proinflammatory cytokines, which leads to increased production of anti-inflammatory cytokines, promotion of a shift toward a predominant Th2 immune response, and secretion of soluble factors that are involved in the suppression of T-cell response (*Blood* 2005; 105: 1815-22, *Circ Res* 2004; 94: 678-85, etc.). Temcell is also considered to exert a therapeutic effect in patients with acute GVHD through similar pharmacological effects.

In Japan, 5371 patients underwent hematopoietic stem cell transplantation in 2012 (of these patients, 3574 underwent allogeneic hematopoietic stem cell transplantation, namely, related bone marrow transplantation, related peripheral blood stem cell transplantation, unrelated bone marrow transplantation, or allogeneic cord blood transplantation) (the national survey report FY 2013 of the Japan Society for Hematopoietic Cell Transplantation [JSHCT]). A total of 22,885 patients underwent allogeneic hematopoietic stem cell transplantation between 1990 and 2007. Of these patients, 12,719 (55.6%) experienced acute GVHD (Grades I-IV) (JSHCT national survey report FY 2008). Assuming that a similar proportion of recipients experience acute GVHD each year, acute GVHD is estimated to develop in approximately 2000 patients per year in recent years.

Temcell was designated as an orphan drug with the intended indication of "acute GVHD" in December 2013 and has been deemed to be classified as an orphan regenerative medical product in accordance with Article 56 of the supplementary provisions to the Act for Partial Revision of the Pharmaceutical Affairs Act (Act No. 84 of 2013) (Drug Designation No. 326 of 2013 [*25 yaku*]).

2.2. Development history, etc.

Acute GVHD following hematopoietic stem cell transplantation is a syndrome characterized by rash, jaundice, and diarrhoea, which occur in the early post-transplant period. According to the hematopoietic cell transplantation guideline—diagnosis and treatment of GVHD, developed by the JSHCT (hereinafter "JSHCT guideline"), corticosteroids are the initial first-line treatment for acute GVHD, but this is ineffective in approximately 50% of patients experiencing acute GVHD. The second-line treatments recommended for patients who have failed to respond to first-line therapy or for patients with flares of their GVHD include steroid pulse therapy and immunosuppressants such as anti-thymocyte globulin (ATG), cyclosporine (CSP),

tacrolimus (TAC), and mycophenolate mofetil (MMF). However, infections etc., commonly occur after second-line treatment due to excessive immunosuppression and the 2-year non-relapse mortality is as high as 56.3% (*Biol Blood Marrow Transplant* 2013; 19: 1183-89). No standard second-line treatment for acute GVHD has therefore been established for patients who have failed to respond to first-line therapy or patients with flares of their GVHD.

The applicant undertook the development of Temcell in Japan by in-licensing the technology for the manufacturing of hMSC product (Prochymal) developed by Osiris Therapeutics Inc. (Osiris), the United State (US). Osiris sold its Prochymal business to Mesoblast Ltd., Australia, in 2013. Prochymal was approved in Canada in May 2012 and in New Zealand in June 2012 for treatment of acute GVHD in children, but has not yet been launched commercially for economic reasons. Prochymal received orphan drug designation in the US and EU and was granted the Rapid Authorization Procedure status in Switzerland.

Temcell was classified as a cell/tissue-based drug when its clinical development was planned, and thus a pre-CTN (clinical trial notification) application for confirmation of the safety and quality of this investigational product was submitted as of August 28, 2006 in accordance with "Quality and Safety Assurance of Cell/Tissue-based Medical Devices or Drugs" (Notification No. 906 of the Pharmaceutical and Medical Safety Bureau, Ministry of Health and Welfare, dated July 30, 1999). After deliberation at a meeting organized by the Committee on Biotechnology of the Pharmaceutical Affairs and Food Sanitation Council on May 29, 2007, the safety and quality of the investigational product were confirmed (Notification No. 0621007 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, dated June 21, 2007). Then, the applicant initiated clinical development in Japan, which led to the filing of application for a marketing approval of a new regenerative medical product, Temcell.

3. Manufacturing Process and Specifications

3.1. Cells as the main constituent of the product

The product consists of undifferentiated allogeneic hMSCs, which are obtained by expansion of the nucleated cell fraction isolated from human bone marrow aspirate in adherent culture (*ex-vivo* cultured hMSCs).

3.1.1. Manufacturing process

3.1.1.1. Manufacturing process

The manufacturing process for *ex-vivo* cultured hMSCs is divided into 2 stages: the production of donor cell bank (DCB) and the production of product dose (PD). The production of DCB consists of receipt of bone marrow aspirate, isolation of [redacted] cells, [redacted], harvesting for DCB, formulation and filling of DCB for cryopreservation, and freezing of DCB. The production of PD consists of thaw of DCB, [redacted], and harvesting for PD. Verification of the manufacturing process for *ex-vivo* cultured hMSCs was performed on a commercial scale [see Section 3.4.2.]

and all process steps were defined as critical steps.

DCB is a critical intermediate and is stored in the vapor phase of liquid nitrogen. Unlike a master cell bank, DCB is not a permanent source of the product and a new DCB is generated from the bone marrow aspirate of a new donor periodically. [redacted] to [redacted] bags of the product are produced from a single DCB.

3.1.1.2. In-process controls

In-process controls for the manufacturing process for *ex-vivo* cultured hMSCs are as shown in Table 3.1 [see Section 3.4.2.].

Table 3.1. In-process controls

	Process step	In-process test
Production of DCB	Isolation of [redacted] cells	[redacted]
	[redacted]	[redacted]
	Harvesting for DCB	[redacted] sterility
	Formulation and filling of DCB for cryopreservation	[redacted]
Production of PD	Thaw of DCB	[redacted]
	[redacted]	[redacted]
	[redacted]	[redacted]
	Harvesting for PD	[redacted] sterility

3.1.2. Adventitious agents safety evaluation

3.1.2.1. Human bone marrow aspirate

As the raw material of Temcell, human bone marrow aspirates are collected from eligible healthy adult donors. A bone marrow aspirate is collected from a donor who is deemed to be eligible after the donor eligibility determination process consisting of selection (Table 3.2), prescreening (questioning about medical history, a history of infections, travel history, etc.), and screening (Table 3.3). The collected bone marrow aspirate is shipped at [redacted]°C to [redacted]°C to the manufacturing facility for Temcell. Donor re-testing for viral infection that is potentially found after the window period is not performed. However, to minimize the risk of viral infection during the period between donor registration and collection of bone marrow aspirate, the donor is questioned about medical history, history of infections, travel history, etc., taking account of the window period. At the same time, blood tests and serology and PCR testing are performed at least [redacted] days prior to collection of bone marrow aspirate [see Section 3.4.1.1.].

When collecting human bone marrow aspirates, Heparin Sodium Injection approved for marketing in the US is used [see Section 3.4.1.2.].

Table 3.2. Donor selection

・Healthy adults aged between [redacted] and [redacted] years
・≤[redacted] previous bone marrow donations
・The latest bone marrow cell count: ≥[redacted] cells/mL
・History of certain infections including CMV: Negative
・Men: BMI ≤[redacted], or body weight ≤[redacted] kg
・Women: BMI ≤[redacted], or body weight ≤[redacted] kg

CMV: Cytomegalovirus, BMI: Body mass index

Table 3.3. Donor screening

Tests	Test results
Blood tests	
Complete blood count	Criteria met
Clinical chemistry	
ABO and Rh blood types	
Human leukocyte antigen (HLA)	
Serology and PCR tests	
Human immunodeficiency virus (HIV-1, HIV-2)	Negative
Hepatitis C virus (HCV)	
Surface antigen of hepatitis B virus	
Anti-hepatitis B virus antibody	
Human T-cell leukemia virus (HTLV-1, HTLV-2)	
Treponema pallidum	
Human immunodeficiency virus (HIV-1)	
Hepatitis B virus DNA	
Hepatitis C virus RNA	
West Nile fever virus	
Human parvovirus B19	
Chagas' disease	
Health examination	
Blood pressure	Criteria met
Body temperature	
Height	
Body weight	
Physical examination	

3.1.2.2. Human or animal derived raw materials (other than human bone marrow aspirates)

Human or animal derived raw materials (other than human bone marrow aspirates) used for the production of *ex-vivo* cultured hMSCs are as shown in Table 3.4. Fetal bovine serum (FBS) is derived from healthy bovine blood sourced from New Zealand or Australia, which is γ-irradiated (≥30 kGy) to inactivate pathogenic agents and tested for adventitious bovine viruses, endotoxins, sterility, and mycoplasma. Trypsin in trypsin-ethylenediaminetetraacetic acid (EDTA) solution is derived from healthy porcine pancreas. Trypsin powder is electron beam-irradiated (≥25 kGy) and trypsin solution is γ-irradiated (≥25 kGy) to inactivate pathogenic agents. Electron beam- and γ-irradiated trypsin solution is tested for porcine parvovirus, endotoxins, sterility, and mycoplasma. All raw materials etc., have been shown to conform to the Standard for Biological Ingredients (MHLW Bulletin No. 210, 2003). Human serum albumin used is a drug product approved for marketing.

Table 3.4. Raw materials of biological origin (other than human bone marrow aspirates) used for the production of *ex-vivo* cultured hMSCs

Raw material	Animal species	Specific part of animal used	Process step
FBS	Bovine	Blood	each step of [redacted], each step of [redacted], [redacted] step
Trypsin-EDTA solution	Porcine	Pancreas	each step of [redacted], [redacted] step, [redacted] step
Human serum albumin	Human	Blood	[redacted] step, [redacted] step, [redacted] step, [redacted]

3.1.3. Manufacturing process development (comparability)

Major changes made to the manufacturing process during the development of *ex-vivo* cultured hMSCs are as follows:

1. Change from the manufacturing process for [redacted] (Process A) to Process B: changes in [redacted] and DCB harvesting method [redacted], [redacted] used for DCB cryopreservation, and DCB container material ([redacted]) for the production of DCB
2. Change from Process B to Process C [redacted]: manufacturing site change, changes in [redacted] and PD harvesting method ([redacted]) for the production of PD
3. Change from Process C to the intended commercial manufacturing process (Process D): changes in [redacted] and DCB harvesting method ([redacted]) for the production of DCB

Following process changes (the above 1 and 2), comparability studies on quality attributes were performed, which demonstrated comparability between pre-change and post-change *ex-vivo* cultured hMSCs. On the other hand, following process changes (the above 3), Process verification was carried out on [redacted] lots of DCB and [redacted] batches of the product and a total of [redacted] batches did not meet the acceptance criteria (Table 3.5). The non-conformances, excluding contamination during collection of bone marrow aspirate and abnormalities in the cell culture equipment, were due to inter-individual donor differences. There were no abnormalities in manufacturing control and the acceptance criteria for other test attributes were met. The applicant concluded that these non-conformances were not attributable to the manufacturing process and that the manufacturing process changes did not affect quality [see Section 3.4.2.].

Table 3.5. Batches that failed to meet the acceptance criteria in process verification following process changes

Batch Number	Process step	Details of non-conformance	Discussion of the cause
[redacted]			

3.1.4. Characterization

Characterization of *ex-vivo* cultured hMSCs was performed using DCB ([redacted]) and *ex-vivo* cultured hMSCs ([redacted]). The characterization identified the cell proliferation profile. The cells were shown to lose proliferative capacity after about [redacted]th cell division from human bone marrow aspirate.

3.1.4.1. Characterization of DCB

The results of isozyme analysis and flow cytometry demonstrated that DCB is of human origin and is CD45-negative, CD105-positive, and CD166-positive. Morphology observation and [redacted] to assess the differentiation capacity into [redacted] cells, morphology observation and [redacted] to assess the differentiation capacity into [redacted] cells, and [redacted] and [redacted] to assess the differentiation capacity into [redacted] cells demonstrated that DCB has the capacity to differentiate into mesodermal cell lineages.

[redacted] demonstrated that DCB expresses [redacted] as tumor necrosis factor (TNF) α-receptor, secretes prostaglandin E_2 (PGE_2), and further increases [redacted] upon [redacted] stimulation.

Culturing of DCB demonstrated that the cells are spindle-shaped and that the cell density is ≥[redacted] cells/cm^2 when confluence is achieved after the period of culture defined in the specification.

Karyotype analyses (G-banding and multicolor fluorescence *in situ* hybridization [FISH]) revealed no abnormalities.

In order to examine chromosomes related to hematologic malignancies, [redacted] for chronic myeloid leukemia and acute myeloid leukemia, [redacted] for myelodysplastic syndrome and acute myeloid leukemia, and [redacted] and [redacted] for acute myeloid leukemia were performed. All of the analyses revealed no abnormalities.

In a soft agar colony formation assay, no colonies were formed. The endotoxin limit (chromogenic technique) was < [redacted] EU/mL. Sterility testing (direct inoculation of the culture medium), mycoplasma testing (culture method and indicator cell culture method), and testing for viruses (electron microscopy, *in vitro* assays, *in vivo* assays, polymerase chain reaction [PCR] assays [human immunodeficiency virus (HIV) 1 and 2, human T-cell leukemia virus (HTLV) 1 and 2, human herpesvirus (HHV) 6 and 8, hepatitis B virus (HBV), hepatitis C virus (HCV), human parvovirus B19 (B19), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human papillomavirus (HPV)]) were all negative.

Culture-expanded cells from DCB were subjected to analysis of the expression of molecules involved in immunogenicity by flow cytometry, which demonstrated that the cells do not express major histocompatibility complex (MHC) class II molecules and express weak levels of MHC class I molecules. On the other hand, the expression of low levels of MHC class II molecules was induced and the expression of MHC class I molecules was increased by stimulation with interferon-γ (IFN-γ). No expression of CD40, CD80, or CD86 cell surface antigen was detected, with or without stimulation with IFN-γ. Moreover, analysis of other cell surface antigens showed the expression of CD73, CD90, CD105, and CD166 and the lack of CD34 and CD45 expression.

3.1.4.2. Characterization of *ex-vivo* cultured hMSCs

The cell size of *ex-vivo* cultured hMSCs was measured (cell size analysis [[redacted]]). The mean cell diameter was [redacted] ± [redacted] μm (mean ± standard deviation [SD]) and the value of a cell diameter corresponding to a cumulative fraction of [redacted] % was [redacted] ± [redacted] μm (mean ± SD).

Morphology observation and [redacted] to assess the differentiation capacity into [redacted] cells, morphology observation and [redacted] to assess the differentiation capacity into [redacted] cells, and [redacted] and [redacted] to assess the differentiation capacity into [redacted] cells demonstrated that *ex-vivo* cultured hMSCs have the capacity to differentiate into mesodermal cell lineages.

The results of T-cell proliferation inhibition assay showed that *ex-vivo* cultured hMSCs inhibit human peripheral blood T-cell proliferation induced by anti-CD3/CD28 stimulation and that the presence of a PGE_2 synthesis inhibitor or an inhibitor of indoleamine 2,3-dioxygenase (IDO) reduces the suppression of T-cell proliferation by *ex-vivo* cultured hMSCs. These results indicated that at least the secretion of PGE_2 or the induction of IDO production is involved in the suppression of T-cell proliferation by *ex-vivo* cultured hMSCs. The addition of IFN-γ, a Toll-like receptor (TLR) 3 agonist, poly (i:c), or a TLR4 agonist, lipopolysaccharide (LPS) resulted in concentration-dependent increases in *IDO1* expression, which had been hardly observed before the addition of these agonists.

As to the expression of molecules related to immune responses and anti-inflammatory factors, *ex-vivo* cultured hMSCs expressed *TLR1*, *TLR3*, *TLR4*, and *TLR6*. However, *TLR2*, *TLR5*, *TLR7*, *TLR8*, *TLR9*, or *TLR10* were

not observed. The addition of poly (i:c) or LPS to *ex-vivo* cultured hMSCs resulted in concentration-dependent increases in the expression of *IL-6* and *IL-8* genes and in the secretion of IL-6 and IL-8 proteins.

The expression of molecules related to migration and the migratory ability were evaluated. The gene expression of cell adhesion molecules, integrin α4 (ITGA4) and integrin β1 (ITGB1), was detected in *ex-vivo* cultured hMSCs, but the expression of ITGA4 protein was not detected. The gene expression of matrix metalloproteinases (MMPs), MMP-2 and MMP-14, and of tissue inhibitors of metalloproteinases (TIMPs), TIMP-1 and TIMP-2, was also detected. On the other hand, the gene expression of chemokine receptor 4 (CXCR4), a chemokine receptor, was not detected, but the protein expression was observed. Furthermore, in an *in vitro* cell migration assay, *ex-vivo* cultured hMSCs exhibited migratory ability in the presence of FBS and the inhibitors of platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF-1), and MMP reduced the migration in a concentration-dependent manner.

A cytokine array detected the secretion of interleukin (IL)-6, -7, and -8, monocyte chemoattractant protein (MCP) 1, and growth-related oncogene (GRO) from *ex-vivo* cultured hMSCs. In DCB, IL-7 and GRO, which were secreted from *ex-vivo* cultured hMSCs, were not detected and the presence of angiogenin was detected.

3.1.5. Manufacturing process evaluation

3.1.5.1. Cytogenetic stability

Karyotype analyses (G-banding, multicolor FISH) revealed no abnormalities in DCB or cells cultured up to [redacted] passages (beyond the passage level of *ex-vivo* cultured hMSCs).

Analysis of chromosomes related to hematologic malignancies in patients with chronic myeloid leukemia, myelodysplastic syndrome, and acute myeloid leukemia revealed no abnormalities in DCB or cells cultured up to [redacted] passages (beyond the passage level of *ex-vivo* cultured hMSCs).

In a soft agar colony formation assay of cells cultured up to [redacted] passages (beyond the passage level of *ex-vivo* cultured hMSCs), no colonies were formed.

3.1.5.2. Cell washing process capability

The efficiency of a cell washing step in the PD harvesting process was assessed by determining the residual concentration of bovine serum albumin (BSA), a typical indicator for media components, in the product by an ELISA. As a result, BSA was demonstrated to be removed to levels below the detection limit (< [redacted] ng/mL) in [redacted] batches of the product.

3.1.6. Control of *ex-vivo* cultured hMSCs

The specifications for DCB were established and its stability was assessed.

3.1.6.1. DCB specifications

The DCB specifications are as shown in Table 3.6 [see Section 3.4.2.].

Table 3.6. DCB specifications

Test		Test procedure
Identity	Cell species	[redacted]
	Cell surface antigen (1)	Flow cytometry [redacted]
	Cell surface antigen (2)	Flow cytometry [redacted]
	Differentiation capacity into [redacted] cells	[redacted]
	Differentiation capacity into [redacted] cells	[redacted]
[redacted]		[redacted]
[redacted]		[redacted]
Chromosome analysis		G-banding ([redacted])
		Multicolor FISH ([redacted])
Soft agar colony formation assay		[redacted]
Endotoxins		Chromogenic technique (Japanese Pharmacopoeia [JP])
Sterility		Direct inoculation of the culture medium
Mycoplasma		Culture method (General Information in JP)
		Indicator cell culture method (General Information in JP)
	Electron microscopy	Electron microscopy
	In vitro assays (Vero, MRC5, and Hs68 cells)	Cytopathic changes Hemagglutination Hemadsorption
Testing for viruses	*In vivo* assays	Adult mice Suckling mice Embryonated eggs ([redacted]) Embryonated eggs ([redacted])
	HIV-1	PCR
	HIV-2	PCR
	HTLV-1/2	PCR
	HHV-6	PCR
	HHV-8	PCR
	HBV	PCR
	HCV	PCR
	B19	PCR
	CMV	PCR
	EBV	PCR
	HPV	PCR
Cell concentration		[redacted]

3.1.6.2. Stability of DCB

Stability studies of DCB are outlined in Table 3.7.

Table 3.7. Outline of stability studies of DCB (Long-term testing)

Batch No.	Process	[redacted]	Storage condition	Testing period (days)	Storage package
[redacted]	A	[redacted]	Liquid nitrogen (vapor phase)	1592	[redacted] cell freezing bag
[redacted]	B	[redacted]		1413	[redacted] cell freezing bag

There were no changes in [redacted] over time for all batches. The product specification tests were conducted on the product derived from stored DCB. The tests revealed no clear changes in the product. Thus, product qulity was not affected by the time period for which stability of DCB has been demonstrated.

Based on the above, DCB is stable for 46 months (1399 days) when sealed in a cell freezing bag and stored in the vapor phase of liquid nitrogen [see Section 3.4.3.].

3.2. Product

3.2.1. Description and composition of the product and formulation development

[redacted] $\times 10^6$ cells are filled in a product bag to account for losses after freezing and thawing so that 72×10^6 viable cells are present in each product bag (10.8 mL) upon thawing. The product contains dimethyl sulfoxide (DMSO) (a stabilizer), human serum albumin (a stabilizer), and bicarbonate Ringer's solution (a suspending agent) as excipients. Human serum albumin and bicarbonate Ringer's solution used are drug products approved for marketing. The primary container is a cell freezing bag with a polyethylene tube and the secondary container is a carton.

3.2.2. Manufacturing process

3.2.2.1. Manufacturing process steps

The product manufacturing process consists of cell suspension preparation, filling and closure, packaging, freezing, and testing. Filling and closure were defined as critical steps. Verification of the manufacturing process was performed on a commercial scale.

3.2.2.2. In-process controls

In-process controls for the manufacturing process are as shown in Table 3.8 [see Section 3.4.2.].

Table 3.8. In-process controls

Process step	In-process test
Cell suspension preparation	[redacted]
Filling and closure	[redacted]

3.2.3. Development of product manufacturing process (comparability)

Major changes made to the manufacturing process during development are as follows:

- Change from Process A to Process B: Changes in the labeled amount (cell count and suspension volume) and the fill amount, the material of the primary container (polyethylene vinyl acetate → polyethylene), the method of sterilization (γ-ray sterilization → ethylene oxide sterilization), and the material of the secondary container (aluminum → paper)
- Change from Process B to Process D: manufacturing site change

Comparability studies on quality attributes were performed, which demonstrated comparability between pre-change and post-change product.

3.2.4. Characterization

Process-related impurities that may remain in the product are FBS and trypsin derived from porcine pancreas. Since the removal efficiencies of these impurities were considered similar, the residual concentration of BSA, a major component of FBS, was determined. This is because the level of FBS remaining in the product is considered to be high and its antigenicity is likely to be higher. As a result, residual BSA was found to be below the detection limit (< [redacted] ng/mL) in all [redacted] batches of the product.

3.2.5. Product control

The product specifications are as shown in Table 3.9 [see Section 3.4.2.].

Table 3.9. Product specifications

Test		Test procedure
Appearance		Visual
Identity	Cell surface antigen (1)	Flow cytometry [redacted]
	Cell surface antigen (2)	Flow cytometry [redacted]
Purity	Cell viability	[redacted]
	Media components	[redacted]
[redacted]		[redacted]
[redacted]		[redacted]
Fill volume		Weight
Foreign insoluble matter		Visual inspection
Endotoxins		Chromogenic technique (JP)
Sterility		Direct inoculation of the culture medium (JP)
Mycoplasma		Culture method (General Information in JP)
		PCR (General Information in JP)
Cell count		[redacted]

3.3. Reference materials

No reference material for DCB or the product has been established.

3.4. Outline of the review by PMDA

Based on the submitted data and the review presented below, PMDA concluded that the quality of Temcell is adequately controlled.

3.4.1. Safety of adventitious agents

3.4.1.1. Retesting for human bone marrow donor screening

Human bone marrow aspirates, which is the raw materials of Temcell, must conform to the Standard for Biological Ingredients. The necessity of retesting after the appropriate window period is specified in Section (3)-c of 1. Standard for raw materials of human cell/tissue-based products, III. General rules for human derived ingredients, the Standard for Biological Ingredients. Since no such retest or relevant control has been performed for the product, PMDA asked the applicant to explain their idea about ensuring the safety of Temcell in relation to adventitious agents.

The applicant's response:

In consideration with the necessity of retesting after the appropriate window period, the applicant confirmed that equivalent controls are used in accordance with Section 3.(4) of the "Implementation of the Standard for Biological Ingredients" (the notification issued jointly by the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW [Notification No. 1002-1] and the Counselor for Medical Device and Regenerative Medicine Product Evaluation, Minister's Secretariat, MHLW [Notification No. 1002-5], dated October 2, 2014) (Implementation Notification). Therefore, there should be no problem with the conformity to the Standard for Biological Ingredients.

PMDA's view:

Since no retesting or relevant control has been performed after the appropriate window period, the possibility of the presence of trace amounts of viruses and other microorganisms that are difficult to be detected by the aspiration and biopsy of human bone marrow cannot be ruled out. The safety of human bone marrow aspirates with regard to adventitious agents is not necessarily ensured. However, since DCB is tested extensively for the presence of virus etc., based on ICH-Q5A, the potential safety risk of residual virus is acceptable.

3.4.1.2. Viral safety of Heparin Sodium Injection used in collecting human bone marrow aspirates

PMDA asked the applicant to explain the conformity of Heparin Sodium Injection, used in collecting human bone marrow aspirates, to the Standard for Biological Ingredients.

The applicant responded that although the Heparin Sodium Injection has been confirmed to be derived from healthy porcine small intestine and has been approved for marketing as a drug by the US Food and Drug Administration, the details of the inactivation/removal of viruses etc., in the process of production, are currently being checked.

After reviewing the applicant's final response, PMDA will determine whether Heparin Sodium Injection conforms to Section (4) of 3. Standards for animal-derived raw materials, IV. General rules for animal-derived ingredients, the Standard for Biological Ingredients. The results will be reported in the Review Report (2).

3.4.2. Conduct of verification

Verification of the manufacturing process for Temcell has been performed on a commercial scale. However, taking account of the points listed below, PMDA considers that no source of variability in the manufacturing process derived from the quality attributes of bone marrow aspirates, the raw materials of Temcell, has been identified at present. Therefore it is not appropriate to conclude that the results of a limited number of process validation studies have demonstrated that the manufacturing process can consistently produce a product of the intended quality. PMDA requested the applicant to review the quality control strategy so that the quality of Temcell will be ensured through verification during production.

- Bone marrow aspirates have not so far been analyzed for quality attributes associated with the production of Temcell and the quality to be controlled at the stage of raw materials and in the manufacturing process has not been defined.
- There were multiple batches of DCB that did not meet the acceptance criteria, which was likely due to inter-individual donor variability in the quality of bone marrow aspirate.
- In the control strategy drafted by the applicant, in-process controls etc., were not based on the potential quality risk associated with variation in the quality attributes of bone marrow aspirate.

The applicant's response:

Since no sources of process variability have been identified at present, the applicant will establish in-process controls as shown in Table 3.10-1, Table 3.10-2, and Table 3.10-3 and the DCB and product specifications as shown in Table 3.11 and Table 3.12 to conduct the verification test during each production, thereby developing the control strategy to ensure the intended product quality.

Table 3.10-1. In-process controls for production of DCB

Process step		In-process controls	
Production of DCB		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		Test on critical intermediate	

Table 3.10-2. In-process controls for production of PD

Process step		In-process controls	
Production of PD		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	
		Parameter	
		In-process test	

Table 3.10-3. In-process controls for product manufacturing process

Process step		In-process controls	
Product manufacturing process	Cell suspension preparation	Parameter	
		In-process test	
	Filling and closure	Parameter	
		In-process test	
	Packaging	In-process test	
	Product freezing	Parameter	
	Product storage	In-process test	

Table 3.11. DCB specifications

Test		Test procedure
Identity	Cell species	[redacted]
	Cell surface antigen (1)	Flow cytometry [redacted]
	Cell surface antigen (2)	Flow cytometry [redacted]
	Differentiation capacity into [redacted] cells	[redacted]
	Differentiation capacity into [redacted] cells	[redacted]
	Differentiation capacity into [redacted] cells	[redacted]
Cell viability		[redacted]
[redacted]		[redacted]
[redacted]		[redacted]
Chromosome analysis		G-banding ([redacted])
		Multicolor FISH ([redacted])
Soft agar colony formation assay		[redacted]
Endotoxins		Chromogenic technique (JP)
Sterility		Direct inoculation of the culture medium
Mycoplasma		PCR
Testing for viruses	Electron microscopy	Electron microscopy
	In vitro assays (Vero, MRC5, and Hs68 cells)	Cytopathic changes Hemagglutination Hemadsorption
	In vivo assays	Adult mice Suckling mice Embryonated eggs ([redacted]) Embryonated eggs ([redacted])
	HIV-1	PCR
	HIV-2	PCR
	HTLV-1/2	PCR
	HHV-6	PCR
	HHV-8	PCR
	HBV	PCR
	HCV	PCR
	B19	PCR
	CMV	PCR
	EBV	PCR
	HPV	PCR
Cell concentration		[redacted]

Table 3.12. Product specifications

Test		Test procedure
Appearance		Visual
Identity	Cell surface antigen (1)	Flow cytometry [redacted]
	Cell surface antigen (2)	Flow cytometry [redacted]
Purity	Cell viability	[redacted]
	Media components	[redacted]
[redacted]		[redacted]
[redacted]		[redacted]
Extractable volume		Test for Extractable Volume (JP)
Foreign insoluble matter		Foreign Insoluble Matter Test for Injections Method 1 (JP)
Endotoxins		Chromogenic technique (JP)
Sterility		Direct inoculation of the culture medium
Mycoplasma		PCR
Cell count		[redacted]

PMDA concluded that the potential quality risk associated with variation in the quality attributes of bone marrow aspirate can be controlled by in-process tests and DCB and product specification tests as presented by the applicant and accepted the Temcell quality control strategy.

3.4.3. Stability of DCB

The applicant's explanation:

Since the product derived from DCB stored up to 1592 days was demonstrated to conform to the specifications, the long-term storage of DCB should have a limited impact on product quality. Hence, there should be no problem with proposing a storage time of 46 months (1399 days) for DCB, based on the stability data (1413 days) to support Process B, where the same container and formulation as those in Process D were used.

PMDA's view:

Given that DCB is generated from the bone marrow aspirate of each donor, adequate assessment of the storage stability of DCB is not ensured solely because post-thaw cell count after long-term storage of DCB and the quality of the product derived from this DCB both meet the acceptance criteria. The storage stability of each DCB generated should first be confirmed and then the quality of the product derived from this DCB should be controlled. Therefore the idea of verification [see Section 3.4.2.] should be employed to ensure that the quality of each batch of DCB after storage as well as that of the product derived from the batch are confirmed. The storage period should be determined after accumulation of information.

3.4.4. Differences between Temcell and Prochymal

The applicant developed Temcell in Japan, by in-licensing the technology of Prochymal developed by Osiris. PMDA asked the applicant to explain quality differences between Temcell and Prochymal, based on the results of process evaluation and characterization studies performed on the transfer of the technology from Osiris, the US.

The applicant's response:

After the in-licensing of the technology from Osiris, the manufacturing process was changed 4 times until Process D was established. Since comparability was demonstrated after process changes, the manufacturing process changes would not cause differences in quality attributes between Temcell and Prochymal. The results of in-process tests and specification tests were also similar between Temcell and Prochymal, thus demonstrating the comparability of Temcell to Prochymal.

PMDA's view:

Change control has been performed after the in-licensing of the technology and comparison of the results of in-process tests and specification tests indicated no apparent differences in quality attributes between Temcell and Prochymal. Nevertheless, it is difficult to conclude from these findings alone that Prochymal is comparable to Temcell manufactured through different processes by another manufacturer, because no adequate analysis of quality attributes has been performed for bone marrow aspirates as the raw materials of Temcell, nor have any sources of process variability been identified [see Section 3.4.2.].

4. Stability

4.1. Product stability

Stability studies of the product are outlined in Table 4.1.

Table 4.1. Outline of long-term stability studies of product

Batch No.	Process	DCB used	Storage condition	Testing period	Storage package
[redacted]	B	[redacted]	Liquid nitrogen (vapor phase)	60 months	A cell freezing bag with a polyethylene tube

No significant changes in quality attributes occurred under the long-term condition throughout the testing period.

Based on the above, a shelf life of 60 months was proposed for the product when packaged in a cell freezing bag with a polyethylene tube and stored in the vapor phase of liquid nitrogen.

4.2. Stability of infusion solutions

The product is thawed prior to infusion and one bag of the product is diluted with 18 mL of physiological saline to prepare an infusion solution. Stability studies of infusion solutions are outlined in Table 4.2.

Table 4.2. Outline of stability studies of infusion solutions

Batch No.	Process	DCB used	Storage conditions	Testing period	Storage package
[redacted]	B	[redacted]	22.4°C-26.7°C, 1000-1100 lx	3, 6, 24, and 30 hours after preparation	A cell freezing bag with a polyethylene tube

[redacted] at 22.4°C to 26.7°C were ≥[redacted]% at ≤[redacted] hours after preparation and ≥[redacted]% at ≤[redacted] hours after preparation, and batch-to-batch variability was observed at ≥[redacted] hours after preparation.

On the basis of the above results, infusion solutions are to be kept at room temperature and are to be infused within 3 hours.

4.3. Outline of the review by PMDA

On basis of the presented data, PMDA concluded that the proposed shelf life of 60 months for the product and the proposed method of use (once diluted, the product must be kept at room temperature and must be infused within 3 hours) are justified.

5. Indication or Performance

5.1. Studies to support the indication or performance

In vitro studies were performed to examine the immunomodulatory effects, migratory ability, and immunogenicity of Temcell. No *in vivo* studies were performed because there is no suitable animal model.

5.1.1. Immunomodulatory effects

5.1.1.1. Inhibition of human T-cell proliferation (CTD 4.2.1.1.1)

Since hMSCs inhibit the proliferation of T-cells activated by antigen-presenting cells, the effect of Temcell on T-cell proliferation induced by anti-CD3/CD28 stimulation was examined. T-cell proliferation induced by anti-CD3/CD28 stimulation was inhibited by 73% by Temcell co-cultured with human peripheral blood mononuclear cells (3 days).

5.1.1.2. Effect of prostaglandin E2 (PGE_2) synthesis inhibitors or an inhibitor of indoleamine 2,3-dioxygenase (IDO) on the suppression of human T-cell proliferation by Temcell (CTD 4.2.1.1.2) (CTD 4.2.1.1.3) (CTD 4.2.1.1.7)

Since activated hMSCs secrete PGE_2 and the IDO metabolite kynurenine, which inhibit T-cell proliferation, the effects of PGE_2 and kynurenine in Temcell-mediated T-cell proliferation inhibitory activity were examined. The levels of PGE_2 in the cell-culture supernatant were determined by ELISA. As a result, the PGE_2 concentration was 2.2 ng/mL after [redacted] hours of culture, which was increased to 3.2 ng/mL in the presence of TNFα, a proinflammatory cytokine ([redacted] ng/mL). While Temcell inhibited T-cell proliferation induced by anti-CD3/CD28 stimulation by 79%, T-cell proliferation was inhibited by 58% and 57% in the presence of PGE_2 synthesis inhibitors, indomethacin ([redacted] μmol/L) and NS398 ([redacted] μmol/L), respectively, showing that the presence of these inhibitors reduced the suppression of T-cell proliferation by Temcell.

In untreated Temcell, there was no expression of *IDO*. The addition of IFN-γ, poly (i:c), or LPS induced *IDO* expression. While Temcell inhibited anti-CD3/CD28-stimulated T-cell proliferation by 82%, T-cell proliferation was inhibited by 64% in the presence of an inhibitor of *IDO*, 1-methyl-DL-tryptophan (1 mmol/L),

showing that the presence of the inhibitor reduced the suppression of T-cell proliferation by Temcell.

5.1.1.3. Regulatory T-cell induction (CTD 4.2.1.1.4) (CTD 4.2.1.1.5)

hMSCs induce the differentiation of CD4-positive T cells ($CD4^+$T cells) into $CD4^+CD25^+$*FoxP3*$^+$ regulatory T cells (*Cell Stem Cell* 2013; 13(4): 392-402, *Immunol Cell Biol.* 2013; 91(1): 19-26). The level of expression of *FoxP3* in $CD4^+$T cells co-cultured with Temcell and how the ratio of CD4- and CD25-positive cells ($CD4^+CD25^+$T cells) was affected by Temcell were determined. When $CD4^+$T cells were co-cultured with Temcell for 3 days, the level of expression of *FoxP3* in $CD4^+$T cells was increased to 2.2- to 3.0-fold that in $CD4^+$T cells cultured alone, and the proportion of $CD4^+CD25^+$T cells increased from 0.68% to 0.79% to 1.09% to 1.14%.

5.1.1.4. TLR family expression (CTD 4.2.1.1.6)

Temcell was analyzed by RT-PCR for the expression of *TLR 1* to *10*. As a result, Temcell was found to express *TLR1*, *TLR3*, *TLR4*, and *TLR6*. The expression of TLR3 and TLR4 is known to be induced by proinflammatory cytokines such as IL-1β and IFN-γ. Since TLR3 and TLR4 were considered to be involved in the immunomodulatory effects of Temcell, the gene expression of proinflammatory cytokines in the presence of a TLR3 agonist, poly (i:c) or a TLR4 agonist, LPS was analyzed by real-time RT-PCR. As a result, the addition of each agonist resulted in concentration-dependent increases in the gene expression of IL-6 and IL-8, proinflammatory cytokines, leading to increased levels of IL-6 and IL-8 in the culture supernatant.

5.1.2. Cell migratory ability

5.1.2.1. Expression of cell migration-related genes (CTD 4.2.1.1.8) (CTD 4.2.1.1.9)

CXCR4, ITGA4, and ITGB1 play an important role in the adhesion of hMSCs to vascular endothelial cells, while MMP and TIMP play an important role in the breakdown of the basement membrane and extracellular matrix for extravascular migration of hMSCs that have adhered to vascular endothelial cells. Temcell was analyzed for the expression of adhesion-related factors by real-time RT-PCR. As a result, Temcell was found to express *ITGA4*, *ITGB1*, *MMP2*, *MMP14*, *TIMP1*, and *TIMP2*. Temcell was analyzed for the expression of CXCR4, ITGA4, and ITGB1 using flow cytometry and the expression of CXCR4 and ITGB1 was detected.

5.1.2.2. Cell migration (CTD 4.2.1.1.10)

The effects of proinflammatory cytokines and growth factors in the migration of Temcell were examined in an *in vitro* cell migration assay. As a result, FBS-induced migration of Temcell was inhibited concentration-dependently by an IGF-1 inhibitor, PPP; a PDGF inhibitor, AG1296; and a MMP inhibitor, GM6001.

5.1.3. Factors involved in immunogenicity

5.1.3.1. Analysis for MHC and co-stimulatory molecules (CTD 4.2.1.1.11)

hMSCs express low levels of MHC, do not express co-stimulatory molecules, and delay or avoid immune rejection by suppressing an allogeneic immune response in a patient via its immunomodulatory effects. Temcell was analyzed by flow cytometry to examine the expression of cell surface antigens (MHC class I and II

molecules) and co-stimulatory molecules required for antigen-specific immune responses (CD40, CD80, CD86). As a result, Temcell was found to express weak levels of MHC class I molecules, which were increased by stimulation with IFN-γ. Temcell did not express MHC class II molecules, but the expression of MHC class II molecules was induced by stimulation with IFN-γ. The expression of CD40, CD80, or CD86 was not detected, with or without stimulation with IFN-γ.

5.2. Outline of the review by PMDA

The applicant's explanation on the mechanism of action of Temcell in the treatment of GVHD, based on the study results:

Temcell acquires the ability to migrate toward the site of inflammation in response to PDGF, IGF-1, etc., released during inflammation associated with GVHD. Temcell adheres to the vessel wall via CXCR4, ITGA4, and ITGB1, and then traverse the vessel wall using expressed MMPs and TIMPs to reach the site of inflammation. Temcell expresses the TNFα receptor, INF-γ receptor, TLR3, and TLR4 and is activated at sites of inflammation upon stimulation with proinflammatory cytokines such as TNFα and IFN-γ, thereby secreting PGE_2 and kynurenine. Secreted PGE_2 and kynurenine inhibit T-cell proliferation. Temcell also suppresses immune responses by inducing the differentiation of T cells ($CD4^+$T cells) into regulatory T cells ($CD4^+CD25^+$ cells). Temcell is considered to exert a therapeutic effect in patients with GVHD through immunosuppressive effects mediated by these multiple mechanisms.

Temcell expresses low levels of MHC (MHC class I and II molecules) involved in immunogenicity and does not express co-stimulatory molecules (CD40, CD80, CD86). This would result in the delay or avoidance of rejection of the transplanted cells by the host immune system and thus the efficacy of Temcell can be expected even in MHC-unmatched recipients.

PMDA's view:

The studies conducted to support the indication or performance did not directly show that Temcell would contribute to improvement of GVHD. Although the results of *in vitro* studies do not contradict the previous reports on the effects of hMSCs, the applicant's explanation about the immunomodulatory effects, migratory ability, and immunogenicity of Temcell based on these results is only an inference. The mechanism of action of Temcell in the treatment of GVHD should continue to be investigated and discussed, with a close attention to new research reports etc., on hMSCs. If there is any report that raises concerns about the safety of Temcell, the information should be provided to healthcare professionals as needed.

6. Biodistribution of the Product

The biodistribution of Temcell was assessed following administration of ^{51}Cr-JR-031 to mice.

6.1. Single-dose biodistribution study (CTD 4.2.2.3.1)

A single dose of ^{51}Cr-JR-031 (20×10^6 cells/kg) was infused into the tail vein of male and female SCID mice and tissue radioactivity concentrations were calculated from radioactivity detected in freeze-dried whole-body

sections at 2, 24, 72, 168, 336, and 672 hours post-infusion. As a result, in males, the highest level of radioactivity was found in the lungs at 2 hours post-infusion and radioactivity was distributed in the spleen, liver, bone marrow, kidneys, heart, adrenal gland, and small intestine. At 24 hours post-infusion, a decrease in lung radioactivity and increases in spleen and liver radioactivity were observed. At 72 hours post-infusion, radioactivity was detected in the spleen, liver, bone marrow, lungs, kidneys, eyeballs, adrenal gland, brain, testes, and large intestine. At 672 hours post-infusion, radioactivity was detected in the spleen, liver, and bone marrow, though lower concentrations than those at 2 hours post-infusion. Radioactivity in blood peaked at 2 hours post-infusion and was detectable up to 336 hours post-infusion and below the lower limit of quantification at 672 hours post-infusion.

In females, as in males, the highest level of radioactivity was found in the lungs at 2 hours post-infusion and radioactivity was distributed in the bone marrow, liver, spleen, kidneys, heart, adrenal gland, and small intestine. At 24 hours post-infusion, a decrease in radioactivity in the lungs and increases in radioactivity in the liver, spleen, and kidneys were observed. At 72 hours post-infusion, radioactivity was detected in the liver, spleen, lungs, bone marrow, kidneys, adrenal gland, ovaries, stomach, uterine, submandibular gland, eyeballs, heart, and brain. At 672 hours post-infusion, radioactivity was detected in the spleen, liver, bone marrow, and lungs. Radioactivity in blood was detectable up to 336 hours post-infusion and below the lower limit of quantification at 672 hours post-infusion.

Tissue concentrations over time calculated from radioactivity distribution in freeze-dried whole-body sections at different time points were as shown in Figure 6.1 and Figure 6.2. If radioactivity was below the lower limit of quantification, no plot was produced.

6.2. Outline of the review by PMDA

The applicant's explanation on the biodistribution of Temcell:

Following intravenous infusion of ^{51}Cr-JR-031 to SCID mice, the highest level of radioactivity was found in the lungs in both males and females at 2 hours post-infusion. Then, radioactivity in the lungs decreased while radioactivity in the spleen and liver increased, indicating the possibility that the cells are redistributed from the lungs to the spleen and liver through blood. Analysis of distribution of radioactivity in freeze-dried whole-body sections showed that radioactivity was detected in the liver, spleen, and bone marrow of male and female mice at 672 hours post-infusion (the last measurement time point). Measurement of radioactivity in excised tissues (CTD 2.6.4, Table 2.6.4-4 and Table 2.6.4-5) showed that radioactivity was detected in both male and female lungs, suggesting that ^{51}Cr-JR-031 remains in the lungs, liver, spleen, and bone marrow for at least 672 hours after infusion.

PMDA concluded that assessment of the biodistribution of Temcell in mice is acceptable.

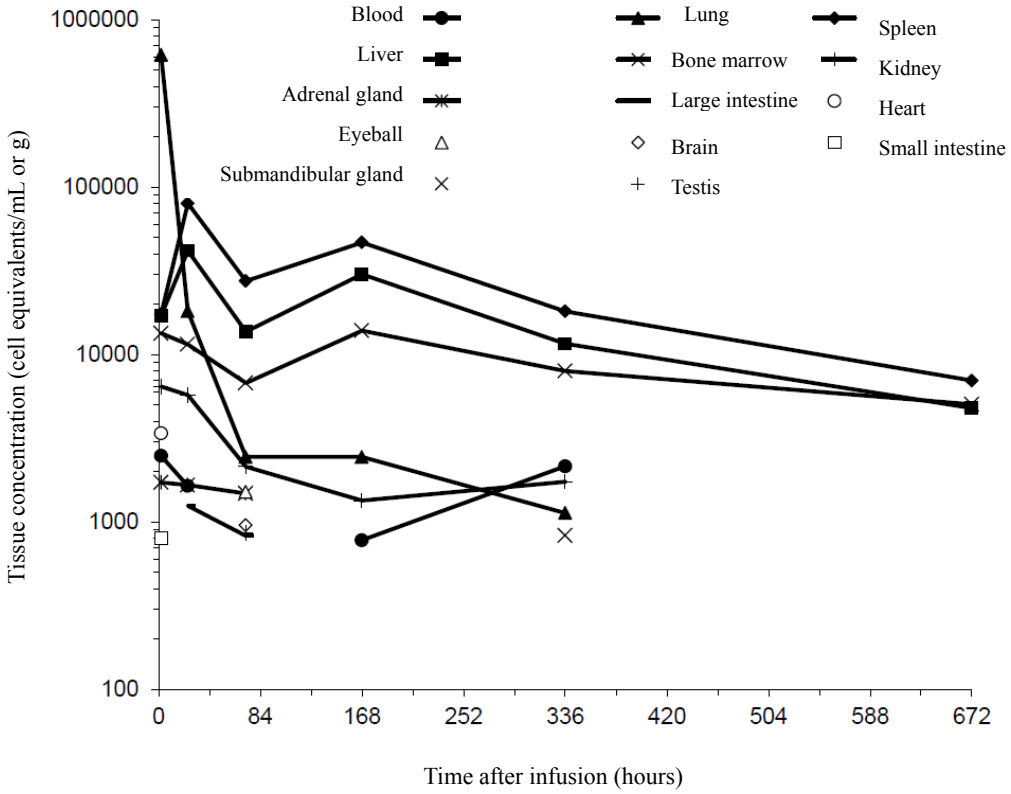

Figure 6.1. Tissue concentrations over time following a single intravenous infusion of ^{51}Cr-JR-031 20 × 10^6 cells/kg in male SCID mice

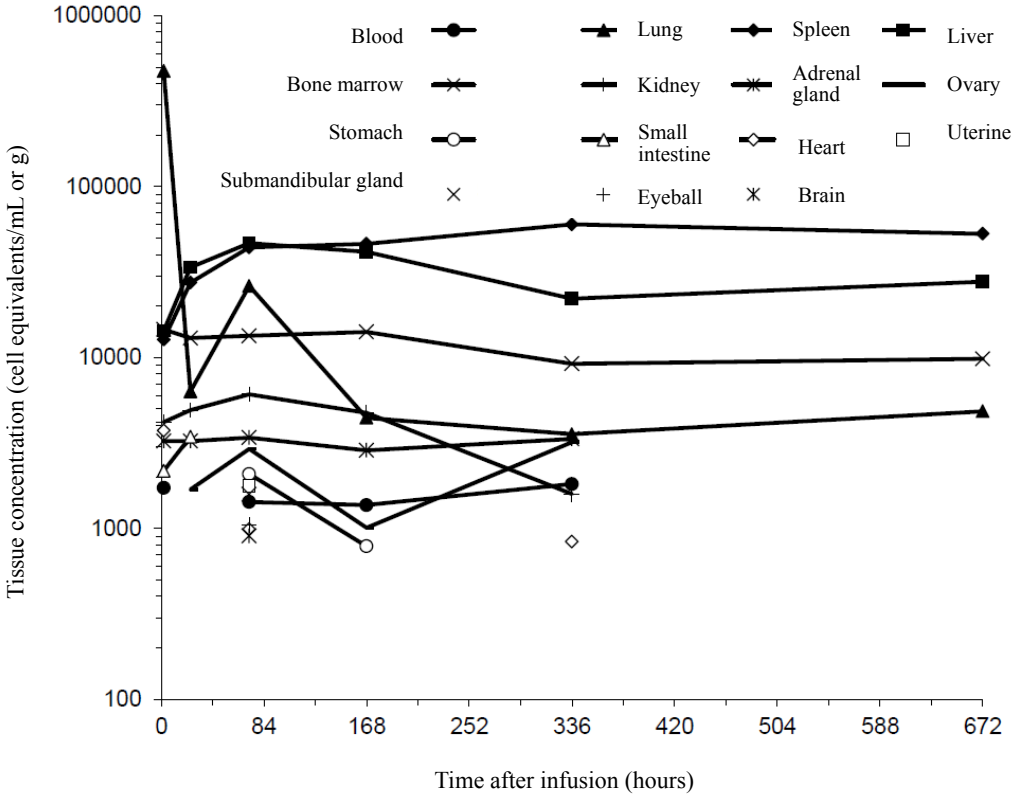

Figure 6.2. Tissue concentrations over time following a single intravenous infusion of ^{51}Cr-JR-031 20 × 10^6 cells/kg in female SCID mice

7. Nonclinical Safety Data

As non-clinical safety data on Temcell, the results from a repeat-dose toxicity study were submitted. Reference data submitted include the results from single-dose toxicity and repeat-dose toxicity studies and other studies, in which ACI rat MSCs (rMSCs) were used.

7.1. Mouse 4-week repeated intravenous dose toxicity study (CTD 4.2.3.2.3)

Male and female SCID mice were intravenously given vehicle[1] or Temcell (2×10^6 or 20×10^6 cells/kg) twice weekly for 4 weeks.[2] As a result, large cells in the alveolar wall were observed in the 20×10^6 cells/kg group, which were considered of little toxicological significance because no toxicological changes such as congestion, hemorrhage, and inflammation were noted. This finding was not observed at 9 weeks after the end of treatment. The no observed adverse effect level (NOAEL) was determined to be 20×10^6 cells/kg.

7.2. Reference data

7.2.1. Rat single intravenous dose toxicity study (CTD 4.2.3.1.1)

Male and female F344 rats were given a single dose of 0 (vehicle[3]), 10×10^6, 40×10^6, or 65×10^6 cells/kg of rMSCs via intravenous infusion.[4] No rMSC-related death occurred. Red urine was observed in the 65×10^6 cells/kg group.

7.2.2. Rat 13-week repeated intravenous dose toxicity study (CTD 4.2.3.2.2)

Male and female F344 rats received 0 (vehicle[5]), 2×10^6, 10×10^6, or 20×10^6 cells/kg of rMSCs intravenously twice weekly for 4 weeks,[6] followed by a no-treatment period of 1 week, then received the same dose once weekly for 4 weeks.[6] As a result, when rMSCs were adminsitered twice weekly, 2 of 20 males in the 10×10^6 cells/kg group and 2 of 20 males and 2 of 20 females in the 20×10^6 cells/kg group died. Surviving animals exhibited decreased activity, loss of righting reflex, and red urine at 10×10^6 cells/kg and pale skin of the whole body, hunched posture, and salivation at 20×10^6 cells/kg, which were not observed when rMSCs were administered once weekly. Interim necropsy following 4 weeks of twice-weekly intravenous dosing revealed cellular embolism in the lungs and thrombus at the administration site (tail vein) at $\geq 2 \times 10^6$ cells/kg, degeneration of spermatocytes and sperm cells in the testis at $\geq 10 \times 10^6$ cells/kg, and thrombus in the lungs at 20×10^6 cells/kg, but all of these findings were mild in severity and reversible at the final necropsy. Interim necropsy also revealed increased spleen weights at $\geq 2 \times 10^6$ cells/kg and increased adrenal weights at $\geq 10 \times 10^6$ cells/kg without associated histopathological changes.

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[1] Bicarbonate Ringer's solution containing 1.7% human serum albumin and 3.75% DMSO
[2] Administration volume, 5 mL/kg; Infusion rate, 1.2 mL/min
[3] Acetate Ringer's solution containing 5% fetal bovine serum and 10% DMSO
[4] Administration volume, 5 mL/kg; Infusion rate, 0.8 mL/min
[5] Acetate Ringer's solution containing 5% rat serum and 10% DMSO
[6] Administration volume, 5 mL/kg; Infused over approximately 2 minutes

7.2.3. Other studies

7.2.3.1. Effects on central nervous system in rats (CTD 4.2.1.3.1)

Male F344 rats were given a single dose of 0 (vehicle[7]), 5×10^6, 15×10^6, or 45×10^6 cells/kg of rMSCs or physiological saline as an intravenous infusion[8] and the effects of rMSCs on general symptoms and behavior were assessed by modified Irwin's test. As a result, no rMSCs-related effects were observed.

7.2.3.2. Effects on respiratory system in rats (CTD 4.2.1.3.2)

Male F344 rats were given a single dose of 0 (vehicle[7]), 5×10^6, 15×10^6, or 45×10^6 cells/kg of rMSCs or physiological saline as an intravenous infusion[8] and the effects of rMSCs on respiratory rate, tidal volume, and minute ventilation were assessed. Although animals in the 45×10^6 cells/kg group exhibited increased respiratory rate and decreased tidal volume, minute ventilation did not decrease. Thus, these findings were not considered serious changes.

7.3. Outline of the review by PMDA

PMDA asked the applicant to explain the tumorigenic risk of Temcell.

The applicant's response:

In a mouse 4-week repeated intravenous dose toxicity study (CTD 4.2.3.2.3), there were no findings related to tumorigenicity of the administered cells. Temcell can be expanded in an undifferentiated state *in vitro*, but is considered to lose proliferative capacity after appropximately [redacted]th cell division from the bone marrow aspirate (a raw material) [see Section 3.1.4.]. It has been reported that hMSCs do not undergo transformation after long-term *in vitro* culture (*Clin Sarcoma Res.* 2013; 3: 10, *Cancer Res.* 2007; 67: 9142-9, etc.). Also in clinical studies of Temcell or Prochymal and clinical trials of other hMSCs (*PLoS One.* 2012; 7: e47559), no adverse events suggestive of tumor formation attributed to the administered cells were observed. The literature reports that hMSCs did not engraft 112 days after clinical infusion (*Stem Cells.* 2012; 30: 1575-8). Furthermore, the specification tests for DCB of Temcell include chromosome analysis and soft agar colony formation assay using cells cultured beyond the passage level of *ex-vivo* cultured hMSCs to make sure that no transformed cells are included [see Section 3.1.5.1.]. Therefore, the tumorigenic risk of Temcell should be low.

PMDA asked the applicant to explain the appropriateness of the 4-week duration of a general toxicity study of Temcell.

The applicant's response:

There was a concern about a strong immune response to xenogeneic cells when assessing the general toxicity of human-derived cells, Temcell, in animals. In a single intravenous dose biodistribution study [see Section 6.1.], Temcell remained in the body of SCID mice for at least 4 weeks. Thus, SCID mice were selected as test animals. However, as 4 weeks of twice-weekly dosing of rMSCs resulted in death in a rat 13-week repeated

[7] Acetate Ringer's solution containing 1.9% rat serum and 3.7% DMSO
[8] Administration volume, 5.62 mL/kg; Infusion rate, 1 mL/min

intravenous dose toxicity study, a study duration of >4 weeks was not selected and thus a duration of 4 weeks (twice-weekly dosing) was selected. Given the results of the single-dose biodistribution study [see Section 6.1.], Temcell may accumulate in the human body when administered with the clinical dosage regimen. The applicant considered that the safety of Temcell in relation to its accumulation can be assessed by including a dose higher than the clinical dose (10 times the clinical dose [the high-dose group]) in a general toxicity study. On the basis of the above, the design for the general toxicity study of Temcell is appropriate and the general toxicity of Temcell can be assessed in the mouse 4-week repeated intravenous dose toxicity study (CTD 4.2.3.2.3).

PMDA's view:
The applicant's response about the tumorigenic risk of Temcell and the duration of the general toxicity study is acceptable. PMDA concluded that there is no particular problem with the non-clinical safety of Temcell.

8. Clinical Data

The efficacy and safety evaluation data submitted consisted of the results from a Japanese phase I/II study (JR-031-201) and its extension study (JR-031-202) and a Japanese phase II/III study (JR-031-301) in patients with acute GVHD refractory to treatment with corticosteroids. The reference data submitted consisted of the results from the following acute GVHD patient studies with Prochymal conducted by Osiris: a foreign phase III study (280), an Expanded Access Program study (275), and Emergency-Use Protocols (208, 215, 216, 220, 221, 222, 224, 225, 227, 230, 231, 270E.8/271). The response criteria are presented in Table 8.1.

Table 8.1. Acute GVHD response criteria

Abbreviation	Definition
CR	Complete response: Resolution of acute GVHD in all involved organs
PR	Partial response: Organ improvement of at least 1 stage without worsening in any other organ system
OR	Overall response: CR or PR
MR	Mixed response: Improvement by at least 1 organ stage in at least 1 evaluable organ with worsening by at least 1 organ stage in at least 1 other organ

8.1. Japanese phase I/II study (CTD 5.3.5.2.1, Study JR-031-201 [[redacted] to [redacted]])

An open-label, uncontrolled study was conducted at 12 sites in Japan to evaluate the safety and efficacy of Temcell in patients with Grades II-IV acute GVHD secondary to allogeneic hematopoietic stem cell transplantation (bone marrow transplantation, peripheral blood stem cell transplantation, cord blood transplantation) who had failed to respond to systemic corticosteroid therapy[9] (target sample size, 20 subjects).

Temcell was given at a dose of 2×10^6 cells/kg twice weekly for 4 weeks (infusions were administered at least 3 days apart, a total of 8 infusions). Subjects who had a PR or MR at 4 weeks after the first infusion were eligible for continued therapy with an additional 4 weekly infusions. Subjects who achieved a CR after the first infusion and who had a subsequent flare of Grade II-IV GVHD by Week 10 were allowed to be retreated with Temcell twice weekly for 4 weeks (infusions were administered at least 3 days apart, for a total of 8 infusions).

[9] Initial treatment of acute GVHD with corticosteroids and response evaluation and grading of acute GVHD were based on the JSHCT guideline.

The duration of the study was 24 weeks.

All of 14 subjects treated with Temcell were included in the safety analysis set, efficacy analysis set, and Full Analysis Set (FAS).

All of the 14 subjects experienced adverse events. Adverse events reported by ≥20% of subjects and those classified as adverse reactions (adverse events other than those assessed by the investigator as causally unrelated to Temcell or adverse events of which causality to Temcell could not be ruled out by the sponsor) are as shown in Table 8.2.

Serious adverse events occurring in 13 subjects were as follows: hepatic function abnormal, white blood cell count decreased, and neutrophil count decreased (5 subjects each); platelet count decreased and haemoglobin decreased (3 subjects each); pneumonia and acute GVHD (2 subjects each); and bacteraemia, herpes zoster, sepsis, cytomegalovirus enterocolitis, anaemia, chronic GVHD, electrolyte imbalance, spasm, pleural effusion, organising pneumonia, venoocclusive liver disease, cholecystocholangitis, hydronephrosis, acute renal failure, multi-organ failure, blood potassium decreased, blood uric acid increased, and γ-glutamyl transferase (γ-GTP) increased (1 subject each). Fatal events occurred in 3 subjects (pneumonia and pleural effusion [1 subject]; hepatic function abnormal and multi-organ failure [1 subject]; and venoocclusive liver disease [1 subject]). Among the non-fatal serious adverse events, those classified as adverse reactions were as follows: hepatic function abnormal, white blood cell count decreased, neutrophil count decreased, platelet count decreased, and haemoglobin decreased (2 subjects each); and bacteraemia, sepsis, hydronephrosis, and γ-GTP increased (1 subject each).

Table 8.2. Adverse events reported by ≥20% of subjects

Adverse event term		Adverse events	Adverse reactions
		N = 14	N = 14
No. of subjects with any event		14 (100)	13 (92.9)
Infections and infestations			
	Bacteraemia	3 (21.4)	1 (7.1)
	Pneumonia	3 (21.4)	1 (7.1)
Blood and lymphatic system disorders			
	Anaemia	3 (21.4)	1 (7.1)
Immune system disorders			
	Chronic GVHD	5 (35.7)	1 (7.1)
Gastrointestinal disorders			
	Vomiting	6 (42.9)	2 (14.3)
	Constipation	4 (28.6)	1 (7.1)
	Diarrhoea	3 (21.4)	0 (0)
Hepatobiliary disorders			
	Hepatic function abnormal	7 (50.0)	4 (28.6)
Skin and subcutaneous tissue disorders			
	Rash	5 (35.7)	1 (7.1)
Musculoskeletal and connective tissue disorders			
	Arthralgia	5 (35.7)	0 (0)
General disorders and administration site conditions			
	Pyrexia	7 (50.0)	6 (42.9)
	Oedema	4 (28.6)	2 (14.3)
Investigations			
	White blood cell count decreased	8 (57.1)	1 (7.1)
	Platelet count decreased	7 (50.0)	2 (14.3)
	Neutrophil count decreased	6 (42.9)	1 (7.1)
	Blood potassium decreased	5 (35.7)	0 (0)
	Blood urine present	5 (35.7)	1 (7.1)
	Haemoglobin decreased	4 (28.6)	1 (7.1)
	Blood magnesium increased	3 (21.4)	1 (7.1)
	Blood pressure decreased	3 (21.4)	1 (7.1)
	Red blood cell count decreased	3 (21.4)	0 (0)

n (%)
MedDRA/J ver. 13.1

No changes were noted in vital signs (pulse rate, body temperature, blood pressure, respiratory rate) or percutaneous oxygen saturation after the first infusion. ECG showed sinus tachycardia in 2 subjects after the first infusion. Ectopic tissue formation was not observed.

The efficacy endpoints were an OR at 4 weeks or 12 weeks post first infusion, a CR of ≥28 days duration, and survival at 24 weeks post first infusion (the end of the observation period). The results are as shown in Table 8.3.

Table 8.3. Summary of results of efficacy endpoints (Study JR-031-201)

Endpoint	No. of evaluable subjects	No. of subjects achieving endpoint	Proportion (%)	95% CI
OR at 4 weeks post first infusion	14	13	92.9	66.1, 99.8
OR at 12 weeks post first infusion	14	13	92.9	66.1, 99.8
CR of ≥28 days duration	14	10	71.4	41.9, 91.6
Survival at 24 weeks post first infusion	14	11	78.6	49.2, 95.3

CI: confidence interval

8.2. Japanese phase I/II extension study (CTD 5.3.5.2.2, Study JR-031-202 [[redacted] to [redacted]])

An extension study was conducted in patients who participated in Study JR-031-201 to evaluate the safety and

efficacy of JR-031 up to 24 months after the first infusion in Study JR-031-201. No additional doses of Temcell were administered in this study.

Of 14 subjects treated with Temcell in Study JR-031-201, 11 subjects entered this study and were included in the safety analysis set while the remaining 3 subjects were excluded from the analysis due to death. Of the 11 subjects, 9 subjects completed the observation period and 2 subjects were withdrawn due to death.

In this study, adverse events occurring beyond 12 months post first infusion in Study JR-031-201 were collected only if they were classified by the investigator as serious or significant adverse events or adverse events for which a causal relationship to the investigational product could not be ruled out. Therefore, adverse events occurring between 24 weeks (the initial period of this study) and 12 months post first infusion in Study JR-031-201 and those occurring between 12 months and 24 months post first infusion were analyzed separately. Adverse events occurred in all of the 11 subjects. Those events are as shown in Table 8.4.

Between 24 weeks and 24 months post first infusion, serious adverse events occurring in 7 subjects were as follows: septic shock, acute myeloid leukaemia recurrent, and electrolyte imbalance (2 subjects each); and herpes zoster, metastatic breast cancer, anaemia, lymphadenitis, hyperglycaemia, decreased appetite, altered state of consciousness, interstitial lung disease, and lipase increased (1 subject each). Fatal events occurred in 2 subjects (metastatic breast cancer and acute myeloid leukaemia recurrent [1 subject each]).

Table 8.4. Adverse events reported by ≥10% of subjects

Adverse event term		Between 24 weeks and 12 months post first infusion in Study JR-031-201	Between 12 months and 24 months post first infusion in Study JR-031-201
		N = 11	N = 9
No. of subjects with any event		11 (100)	2 (22.2)
Infections and infestations			
	Pneumonia	3 (27.3)	1 (11.1)
	Septic shock	2 (18.2)	1 (11.1)
	Sinusitis	2 (18.2)	0 (0)
	Upper respiratory tract infection	2 (18.2)	0 (0)
	Oral herpes	2 (18.2)	0 (0)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)			
	Acute myeloid leukaemia recurrent	1 (9.1)	1 (11.1)
Immune system disorders			
	Chronic GVHD	2 (18.2)	0 (0)
Metabolism and nutrition disorders			
	Electrolyte imbalance	2 (18.2)	0 (0)
Gastrointestinal disorders			
	Constipation	2 (18.2)	0 (0)
	Haemorrhoids	2 (18.2)	0 (0)
	Vomiting	2 (18.2)	0 (0)
Musculoskeletal and connective tissue disorders			
	Back pain	2 (18.2)	0 (0)
General disorders and administration site conditions			
	Pyrexia	4 (36.4)	0 (0)
	Oedema	2 (18.2)	0 (0)
Investigations			
	Weight decreased	2 (18.2)	0 (0)
	White blood cell count decreased	1 (9.1)	1 (11.1)

n (%)
MedDRA/J ver. 15.0

Ectopic tissue formation was not observed during the study period (approximately 18 months).

8.3. Japanese phase II/III study (CTD 5.3.5.2.3, Study JR-031-301 [[redacted] to [redacted]])

An open-label, uncontrolled study was conducted at 18 sites in Japan to evaluate the efficacy and safety of Temcell in patients with Grade III or IV acute GVHD secondary to allogeneic hematopoietic stem cell transplantation (bone marrow transplantation, peripheral blood stem cell transplantation, and cord blood transplantation) who had failed to respond to systemic corticosteroid therapy[10] (target sample size, 25 subjects).

Temcell was given at a dose of 2×10^6 cells/kg twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions). Subjects who had a PR or MR at 4 weeks after the first infusion were eligible for continued therapy with an additional 4 weekly infusions. Subjects who achieved a CR after the first infusion and who had a subsequent flare of Grades II-IV GVHD by Week 10 were allowed to be retreated with Temcell twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions). The duration of the study was 52 weeks.

All of 25 subjects treated with Temcell were included in the efficacy analysis set, safety analysis set, and FAS. Nineteen subjects completed 4-week treatment with Temcell (i.e., a total of 8 infusions).

The proportion of subjects with a CR of ≥28 days duration (primary efficacy endpoint) was 12 of 25 subjects (48.0% [95% confidence interval (CI); 27.8, 68.7]) and the lower bound of the 95% confidence interval was below the historical response rate (30%).

Safety data were analyzed. All of the 25 subjects experienced adverse events. Table 8.5 shows adverse events reported by ≥20% of subjects and those classified as adverse reactions.

Serious adverse events occurring in 22 subjects were as follows: sepsis (7 subjects); anaemia (5 subjects); white blood cell count decreased (4 subjects); platelet count decreased (4 subjects); pneumonia (3 subjects); thrombotic microangiopathy, bone marrow failure, chronic GVHD, hypokalaemia, gastrointestinal haemorrhage, hepatic function abnormal, multi-organ failure, and γ-GTP increased (2 subjects each); and infection, herpes zoster, septic shock, pyelocystitis, acute myeloid leukaemia recurrent, pancytopenia, thrombotic thrombocytopenic purpura, liver GVHD, hyperglycaemia, malnutrition, hypertensive encephalopathy, cardiac arrest, hypoxia, interstitial lung disease, ileus paralytic, pancreatitis acute, eczema, renal disorder, cystitis haemorrhagic, haematuria, blood bilirubin increased, compression fracture, and delayed engraftment (1 subject each). Fatal events occurred in 12 subjects (thrombotic microangiopathy, sepsis, and multi-organ failure [2 subjects each]; and pneumonia, liver GVHD, gastrointestinal haemorrhage, thrombotic thrombocytopenic purpura, cardiac arrest, and acute myeloid leukaemia recurrent [1 subject each]). Among the

[10] Initial treatment of acute GVHD with corticosteroids and response evaluation and grading of acute GVHD were based on the JSHCT guideline.

non-fatal serious adverse events, those classified as adverse reactions were as follows: anaemia and platelet count decreased (3 subjects each); hepatic function abnormal and γ-GTP increased (2 subjects each); and sepsis, hypoxia, blood bilirubin increased, gastrointestinal haemorrhage, eczema, white blood cell count decreased, pancreatitis acute, renal disorder, bone marrow failure, haematuria, pneumonia, and hypokalaemia (1 subject each).

Table 8.5. Adverse events reported by ≥20% of subjects (FAS)

Adverse event term		Adverse events	Adverse reactions
		N = 25	N = 25
No. of subjects with any event		25 (100)	20 (80.0)
Infections and infestations			
	Sepsis	7 (28.0)	1 (4.0)
	Infection	5 (20.0)	1 (4.0)
Blood and lymphatic system disorders			
	Anaemia	8 (32.0)	2 (8.0)
	Thrombotic microangiopathy	6 (24.0)	2 (8.0)
Immune system disorders			
	Chronic GVHD	6 (24.0)	1 (4.0)
Vascular disorders			
	Hypertension	7 (28.0)	3 (12.0)
Gastrointestinal disorders			
	Constipation	5 (20.0)	0 (0)
Hepatobiliary disorders			
	Hepatic function abnormal	6 (24.0)	4 (16.0)
Investigations			
	White blood cell count decreased	12 (48.0)	5 (20.0)
	Platelet count decreased	10 (40.0)	6 (24.0)
	C-reactive protein increased	6 (24.0)	1 (4.0)
	γ-GTP increased	6 (24.0)	3 (12.0)
	Blood potassium decreased	5 (20.0)	1 (4.0)
	White blood cell count increased	5 (20.0)	3 (12.0)

n (%)
MedDRA/J ver. 16.1

No changes were noted in vital signs (pulse rate, body temperature, blood pressure, respiratory rate) or percutaneous oxygen saturation after the first infusion. ECG showed abnormal findings in 2 subjects after the first infusion (complete right bundle branch block; and premature ventricular contraction and sinus tachycardia). Ectopic tissue formation was not observed.

8.4. Reference data (Foreign studies with Prochymal)

8.4.1. Foreign phase III study in patients with acute GVHD (CTD 5.3.5.1.1, Study 280 [[redacted] to [redacted]])

A randomized, double-blind, placebo-controlled study was conducted at 72 sites in 6 countries including the US to evaluate the efficacy and safety of Prochymal in patients with Grades B-D acute GVHD (assessed using the International Bone Marrow Transplant Registry [IBMTR] grading system) secondary to allogeneic hematopoietic stem cell transplantation or donor lymphocyte infusion who had failed to respond to systemic corticosteroid therapy.

Prochymal at a dose of 2 × 10^6 cells/kg or placebo[11] was given twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions). Patients who had a PR or MR at Study Day 32 (±2 days) were eligible for continued therapy with an additional 4 weekly infusions. Subjects who achieved a CR after the first infusion and who had a subsequent flare of GVHD (IBMTR Grades B-D) by Study Day 72 were allowed to be retreated twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions). Subjects in both the Prochymal and placebo groups concomitantly received second-line therapy determined by their medical institutions. The duration of the study was 180 days.

A total of 260 subjects enrolled in Study 280 were randomized to the Prochymal (173 subjects) or placebo (87 subjects) group and were included in the Intent-To-Treat (ITT) population for analysis of the primary efficacy endpoint. Of the 260 randomized subjects, 223 subjects completed study treatment and 37 subjects (22 and 15 subjects in the Prochymal group and the placebo group, respectively) discontinued treatment due to consent withdrawal (11 subjects [7 vs. 4 subjects, respectively]), adverse events (5 subjects [1 vs. 4 subjects, respectively]), lost to follow-up (1 subject [1 vs. 0 subjects, respectively]), and others (20 subjects [13 vs. 7 subjects, respectively]). Further, 244 patients who received at least one dose of Prochymal or placebo (163 vs. 81 subjects, respectively) were included in the modified intent-to-treat (mITT) population for analyses of secondary efficacy endpoints and safety.

The proportion of subjects who achieved a CR of ≥28 days duration in the ITT population (primary efficacy endpoint) was 35% (60 of 173) of subjects in the Prochymal group and 30% (26 of 87) of subjects in the placebo group and the results of secondary endpoints in the mITT population were as shown in Table 8.6.

Table 8.6. Summary of results of efficacy endpoints (Study 280)

Endpoint		Prochymal	Placebo	*P*-value
ITT population		N = 173	N = 87	
	CR of ≥28 days duration	60 (34.7)	26 (29.9)	0.423
mITT population		N = 163	N = 81	
	OR at 28 days post first infusion	94 (57.7)	41 (50.6)	0.224
	Survival at 100 days post first infusion	85 (52.1)	41 (50.6)	0.780
	Survival at 180 days post first infusion	56 (34.4)	34 (42.0)	0.274

n (%)

Safety data were analyzed. Adverse events occurred in all of 244 subjects in the mITT population. Adverse events reported by ≥10% of subjects are as shown in Table 8.7. Serious adverse events occurred in 217 subjects (146 and 71 subjects in the Prochymal group and the placebo group, respectively) and the main events were GVHD (33 subjects [24 vs. 9 subjects, respectively]), sepsis (20 subjects [14 vs. 6 subjects, respectively]), gastrointestinal haemorrhage (17 subjects [12 vs. 5 subjects, respectively]), dyspnoea (15 subjects [10 vs. 5 subjects, respectively]), acute gut GVHD (14 subjects [11 vs. 3 subjects, respectively]), pneumonia (13 subjects [10 vs. 3 subjects, respectively]), and bacteraemia (12 subjects [9 vs. 3 subjects, respectively]). There were 149 deaths (105 vs. 44 subjects, respectively) and the events that led to death were infections and infestations (54 subjects [42 vs. 12 subjects, respectively]) and immune system disorders (28 subjects [23 vs. 5 subjects,

[11] Plasma-Lyte A containing 1.9% human serum and 3.75% DMSO

respectively]). Adverse events leading to treatment discontinuation occurred in 26 subjects (15 vs. 11 subjects, respectively) and the main events were hypoxia (3 subjects [3 vs. 0 subjects, respectively]), GVHD (2 subjects [1 vs. 1 subject, respectively]), haemorrhage intracranial (2 subjects [1 vs. 1 subject, respectively]), and hypotension (2 subjects [1 vs. 1 subject, respectively]).

Table 8.7. Adverse events reported by ≥10% of subjects (mITT)

Adverse event term	Study 280	
	Prochymal	Placebo
	N = 163	N = 81
Oedema peripheral	58 (35.6)	27 (33.3)
Abdominal pain	37 (22.7)	14 (17.3)
Thrombocytopenia	36 (22.1)	18 (22.2)
Hypokalaemia	35 (21.5)	11 (13.6)
Diarrhoea	34 (20.9)	15 (18.5)
Hyperglycaemia	33 (20.2)	11 (13.6)
Pyrexia	33 (20.2)	14 (17.3)
GVHD	32 (19.6)	12 (14.8)
Dyspnoea	30 (18.4)	10 (12.3)
Hypertension	29 (17.8)	6 (7.4)
Confusional state	28 (17.2)	6 (7.4)
Fatigue	27 (16.6)	11 (13.6)
Hypotension	27 (16.6)	16 (19.8)
Nausea	27 (16.6)	16 (19.8)
CMV infection	26 (16.0)	11 (13.6)
Hypomagnesaemia	26 (16.0)	9 (11.1)
Asthenia	25 (15.3)	9 (11.1)
Anaemia	23 (14.1)	12 (14.8)
Anxiety	23 (14.1)	5 (6.2)
Gastrointestinal haemorrhage	23 (14.1)	9 (11.1)
Insomnia	23 (14.1)	6 (7.4)
Tachycardia	23 (14.1)	8 (9.9)
Vomiting	23 (14.1)	17 (21.0)
Steroid myopathy	22 (13.5)	11 (13.6)
Pneumonia	21 (12.9)	6 (7.4)
Staphylococcal bacteraemia	21 (12.9)	5 (6.2)
Tremor	21 (12.9)	5 (6.2)
Cough	20 (12.3)	10 (12.3)
Hyperkalaemia	20 (12.3)	4 (4.9)
Hypoxia	20 (12.3)	6 (7.4)
Headache	19 (11.7)	8 (9.9)
Neutropenia	19 (11.7)	6 (7.4)
Rash	19 (11.7)	5 (6.2)
Sepsis	19 (11.7)	6 (7.4)
Depression	18 (11.0)	7 (8.6)
Hyperbilirubinaemia	18 (11.0)	12 (14.8)
Generalised oedema	17 (10.4)	7 (8.6)
Hypoalbuminaemia	14 (8.6)	10 (12.3)
Arthralgia	13 (8.0)	9 (11.1)
Dry mouth	12 (7.4)	9 (11.1)

n (%)
MedDRA/J ver. 11.0

No changes were noted in vital signs (pulse rate, body temperature, blood pressure, respiratory rate) or percutaneous oxygen saturation after the first infusion. ECG revealed no abnormal findings. No ectopic tissue formation was detected by CT imaging.

8.4.2. Expanded Access Program study in pediatric patients with acute GVHD (CTD 5.3.5.2.4, Study 275 [[redacted] to [redacted]])

An Expanded Access Program study was conducted at 36 sites in 6 countries including the US to evaluate the efficacy and safety of Prochymal in pediatric patients with Grades B-D acute GVHD (assessed using the IBMTR grading system)secondary to allogeneic hematopoietic stem cell transplantation or donor lymphocyte infusion who had failed to respond to systemic corticosteroid therapy.

Prochymal was given at a dose of 2×10^6 cells/kg twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions). Subjects who had a PR or MR at Study Day 28 (±2 days) were eligible for continued therapy with an additional 4 weekly infusions. Subjects who achieved a CR after the first infusion and who had a subsequent flare of GVHD (IBMTR Grades B-D) by Study Day 72 were allowed to be retreated twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions).

All of 75 subjects enrolled in the study[12] were included in the efficacy analysis set and safety analysis set. Seventy-four subjects completed study treatment and 1 subject discontinued treatment due to a serious adverse event.

Only serious adverse events were collected for safety data. Serious adverse events occurred in 46 subjects and the main events were dyspnoea (7 subjects), multi-organ failure (6 subjects), GVHD (3 subjects), and hypertension (3 subjects). There were 30 deaths. The main events were dyspnoea (5 subjects), multi-organ failure (4 subjects), mucormycosis (2 subjects), aspergillosis (2 subjects), and GVHD (2 subjects). Adverse events leading to treatment discontinuation occurred in 11 subjects and a causal relationship to Prochymal could not be ruled out for those experienced by 4 subjects. Assessment of vital signs (pulse rate, body temperature, blood pressure, respiratory rate, percutaneous oxygen saturation) showed hypertension in 3 subjects and tachycardia in 1 subject. ECG revealed no abnormal findings.

8.5. Outline of the review by PMDA

8.5.1. Clinical positioning

The applicant's explanation on the clinical positioning of hMSCs in the treatment of acute GVHD:

According to the JSHCT guideline, corticosteroids are a first-line agent for the initial treatment of acute GVHD (which is ineffective in approximately 50%). In patients who have failed to respond to first-line therapy, steroid pulse therapy and immunosuppressants such as ATG, TAC, and MMF are recommended as second-line therapy. However, infections are common following second-line therapy due to excessive immunosuppression and the non-relapse mortality rate at 2 years after treatment is as high as 56.3% (*Biol Blood Marrow Transplant* 2013; 19: 1183-89). As described above, there are no established second-line therapies for acute GVHD and expectations for the development of a novel therapy are high. hMSCs have been reported to reduce T-cell

[12] Data as of [redacted]. A total of 242 patients were enrolled by [redacted] and the study is ongoing.

activation and thus suppress immune responses by mechanisms such as inhibition of T-cell secretion of pro-inflammatory cytokines, increased production of anti-inflammatory cytokines, promotion of a shift toward a predominant Th2 immune response, and secretion of soluble factors that are involved in the suppression of T-cell response. Thus, hMSCs therapy is expected to be effective in treating an inflammatory, immune-mediated disease.

PMDA examined how the positioning of hMSCs therapy for acute GVHD is defined in several Japanese and foreign textbooks and guidelines for the diagnosis and management of acute GVHD. The summary is as follows:

- The guidelines developed by the European Group for Blood and Marrow Transplantation and the European Leukemia Net (*Bone Marrow Transplant* 2014; 49: 168-173): hMSCs therapy is positioned as a second-line treatment.
- The guidelines developed by the British Committee for Standards in Haematology and the British Society of Blood and Marrow Transplantation (*Br J Haematol*. 2012; 158: 30-45): hMSCs therapy is positioned as a third-line treatment.
- JSHCT guideline: Not mentioned.
- The guideline developed by the American Society for Blood and Marrow Transplantation (*Biol Blood Marrow Transplant* 2012; 18: 1150-63): Not mentioned.
- Williams Hematology (8th Edition): Describes the ongoing multiple early clinical trials and large clinical trials with hMSCs as the recent trend in the treatment of acute GVHD.
- Thomas' Hematopoietic Cell Transplantation Stem Cell Transplantation: Mentions that the development of hMSCs as a treatment for acute GVHD is under way.
- Wintrobe's Clinical Hematology (13th Edition): Not mentioned.

PMDA's view on the clinical positioning of Temcell:
While corticosteroids have been established as first-line treatment for acute GVHD, there is no established standard second-line treatment for patients who have failed to respond to first-line therapy or patients with flares of GVHD. Based on the benefits and risks of Temcell that were demonstrated in the submitted clinical data [see Sections 8.5.2. and 8.5.3.], Temcell can be positioned as a second-line therapy for patients with acute GVHD who have failed to respond to corticosteroids as the first-line therapy.

8.5.2. Efficacy

8.5.2.1. Efficacy endpoint

The applicant explanation on the appropriateness of selecting "a CR of ≥28 days duration" as the primary endpoint of Study JR-031-301:
Since the symptoms may recur after treatment of acute GVHD, an assessment of therapeutic effects at a specific time point only is not appropriate and the persistence of efficacy is important. The clinical significance of CR is clearer than that of PR. Thus, "a CR sustained for a certain period of time" should be assessed. On the basis of these considerations and referring also to the primary endpoint of "a CR of ≥28 days duration within 100

days post first infusion" for Study 280 with Prochymal, "a CR of ≥28 days duration" was chosen as the primary endpoint for Study JR-031-301.

PMDA's view:

Since the worsening of acute GVHD and related pathology may result in poor prognosis, amelioration of recurrent symptoms of acute GVHD is important in the treatment of acute GVHD. Thus, "a CR of ≥28 days duration," which enables persistence of efficacy to be assessed, has a certain significance in efficacy evaluation in the treatment of acute GVHD and this outcome as the primary endpoint for an exploratory study of Temcell is acceptable. However, a discussion of the effect on survival prognosis is also clinically important, and the efficacy of Temcell will therefore be assessed comprehensively, taking also account of overall survival, etc.

8.5.2.2. Results of efficacy evaluation

In Study JR-031-301, 12 of 25 subjects (48.0% [95% CI; 27.8, 68.7]) achieved the primary endpoint of "a CR of ≥28 days duration" and the lower bound of the 95% confidence interval was less than the pre-defined historical response rate of 30%. PMDA asked the applicant to explain the efficacy of Temcell based on the results from Study JR-031-301.

The applicant's explanation:

The historical response rate used for comparison with the results from Study JR-031-301 was based on the results from Study 280 with Prochymal. Namely, 29.7% of subjects with IBMTR Grade C or D acute GVHD in the placebo group of Study 280 achieved "a CR of ≥28 days duration" and a historical response rate of 30% was determined based on this value. The lower bound of the 95% confidence interval for this proportion in Study JR-031-301 was less than the determined historical response rate. The ex-post discussion of these results revealed differences in grading of individual organ stages between the two grading systems (Table 8.8). While the patients had IBMTR Grade C or D acute GVHD in Study 280, the patients had Grade III or IV acute GVHD classified according to the JSHCT guideline in Study JR-031-301. The criteria for staging each organ system are similar except for erythroderma (skin disorder), which is classified as stage 4 under the JSHCT guideline but classified as stage 3 according to the IBMTR grading system.

Table 8.8. Acute GVHD grading

JSHCT guideline				IBMTR grading system			
Grade	Organ stage			Grade	Organ stage		
	Skin	Liver	Gut		Skin	Liver	Gut
I	1-2	0	0	A	1	0	0
II	3	1	1	B	2	1-2	1-2
III	—	2-3	2-4	C	3	3	3
IV	4	4	—	D	4	4	4

In view of differences in the eligibility criteria between Studies 280 and JR-031-301, a subgroup of patients with a similar severity of acute GVHD to patients in Study JR-031-301 were identified from among patients included in Study 280 (a subgroup of patients with Grade III or IV acute GVHD classified according to the JSHCT guideline in the mITT population of Study 280: "Study 280 Grade-III/IV subpopulation") and they

were reanalyzed. As a result, the proportion of placebo-treated patients in this subpopulation who achieved "a CR of ≥28 days duration" was 21.6% (Table 8.9). In clinical studies that evaluated the efficacy of ATG or MMF as second-line therapy for steroid-resistant acute GVHD, the proportion of patients with "a CR of ≥28 days duration" was 20.3% (16 of 79 patients) (*Biol Blood Marrow Transplant* 2002; 8: 40-46) and 15.4% (2 of 13 patients) (*Eur J Haematol* 2004; 73: 56-61), respectively. Given the above findings, the historical response rate determined for comparison with the data from Study JR-031-301 is considered to have been higher than the proportion of patients achieving "a CR of ≥28 days duration" in clinical practice among patients with Grade III or IV acute GVHD classified according to the JSHCT guideline. Then, the lower bound of the 95% confidence interval for the proportion of patients achieving "a CR of ≥28 days duration" was greater in Study JR-031-301 (27.8%) than in the placebo group in the Study 280 III/IV subpopulation (21.6%).

Since American hematologists' report (*Biol. Blood Marrow Transplant* 2009; 15: 777-89) recommends that overall survival, non-relapse mortality, etc., should also be assessed for the overall evaluation of therapeutic effect in patients with acute GVHD, the results of the secondary endpoints of survival in Study JR-031-301 (survival at 180 days and 52 weeks post first infusion) were compared with those in the Study 280 III/IV subpopulation. As shown in Table 8.9, the survival rate at 180 days post first infusion was 60.0% (15 of 25) of subjects in Study JR-031-301, which was higher than that in the placebo group in the Study 280 Grade-III/IV subpopulation (31.4% [16 of 51] of subjects).

Table 8.9. CR of ≥28 days duration and survival at 180 days or 52 weeks post first infusion

Analysis population		N	CR of ≥28 days duration	Survival	
				180 days	52 weeks
Study JR-031-301		25	48.0 [27.8, 68.7] (12)	60.0 [38.7, 78.9] (15)	52.0 [31.3, 72.2] (13)
Study 280 Grade-III/IV subpopulation	Prochymal group	117	30.8 [22.4, 39.1] (36)	26.5 [18.5, 34.5] (31)	-
	Placebo group	51	21.6 [10.3, 32.9] (11)	31.4 [18.6, 44.1] (16)	-

Proportion of patients who achieved endpoint [95% CI] (n)

Therefore, the lower bound of the 95% confidence interval for the primary endpoint in Study JR-031-301 was not greater than the pre-defined historical response rate of 30%, but was higher than the proportion of patients who achieved "a CR of ≥28 days duration" in the placebo group in the Study 280 Grade-III/IV subpopulation, and the survival rate at 180 days post first infusion in Study JR-031-301 was higher than that in the placebo group in the Study 280 Grade-III/IV subpopulation, demonstrating the efficacy of Temcell in the treatment of acute GVHD.

Data on CR of ≥28 days duration and survival at Week 24 were collected and analyzed by the underlying disease, conditioning regimen for transplantation, stem cell source, degree of human leukocyte antigen (HLA) matching, GVHD prophylactic agents, and grade of GVHD in Studies JR-031-201 and JR-031-301. The results are as shown in Table 8.10. There seem to be no differences in efficacy according to patient characteristics.

Table 8.10. CR of ≥28 days duration and survival at Week 24 by background factors in Studies JR-031-201 and JR-031-301

Patient characteristics		CR of ≥28 days duration		Survival at Week 24	
		Study JR-031-201	Study JR-031-301	Study JR-031-201	Study JR-031-301
Underlying disease	Acute myeloid leukemia	75.0 (3/4)	42.9 (3/7)	75.0 (3/4)	57.1 (4/7)
	Acute lymphocytic leukemia	33.3 (1/3)	33.3 (3/9)	66.7 (2/3)	55.6 (5/9)
	Myelodysplastic syndrome	33.3 (1/3)	66.7 (2/3)	66.7 (2/3)	66.7 (2/3)
	Chronic myeloid leukemia	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)
	Others	75.0 (3/4)	57.1 (4/7)	100 (4/4)	57.1 (4/7)
Conditioning regimen for transplantation	Myeloablative	54.5 (6/11)	35.7 (5/14)	72.7 (8/11)	57.1 (8/14)
	Non-myeloablative	66.7 (2/3)	63.6 (7/11)	100 (3/3)	63.6 (7/11)
Stem cell source	Bone marrow transplantation	55.6 (5/9)	42.9 (6/14)	77.8 (7/9)	57.1 (8/14)
	Peripheral blood stem cell transplantation	100 (1/1)	66.7 (4/6)	100 (1/1)	66.7 (4/6)
	Cord blood transplantation	50.0 (2/4)	40.0 (2/5)	75.0 (3/4)	60.0 (3/5)
Degree of human leukocyte antigen (HLA) matching	Fully matched	83.3 (5/6)	45.5 (5/11)	66.7 (4/6)	63.6 (7/11)
	Patially mismatched	37.5 (3/8)	50.0 (7/14)	87.5 (7/8)	57.1 (8/14)
GVHD prophylactic agents [a)]	CSP	66.7 (4/6)	75.0 (3/4)	66.7 (4/6)	100 (4/4)
	TAC	50.0 (5/10)	42.9 (9/21)	70.0 (7/10)	52.4 (11/21)
	MTX	60.0 (6/10)	55.0 (11/20)	80.0 (8/10)	65.0 (13/20)
	MMF	100 (1/1)	0 (0/0)	100 (1/1)	0 (0/0)
	Others	0 (0/0)	60.0 (3/5)	0 (0/0)	60.0 (3/5)
Grade of GVHD	II	66.7 (6/9)	0 (0/0)	77.8 (7/9)	0 (0/0)
	III	40.0 (2/5)	50.0 (11/22)	80.0 (4/5)	59.1 (13/22)
	IV	0 (0/0)	33.3 (1/3)	0 (0/0)	66.7 (2/3)

Proportion of patients who achieved endpoint (%) (No. of subjects who achieved endpoint/No. of evaluable subjects)
MTX: methotrexate

[a)] Some patients used more than one prophylactic agent.

PMDA's view on the efficacy of Temcell:

In order to examine the applicant's explanation about determination of the historical response rate, acute GVHD in Studies JR-031-301 and 280 (Grade C or D in the placebo group) was graded using the JSHCT guideline and IBMTR grading system (Table 8.11). In Study JR-031-301, 9 subjects each (36.0%) had IBMTR Grade B and D acute GVHD and the proportion of patients with Grade B or D acute GVHD was higher than that in Study 280. In Study 280, 18 subjects (28.1%) had Grade II acute GVHD and 3 subjects (4.7%) had Grade IV acute GVHD classified according to the JSHCT guideline and the proportion of patients with Grade II acute GVHD was higher and the proportion of patients with Grade IV acute GVHD was lower in Study 280 compared to Study JR-031-301.

Table 8.11. Acute GVHD grading in patients in Studies JR-031-301 and 280

		Study JR-031-301	Study 280 (Grade C or D in the placebo group)	
			ITT population	mITT population
N		25	64	60
JSHCT guideline	II		18 (28.1)	16 (26.7)
	III	22 (88.0)	43 (67.2)	42 (70.0)
	IV	3 (12.0)	3 (4.7)	2 (3.3)
IBMTR grading system	B	9 (36.0)		
	C	7 (28.0)	50 (78.1)	46 (76.7)
	D	9 (36.0)	14 (21.9)	14 (23.3)

n (%)

Based on the above, 12 of 25 subjects (48.0% [95% CI; 27.8, 68.7]) achieved the primary endpoint of "a CR of ≥28 days duration" in Study JR-031-301 and the lower bound of the 95% confidence interval was less than the pre-defined historical response rate of 30%. However, differences in the definition of acute GVHD Grades between Studies JR-031-301 and 280 may have affected the results in the placebo group of Study 280used to determine the historical response rate, resulting in failure to determine the historical response rate appropriately.

As a result of an overall evaluation, PMDA considers that Study JR-031-301 demonstrated a certain level of efficacy of Temcell taking into account the seriousness of acute GVHD, the applicant's explanation, and also the following reasons.

- There is no established initial treatment for patients with acute GVHD who have failed to respond to corticosteroids.
- There were subjects treated with Temcell who achieved "a CR of ≥28 days duration" in Study JR-031-301.
- The proportion of subjects who achieved "a CR of ≥28 days duration" in Study JR-031-301 was not inferior to those with currently available second-line agents, ATG and MMF, reported in the literature (ATG, 20.3% [16 of 79 patients] [*Biol Blood Marrow Transplant* 2002; 8: 40-46]; MMF, 15.4% [2 of 13 patients] [*Eur J Haematol* 2004; 73: 56-61]).

The survival rate at 180 days post first infusion in Study JR-031-301 was not inferior to that in the placebo group in the Study 280 Grade-III/IV subpopulation This also indicates no results that deny the efficacy of Temcell in the treatment of acute GVHD.

The above conclusions by PMDA will be discussed at the Expert Discussion.

8.5.3. Safety

The applicant explained the safety of Temcell as follows:

A summary of safety up to Week 52 in Studies JR-031-201 and JR-031-202 (JR-031-201/202) and JR-031-301 is as shown in Table 8.12. The incidences of adverse events and adverse reactions up to Week 52 are as shown in Table 8.13.

Table 8.12. Summary of safety in Studies JR-031-201/202 and JR-031-301

Study identifier	JR-031-201/202	JR-031-301
	N = 14	N = 25
Adverse events	14 (100)	25 (100)
Adverse reactions	13 (92.9)	22 (88.0)
Serious adverse events	13 (92.9)	22 (88.0)
Deaths	5 (35.7)	12 (48.0)
Adverse events leading to treatment discontinuation	1 (7.1)	4 (16.0)

n (%)

Table 8.13. Adverse events reported by ≥10% of subjects in Studies JR-031-201/202 and JR-031-301

Adverse event term		Adverse events	Adverse reactions
		N = 39	N = 39
No. of subjects with any event		39 (100)	35 (89.7)
Infections and infestations			
	Pneumonia	9 (23.1)	4 (10.3)
	Sepsis	8 (20.5)	3 (7.7)
	Bacteraemia	6 (15.4)	1 (2.6)
	Herpes zoster	6 (15.4)	1 (2.6)
	Infection	6 (15.4)	1 (2.6)
	Cytomegalovirus viraemia	6 (15.4)	3 (7.7)
Blood and lymphatic system disorders			
	Anaemia	11 (28.2)	5 (12.8)
	Thrombotic microangiopathy	7 (17.9)	3 (7.7)
Immune system disorders			
	Chronic GVHD	13 (33.3)	2 (5.1)
Metabolism and nutrition disorders			
	Hypocalcaemia	4 (10.3)	0 (0)
	Hypokalaemia	4 (10.3)	2 (5.1)
	Hypomagnesaemia	4 (10.3)	1 (2.6)
Psychiatric disorders			
	Delirium	4 (10.3)	1 (2.6)
Nervous system disorders			
	Headache	5 (12.8)	1 (2.6)
	Somnolence	5 (12.8)	1 (2.6)
Vascular disorders			
	Hypertension	8 (20.5)	4 (10.3)
Gastrointestinal disorders			
	Constipation	10 (25.6)	1 (2.6)
	Vomiting	9 (23.1)	2 (5.1)
	Diarrhoea	6 (15.4)	0 (0)
	Nausea	6 (15.4)	2 (5.1)
	Stomatitis	6 (15.4)	2 (5.1)
	Abdominal pain	5 (12.8)	1 (2.6)
	Haemorrhoids	4 (10.3)	0 (0)
Hepatobiliary disorders			
	Hepatic function abnormal	13 (33.3)	8 (20.5)
Skin and subcutaneous tissue disorders			
	Decubitus ulcer	6 (15.4)	0 (0)
	Rash	5 (12.8)	1 (2.6)
	Skin exfoliation	4 (10.3)	1 (2.6)
Musculoskeletal and connective tissue disorders			
	Arthralgia	6 (15.4)	0 (0)
Renal and urinary disorders			
	Renal impairment	6 (15.4)	1 (2.6)
	Cystitis haemorrhagic	5 (12.8)	3 (7.7)
	Renal disorder	5 (12.8)	1 (2.6)
General disorders and administration site conditions			
	Pyrexia	10 (25.6)	7 (17.9)
	Oedema	9 (23.1)	4 (10.3)
Investigations			
	White blood cell count decreased	20 (51.3)	7 (17.9)
	Platelet count decreased	17 (43.6)	11 (28.2)
	Blood potassium decreased	10 (25.6)	1 (2.6)
	Blood urine present	8 (20.5)	3 (7.7)
	γ-GTP increased	7 (17.9)	5 (12.8)
	Neutrophil count decreased	7 (17.9)	2 (5.1)
	C-reactive protein increased	6 (15.4)	1 (2.6)
	Alanine aminotransferase increased	5 (12.8)	1 (2.6)
	Blood lactate dehydrogenase increased	5 (12.8)	5 (12.8)
	White blood cell count increased	5 (12.8)	3 (7.7)
	Blood pressure decreased	4 (10.3)	2 (5.1)
	Haemoglobin decreased	4 (10.3)	3 (7.7)
	Lymphocyte count decreased	4 (10.3)	2 (5.1)

	Weight decreased	4 (10.3)	0 (0)

n (%)
MedDRA/J ver. 16.1

Table 8.14 shows adverse events reported by ≥5% more subjects in the Prochymal group than in the placebo group in Study 280. Confusional state was the only event reported by ≥10% more subjects in the Prochymal group than in the placebo group. This ≥10% difference in the incidence of confusional state was considered attributable to more subjects with confusional state using morphine to treat pain in the Prochymal group (15 of 30 subjects in the Prochymal group, 1 of 6 subjects in the placebo group). Although the reasons for higher incidences of other adverse events noted in the Prochymal group are not clear, a causal relationship to Prochymal was ruled out except for staphylococcal bacteraemia, enterococcal bacteraemia, fluid overload, tremor, and dyspnoea (1 subject each). The incidences of these adverse events in the combined data from Studies JR-031-201/202 and JR-031-301 are also presented in Table 8.14.

Table 8.14. Adverse events reported by ≥5% more subjects in the Prochymal group than in placebo group in Study 280 and the incidences of these adverse events in combined data from Studies JR-031-201/202 and JR-031-301

Adverse event term	Study 280		Study JR-031-201/202 Study JR-031-301
	Prochymal	Placebo	
	N = 163	N = 81	N = 39
Pneumonia	22 (13.5)	6 (7.4)	9 (23.1)
Staphylococcal bacteraemia	21 (12.9)	5 (6.2)	0 (0)
Enterococcal bacteraemia	12 (7.4)	1 (1.2)	0 (0)
Hypokalaemia	37 (22.7)	12 (14.8)	4 (10.3)
Hyperglycaemia	35 (21.5)	11 (13.6)	3 (7.7)
Hyperkalaemia	23 (14.1)	4 (4.9)	1 (2.6)
Decreased appetite	18 (11.0)	3 (3.7)	2 (5.1)
Fluid overload	17 (10.4)	4 (4.9)	0 (0)
Confusional state	30 (18.4)	6 (7.4)	0 (0)
Insomnia	23 (14.1)	6 (7.4)	2 (5.1)
Anxiety	23 (14.1)	5 (6.2)	1 (2.6)
Tremor	21 (12.9)	5 (6.2)	1 (2.6)
Dizziness	9 (5.5)	0 (0)	0 (0)
Hypertension	30 (18.4)	7 (8.6)	8 (20.5)
Dyspnoea	30 (18.4)	10 (12.3)	1 (2.6)
Abdominal distension	16 (9.8)	2 (2.5)	1 (2.6)
Haematochezia	9 (5.5)	0 (0)	1 (2.6)
Rash	19 (11.7)	5 (6.2)	5 (12.8)
Mucosal inflammation	12 (7.4)	1 (1.2)	0 (0)
Blood creatinine increased	11 (6.7)	1 (1.2)	1 (2.6)

n (%)
MedDRA/J ver.16.1

PMDA's view on the safety of Temcell:

In light of fatal and non-fatal serious adverse events reported in Studies JR-031-201/202 and JR-031-301, and adverse events reported at a higher incidence in the Prochymal group than in the placebo group in Study 280, PMDA conducted a safety review, focusing on the occurrence of hepatic dysfunction, infections, relapse of the underlying disease, the risk of promoting growth of malignant tumors other than the underlying disease, the tumorigenic and carcinogenic risk, the risks associated with intravenous infusion of allogeneic cells (events possibly associated with circulatory disorder due to cellular embolism and thrombogenesis, events possibly

associated with intravascular hemolysis, events possibly associated with an immune response), gastrointestinal haemorrhage, skin disorder, electrolyte abnormality, hyperglycaemia, hypertension, and renal impairment. The safety review indicates that attention should be paid to the possible occurrence of the above adverse events associated with Temcell, as described in the sections below. Thus, Temcell should be used by or under the supervision of a physician with adequate knowledge and experience in hematopoietic stem cell transplantation at a medical institution with adequate facilities for the treatment of emergencies and in a setting where appropriate measures are taken such as laboratory monitoring and management. If such measures are taken actually, Temcell should be tolerable. However, because of limited clinical experience with Temcell, the applicant should provide information on the occurrence and management of adverse events to healthcare professionals and ensure that post-marketing safety information is collected adequately and the obtained information is appropriately feedbacked to healthcare professionals.

The above conclusions by PMDA in "8.5.3. Safety" will be discussed at the Expert Discussion.

8.5.3.1. Hepatic dysfunction

The applicant's explanation on hepatic dysfunction associated with Temcell:

Of 17 deaths reported in Studies JR-031-201/202 and JR-031-301, 7 deaths (2 in Study JR-031-201/202 and 5 in Study JR-031-301) resulted from serious worsening of liver function with blood bilirubin elevation after the first infusion. The details of the 7 deaths are as shown in Table 8.15.

Table 8.15. The details of deaths due to serious worsening of liver function

Study identifier	Age	Sex	Total number of infusions	Fatal adverse event	No. of days from first infusion to onset of fatal adverse event	Diagnosis based on liver pathological findings
JR-031-201/202 [a)]	[redacted]	M	3	Venoocclusive liver disease	6	Suspected veno-occlusive disease
	[redacted]	M	5	Hepatic function abnormal and multi-organ failure	20 and 35	Ischemic bile duct and liver disorder
JR-031-301 [b)]	[redacted]	F	9	Liver GVHD	21	-
	[redacted]	M	7	Thrombotic microangiopathy	17	-
	[redacted]	M	7	Sepsis	66	-
	[redacted]	M	8	Sepsis	27	-
	[redacted]	M	12	Multi-organ failure	54	-

[a)] MedDRA/J ver. 13.1
[b)] MedDRA/J ver. 16.1

The occurrence of hepatic dysfunction-related adverse events in Studies JR-031-201/202 and JR-031-301 is as shown in Table 8.16. Besides the fatal adverse events above, serious adverse events reported were hepatic function abnormal in 6 subjects (15.4%) and cholecystocholangitis in 1 subject (2.6%). The occurrence of these hepatic dysfunction-related adverse events in Study 280 is also presented in Table 8.16.

Table 8.16. Hepatic dysfunction-related adverse events

Adverse event term		Study JR-031-201/202 Study JR-031-301	Study 280 [a)]	
			Prochymal	Placebo
		N = 39	N = 153	N = 77
Hepatobiliary disorders		22 (56.4)	43 (28.1)	22 (28.6)
	Hepatic function abnormal	13 (33.3)	2 (1.3)	1 (1.3)
	Liver disorder	3 (7.7)	3 (2.0)	0 (0)
	Drug-induced liver injury	3 (7.7)	0 (0)	0 (0)
	Cholecystitis acute	1 (2.6)	2 (1.3)	2 (2.6)
	Cholelithiasis	1 (2.6)	2 (1.3)	0 (0)
	Venoocclusive liver disease	1 (2.6)	1 (0.7)	0 (0)
	Cholecystitis chronic	1 (2.6)	0 (0)	0 (0)
	Hyperplastic cholecystopathy	1 (2.6)	0 (0)	0 (0)
	Cholecystocholangitis	1 (2.6)	0 (0)	0 (0)
	Hyperbilirubinaemia	0 (0)	18 (11.8)	12 (15.6)
	Jaundice	0 (0)	11 (7.2)	3 (3.9)
	Hepatic failure	0 (0)	3 (2.0)	1 (1.3)
	Cholecystitis	0 (0)	2 (1.3)	0 (0)
	Gallbladder enlargement	0 (0)	2 (1.3)	0 (0)
	Bile duct stone	0 (0)	1 (0.7)	1 (1.3))
	Hepatomegaly	0 (0)	1 (0.7)	1 (1.3)
	Hepatic lesion	0 (0)	1 (0.7)	1 (1.3)
	Cholestasis	0 (0)	1 (0.7)	0 (0)
	Hepatic necrosis	0 (0)	1 (0.7)	0 (0)
	Hepatorenal syndrome	0 (0)	1 (0.7)	0 (0)
	Bile duct obstruction	0 (0)	1 (0.7)	0 (0)
	Hypertransaminasaemia	0 (0)	1 (0.7)	0 (0)
	Hepatorenal failure	0 (0)	0 (0)	2 (2.6)
	Cholangitis	0 (0)	0 (0)	1 (1.3)
	Hepatitis	0 (0)	0 (0)	1 (1.3)
	Hepatosplenomegaly	0 (0)	0 (0)	1 (1.3)

n (%)
MedDRA/J ver. 16.1
[a)] A subgroup of patients with Grades II-IV GVHD classified according to the JSHCT guideline in the mITT population.

Baseline characteristics of subjects who died due to worsening of liver function in Studies JR-031-201/202 and JR-031-301 were examined. As a result, no factors including the underlying disease, graft source, and GVHD prophylactic agents were clearly associated with worsening of liver function, nor was there any trend towards an increased risk of worsening of liver function in any population with a specific characteristic.

PMDA's view:

While hepatic dysfunction can occur as liver GVHD (including chronic GVHD), complications associated with hematopoietic stem cell transplantation (venoocclusive liver disease, thrombotic microangiopathy, infection, etc.), or drug-induced liver injury associated with drugs other than Temcell, some subjects experienced hepatic dysfunction following the administration of Temcell and had fatal outcome. Thus, the administration of Temcell should be considered carefully. The obtained information should be provided to healthcare professionals through the package insert and information materials, and liver function monitoring should be performed appropriately following the administration of Temcell. In addition, Temcell should be used in a setting where in the event of hepatic dysfunction, various laboratory tests including histopathological examination are performed to identify its cause and hepatic dysfunction can be treated in response to the cause, in cooperation

with a physician with adequate knowledge and experience in liver disease.

8.5.3.2. Infections

The applicant's explanation on infections:

The occurrence of infection-related adverse events in Studies JR-031-201/202 and JR-031-301 is as shown in Table 8.17. Fatal adverse events were sepsis (2 subjects) and pneumonia (2 subjects). Other non-fatal serious adverse events were sepsis (6 subjects); pneumonia, herpes zoster, and septic shock (3 subjects each); and bacteraemia, infection, cytomegalovirus enterocolitis, and pyelocystitis (1 subject each). The occurrence of infection-related adverse events in Study 280 is also presented in Table 8.17.

Table 8.17. Infection-related adverse events reported by ≥5% of subjects in Studies JR-031-201/202, JR-031-301, and 280

Adverse event		Study JR-031-201/202 Study JR-031-301	Study 280 [a)]	
			Prochymal	Placebo
		N = 39	N = 153	N = 77
Infections and infestations		36 (92.3)	138 (90.2)	64 (83.1)
	Pneumonia	9 (23.1)	21 (13.7)	6 (7.8)
	Sepsis	8 (20.5)	19 (12.4)	6 (7.8)
	Bacteraemia	6 (15.4)	14 (9.2)	8 (10.4)
	Cytomegalovirus viraemia	6 (15.4)	12 (7.8)	8 (10.4)
	Herpes zoster	6 (15.4)	3 (2.0)	1 (1.3)
	Infection	6 (15.4)	2 (1.3)	0 (0)
	Sinusitis	3 (7.7)	7 (4.6)	4 (5.2)
	Septic shock	3 (7.7)	6 (3.9)	2 (2.6)
	Bacterial infection	3 (7.7)	6 (3.9)	1 (1.3)
	Oral herpes	3 (7.7)	3 (2.0)	0 (0)
	Nasopharyngitis	3 (7.7)	0 (0)	2 (2.6)
	Cellulitis	2 (5.1)	9 (5.9)	2 (2.6)
	Oral candidiasis	2 (5.1)	8 (5.2)	2 (2.6)
	Device related infection	2 (5.1)	6 (3.9)	4 (5.2)
	Upper respiratory tract infection	2 (5.1)	1 (0.7)	4 (5.2)
	Gastroenteritis	2 (5.1)	1 (0.7)	1 (1.3)
	Otitis externa	2 (5.1)	1 (0.7)	1 (1.3)
	Bronchitis	2 (5.1)	0 (0)	2 (2.6)
	Paronychia	2 (5.1)	0 (0)	1 (1.3)
	Pseudomembranous colitis	2 (5.1)	0 (0)	0 (0)
	Enteritis infectious	2 (5.1)	0 (0)	0 (0)
	CMV infection	1 (2.6)	25 (16.3)	10 (13.0)
	Klebsiella infection	1 (2.6)	8 (5.2)	3 (3.9)
	Staphylococcal bacteraemia	0 (0)	20 (13.1)	4 (5.2)
	Staphylococcal infection	0 (0)	18 (11.8)	5 (6.5)
	BK virus infection	0 (0)	14 (9.2)	5 (6.5)
	Enterococcal infection	0 (0)	12 (7.8)	7 (9.1)
	Enterococcal bacteraemia	0 (0)	12 (7.8)	1 (1.3)
	Adenovirus infection	0 (0)	8 (5.2)	5 (6.5)
	Candida infection	0 (0)	8 (5.2)	2 (2.6)
	Enterococcal sepsis	0 (0)	5 (3.3)	4 (5.2)
	Parainfluenzae virus infection	0 (0)	2 (1.3)	7 (9.1)
	Urinary tract infection bacterial	0 (0)	2 (1.3)	4 (5.2)
	Viral haemorrhagic cystitis	0 (0)	1 (0.7)	6 (7.8)

n (%)
MedDRA/J ver. 16.1
[a)] A subgroup of patients with Grades II-IV GVHD classified according to the JSHCT guideline in the mITT population

PMDA's view:

Infections are complications associated with hematopoietic stem cell transplantation or events associated with the use of immunosuppressive drugs including steroids. Death occurred following the administration of Temcell and the incidence of infections was higher in the Prochymal group than in the placebo group in Study 280. Thus, the obtained information should be provide to healthcare professionals through package insert and information materials to make them aware of the risk of infections.

8.5.3.3. Relapse of the underlying disease

The applicant's explanation on the relapse of the underlying disease:

In Studies JR-031-201/202 and JR-031-301, relapse of the underlying disease occurred in 3 of 11 subjects (27.3%) for acute myeloid leukemia, 0 of 12 subjects (0%) for acute lymphocytic leukemia, 0 of 6 subjects (0%) for myelodysplastic syndrome, and 0 of 11 subjects (0%) for others. In Study 280 (mITT), relapse of the underlying disease occurred in 13 of 163 subjects (8.0%) in the Prochymal group and 8 of 81 subjects (9.9%) in the placebo group, including relapse of acute myeloid leukemia in 7 of 163 subjects (4.3%) in the Prochymal group and 3 of 81 subjects (3.7%) in the placebo group. There were no apparent differences among overall events in terms of relapse of the underlying disease. According to studies on the risk of relapse of the underlying disease in patients with acute GVHD treated with hMSCs (*Leuk Lymphoma* 2015; 26: 1-14, *Biol Blood Marrow Transplant* 2015; 21: 97-104), the incidence of relapse of the underlying disease was not different between the hMSCs and non-hMSCs groups.

PMDA's view:

Relapse of the underlying disease is a critical event affecting acute GVHD treatment strategy and survival prognosis. Adequate attention should be paid to relapse of the underlying disease when Temcell is used. As shown in the data presented by the applicant, knowledge of the risk of relapse of the underlying disease after treatment with hMSCs is currently accumulating gradually. Therefore, the obtained information should be provided to healthcare professionals using the package insert and information materials, and Temcell should be used in patients by or under the supervision of a physician with knowledge about hematopoietic stem cell transplantation while the conditions of the patients should be monitored appropriately.

8.5.3.4. Risk of promoting growth of malignant tumors other than the underlying disease

The applicant's explanation on the risk of promoting growth of malignant tumors other than the underlying disease:

In Study JR-031-201/202, one event of malignant tumor was reported. The patient was found to have metastatic liver tumor after receiving a total of 12 infusions of Temcell. The patient was diagnosed with breast cancer metastasis to the liver by liver biopsy and then died. The patient had been diagnosed with breast cancer 2 years prior to bone marrow transplantation and its causal relationship to Temcell was ruled out.

PMDA's view:

Although its causal relationship to Temcell was ruled out, attention should be paid to the growth promotion of malignant tumors other than the underlying disease after treatment with Temcell because (1) some subjects experienced metastasis/relapse of previous malignant tumors after treatment with Temcell and (2) various cytokines and chemokines produced by hMSCs have been reported to promote tumor growth (*Cell Stem Cell* 2012; 11: 812-824). Information materials should be provided to advise physicians to ask about each patient's history of malignant tumors other than the underlying disease prior to the use of Temcell and to periodically assess the relevant patient for metastasis/relapse of previous malignant tumors after treatment with Temcell.

8.5.3.5. Tumorigenic and carcinogenic risk

The applicant's explanation on the tumorigenic and carcinogenic risk:

Following treatment with Temcell, study subjects underwent CT scan (thoracoabdominal region, pelvis) and thoracoabdominal X-ray in Study JR-031-201/202 and thoracoabdominal X-ray in Study JR-031-301, which revealed no ectopic tissue formation. Although mass was found in 1 of approximately 500 subjects in clinical studies of Prochymal including Study 280 (Study 208, CTD 5.3.5.2.5), DNA from the Prochymal MSC donor was not detected in the mass and DNA from the hematopoietic stem cell (cord blood transplantation) donor and from the subject were detected.

The following findings from non-clinical studies of Temcell have been reported:

- Temcell loses proliferative capacity after about [redacted]th cell division from the bone marrow aspirate (the raw material) [see Section 3.1.4.].
- DCB from each donor was tested by chromosome analyses and soft agar colony formation assay using cells cultured beyond the passage level of *ex-vivo* cultured hMSCs. As a result, no transformed cells were detected [see Section 3.1.5.1.].
- An SCID mouse 4-week repeated intravenous dose toxicity study showed no tumorigenic or carcinogenic findings [see Section 7.1.].

PMDA's view:

Although ectopic tissue formation was not observed in Study JR-031-201/202 or JR-031-301, it is necessary to continue to collect information on ectopic tissue formation following treatment with Temcell and then assess patients for it periodically because (1) the number of patients treated with Temcell is very limited and (2) Temcell has the capacity to differentiate into various tissues.

8.5.3.6. Risks associated with intravenous infusion of allogeneic cells

Since Temcell is an allogeneic hMSC product, PMDA examined events possibly associated with circulatory disorder due to cellular embolism and thrombogenesis, events possibly associated with intravascular hemolysis, and events possibly associated with an immune response as the risks associated with intravenous infusion of

hMSCs[13] and the risk of allergic reactions associated with the administration of allogeneic cells.

Events possibly associated with circulatory disorder due to cellular embolism and thrombogenesis

Table 8.18 shows adverse events possibly associated with pulmonary circulatory disorder[14] that occurred on the day of or the following day of infusion of JR-031 or Prochymal. In Study JR-031-201, 1 subject experienced severe oxygen saturation decreased, which was considered to be caused by compressive atelectasis due to ascites associated with venoocclusive liver disease, and its causal relationship to Temcell was ruled out. In Study 280, the incidence of severe adverse events was higher in the Prochymal group than in the placebo group, and 7 subjects had severe hypoxia.

Table 8.18. Adverse events possibly associated with pulmonary circulatory disorder

Adverse event		Study JR-031-201 and Study R-031-301	Study 280	
			Prochymal	Placebo
		N = 39	N = 163	N = 81
Adverse events classified as pulmonary circulatory disorder		5 (12.8)	43 (26.4)	17 (21.0)
	Dyspnoea	1 (2.6)	8 (4.9)	2 (2.5)
	Hypoxia	1 (2.6)	8 (4.9)	1 (1.2)
	Oxygen saturation decreased	1 (2.6)	2 (1.2)	0 (0)
	Presyncope	1 (2.6)	0 (0)	0 (0)
	Sputum retention	1 (2.6)	0 (0)	0 (0)
	Tachycardia	0 (0)	8 (4.9)	1 (1.2)
	Hypotension	0 (0)	5 (3.1)	5 (6.2)
	Cough	0 (0)	5 (3.1)	3 (3.7)
	Hiccups	0 (0)	4 (2.5)	0 (0)
	Syncope	0 (0)	3 (1.8)	0 (0)
	Atrial fibrillation	0 (0)	3 (1.8)	1 (1.2)
	Dyspnoea exertional	0 (0)	3 (1.8)	1 (1.2)
	Respiratory failure	0 (0)	3 (1.8)	1 (1.2)
	Chest pain	0 (0)	3 (1.8)	0 (0)
	Supraventricular tachycardia	0 (0)	2 (1.2)	1 (1.2)
	Atrial flutter	0 (0)	2 (1.2)	0 (0)
	Dizziness postural	0 (0)	1 (0.6)	1 (1.2)
	Productive cough	0 (0)	1 (0.6)	1 (1.2)
	Dizziness	0 (0)	1 (0.6)	0 (0)
	Hypoventilation	0 (0)	1 (0.6)	0 (0)
	Respiratory alkalosis	0 (0)	1 (0.6)	0 (0)
	Respiratory depression	0 (0)	1 (0.6)	0 (0)
	Respiratory distress	0 (0)	1 (0.6)	0 (0)
	Tachypnoea	0 (0)	1 (0.6)	0 (0)
	Haemoptysis	0 (0)	0 (0)	1 (1.2)
	Orthostatic hypotension	0 (0)	0 (0)	1 (1.2)
	Rales	0 (0)	0 (0)	1 (1.2)

n (%)
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Table 8.19 shows adverse events possibly associated with local circulatory disorder due to cellular embolism and thrombogenesis[15] that occurred on the day of or the following day of infusion of JR-031 or Prochymal. In Studies JR-031-201 and JR-031-301, a total of 6 subjects (15.4%) had those events, including thrombotic

[13] hMSCs to be administered are cells that normally do not circulate in large amounts.
[14] Events in the MedDRA HLGTs "pulmonary vascular disorders", "respiratory disorders NEC (excluding "respiratory tract disorders NEC [HLT]" and "upper respiratory tract signs and symptoms [HLT])", "cardiac arrhythmias", and "decreased and nonspecific blood pressure disorders and shock" and HLTs "dyspnoeas" and "blood gas and acid base analyses"
[15] Events in the MedDRA SMQ "embolic and thrombotic events"

microangiopathy in 4 subjects.

Table 8.19. Adverse events possibly associated with local circulatory disorder due to cellular embolism and thrombogenesis

Adverse event		Study JR-031-201 and Study JR-031-301	Study 280	
			Prochymal	Placebo
		N = 39	N = 163	N = 81
Adverse events classified as cellular embolism and local circulatory disorder		6 (15.4)	5 (3.1)	2 (2.5)
	Thrombotic microangiopathy	4 (10.3)	2 (1.2)	0 (0)
	Disseminated intravascular coagulation	1 (2.6)	0 (0)	0 (0)
	Venoocclusive liver disease	1 (2.6)	0 (0)	0 (0)
	Thrombotic thrombocytopenic purpura	0 (0)	1 (0.6)	1 (1.2)
	Hemiparesis	0 (0)	1 (0.6)	0 (0)
	Deep vein thrombosis	0 (0)	1 (0.6)	0 (0)
	Infusion site thrombosis	0 (0)	0 (0)	1 (1.2)

n (%)
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Events possibly associated with intravascular hemolysis[16]

Table 8.20 shows adverse events possibly associated with intravascular haemolysis that occurred on the day of or the following day of infusion of JR-031 or Prochymal. In Study JR-031-201, severe anaemia occurred in 1 subject and its causal relationship to Temcell could not be ruled out. In Study 280, severe blood bilirubin increased and hyperbilirubinaemia occurred in 4 and 2 subjects, respectively, in the Prochymal group and a causal relationship was ruled out except for 1 event of hyperbilirubinaemia.

Table 8.20. Adverse events possibly associated with intravascular haemolysis

Adverse event		Study JR-031-201 and Study JR-031-301	Study 280	
			Prochymal	Placebo
		N = 39	N = 163	N = 81
Adverse events classified as intravascular hemolysis		12 (30.8)	41 (25.2)	16 (19.8)
	Anaemia	4 (10.3)	8 (4.9)	6 (7.4)
	Blood lactate dehydrogenase increased	4 (10.3)	7 (4.3)	0 (0)
	γ-GTP increased	3 (7.7)	1 (0.6)	0 (0)
	Blood bilirubin increased	2 (5.1)	6 (3.7)	1 (1.2)
	Haemoglobin decreased	2 (5.1)	2 (1.2)	1 (1.2)
	Transaminases increased	1 (2.6)	2 (1.2)	2 (2.5)
	Haptoglobin decreased	1 (2.6)	0 (0)	0 (0)
	Hyperbilirubinaemia	0 (0)	6 (3.7)	4 (4.9)
	Liver function test abnormal	0 (0)	6 (3.7)	2 (2.5)
	Aspartate aminotransferase increased	0 (0)	4 (2.5)	0 (0)
	Alanine aminotransferase increased	0 (0)	4 (2.5)	2 (2.5)
	Jaundice	0 (0)	3 (1.8)	1 (1.2)
	Splenomegaly	0 (0)	2 (1.2)	0 (0)
	Hepatic enzyme increased	0 (0)	2 (1.2)	0 (0)
	Blood lactate dehydrogenase abnormal	0 (0)	1 (0.6)	0 (0)
	Haemoglobin abnormal	0 (0)	1 (0.6)	0 (0)
	Haemolysis	0 (0)	1 (0.6)	0 (0)
	Ocular icterus	0 (0)	1 (0.6)	0 (0)

n (%)
MedDRA/J ver. 16.1

[16] Events in the MedDRA HLTs “haemolyses NEC”, “anaemias haemolytic NEC”, “anaemias NEC”, and “liver function analyses” and PTs “splenomegaly”, “haemoglobin abnormal”, “haemoglobin decreased”, “blood lactate dehydrogenase abnormal”, “blood lactate dehydrogenase increased”, “haptoglobin abnormal”, “haptoglobin decreased”, and “urobilinogen urine increased”

Events possibly associated with an immune response[17]

Table 8.21 shows adverse events possibly associated with an immune response that occurred on the day of or the following day of infusion of JR-031 or Prochymal. In Studies JR-031-201 and JR-031-301, all subjects were premedicated with hydrocortisone sodium succinate (or phosphate) (the adult dose, 100-200 mg) and/or chlorpheniramine maleate (the adult dose, 5-10 mg) 30 minutes to 1 hour prior to the administration of Temcell in order to prevent allergic reactions. Approximately 70% of the subjects received both medications. In Study JR-031-201, rash was reported as a severe adverse event in 1 subject. This subject had rash generalised and its causal relationship to Temcell could not be ruled out. In Study 280, rash occurred in 7 subjects (4.3%) in the Prochymal group, but there were no apparent differences between the Prochymal and placebo groups for the incidence and severity of overall adverse events possibly associated with an immune response.

Table 8.21. Adverse events possibly associated with an immune response

Adverse event		Study JR-031-201 and Study JR-031-301	Study 280	
			Prochymal	Placebo
		N = 39	N = 163	N = 81
Adverse events classified as immune responses		6 (15.4)	26 (16.0)	13 (16.0)
	Rash	1 (2.6)	7 (4.3)	2 (2.5)
	Stomatitis	1 (2.6)	2 (1.2)	1 (1.2)
	Swelling face	1 (2.6)	1 (0.6)	0 (0)
	Allergic transfusion reaction	1 (2.6)	0 (0)	1 (1.2)
	Eyelid oedema	1 (2.6)	0 (0)	0 (0)
	Skin exfoliation	1 (2.6)	0 (0)	0 (0)
	Generalised oedema	0 (0)	4 (2.5)	3 (3.7)
	Pruritus	0 (0)	4 (2.5)	0 (0)
	Erythema	0 (0)	3 (1.8)	2 (2.5)
	Respiratory failure	0 (0)	3 (1.8)	1 (1.2)
	Blister	0 (0)	2 (1.2)	1 (1.2)
	Conjunctivitis	0 (0)	2 (1.2)	0 (0)
	Pneumonitis	0 (0)	1 (0.6)	1 (1.2)
	Dermatitis	0 (0)	1 (0.6)	0 (0)
	Flushing	0 (0)	1 (0.6)	0 (0)
	Rash erythematous	0 (0)	1 (0.6)	0 (0)
	Respiratory distress	0 (0)	1 (0.6)	0 (0)
	Wheezing	0 (0)	1 (0.6)	0 (0)
	Localised oedema	0 (0)	0 (0)	2 (2.5)
	Photosensitivity reaction	0 (0)	0 (0)	1 (1.2)

n (%)
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PMDA's view:

It is necessary to continue to collect information on the risks associated with intravenous infusion of allogeneic cells such as hMSCs. In addition, Temcell should be used by or under the supervision of a physician with adequate knowledge and experience in hematopoietic stem cell transplantation at a medical institution with adequate facilities for the treatment of emergencies to ensure that if symptoms considered related to intravenous infusion of Temcell occur, treatment discontinuation can be decided immediately and adverse events can be treated promptly. Currently available information should be provided to healthcare professionals using the package insert and information materials, and information should be continuously collected through post-marketing surveillance etc. Whenever new findings become available, the information should be provided to

[17] Events in the MedDRA SMQ "hypersensitivity"

patients and healthcare providers without delay.

8.5.3.7. Others

Among fatal and non-fatal serious adverse events noted in Studies JR-031-201/202 and JR-031-301, gastrointestinal haemorrhage, skin disorder, electrolyte abnormality, hyperglycaemia, hypertension, and renal impairment are summarized in Table 8.22.

Table 8.22. Occurrence of gastrointestinal haemorrhage, skin disorder, electrolyte abnormality, hyperglycaemia, hypertension, and renal impairment in Studies JR-031-201/202 and JR-031-301

Age	Sex	Total number of infusions	Preferred term	No. of days from first infusion to onset of event	Outcome	Causal relationship
[redacted]	F	11	Gastrointestinal haemorrhage	68	Fatal	Yes
[redacted]	M	8	Eczema	132	Unresolved	Yes
			Gastrointestinal haemorrhage	120	Resolved	Yes
			Gastrointestinal haemorrhage	160	Resolved	Yes
			Gastrointestinal haemorrhage	295	Resolved	Yes
[redacted]	F	12	Blood potassium decreased	74	Resolved	No
[redacted]	F	8	Hypokalaemia	3	Resolved	No
			Hyperglycaemia	64	Resolved	No
[redacted]	M	11	Hypokalaemia	53	Resolved	Yes
[redacted]	F	7	Hyperglycaemia	241	Resolving	No
[redacted]	F	7	Hypertensive encephalopathy	182	Resolved	No
[redacted]	M	3	Acute renal failure	13	Unresolved	No
[redacted]	M	12	Renal disorder	8	Resolved	Yes

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PMDA's view:

Although these adverse events are events that can occur as complications associated with hematopoietic stem cell transplantation, gastrointestinal haemorrhage led to death and eczema and renal impairment were unresolved in Studies JR-031-201/202 and JR-031-301. For this reason, currently available information on these events should be provided with information materials.

8.5.4. Indication or performance

The proposed indication or performance for Temcell were "acute GVHD following hematopoietic stem cell transplantation."

The applicant's explanation on the indication or performance for Temcell:

Study JR-031-301 demonstrated a certain level of efficacy of Temcell in patients with Grade III or IV acute GVHD who had failed to respond to systemic corticosteroid therapy. According to the Japanese guideline, corticosteroids have been established as first-line treatment for acute GVHD. Given this fact, Temcell will not be used as first-line therapy. The inclusion of the statement "patients who have failed to respond to systemic corticosteroid therapy" in the indication or performance for Temcell is considered of little significance and the

appropriate statement for the indication or performance is “acute GVHD following hematopoietic stem cell transplantation.” The indication is the same as that for ATG, whose clinical positioning is the same as that of Temcell.

PMDA’s view on the indication or performance for Temcell:
Although corticosteroids have been established as first-line treatment for acute GVHD, patients with acute GVHD refractory to treatment with systemic corticosteroid therapy have a poor prognosis and steroid pulse therapy and immunosuppressive agents such as ATG, CSP, TAC, and MMF as second-line therapies cannot achieve satisfactory treatment outcomes. Taking account of this point and based on the benefits and risks of Temcell demonstrated in clinical studies, Temcell is expected to be a therapeutic option for patients with acute GVHD who have failed to respond to corticosteroids [see Section 8.5.1.] and the indication or performance for Temcell should be “acute GVHD following hematopoietic stem cell transplantation” and the package insert should include the precautionary statement that Temcell should be used in patients who have failed to respond adequately to corticosteroids.

The intended patient population should also be defined. Since the study population for JR-031-301 was patients with Grade III or IV acute GVHD classified according to the JSHCT guideline, the study populations should be described in the Clinical Studies section of the package insert and the following statements should be included in the Precautions for Indication or Performance section.

- Temcell should be used in patients who have failed to respond adequately to corticosteroids.
- Patients for use of Temcell should be selected by physicians who have a full knowledge of the content of the Clinical Studies section, e.g., the severity of acute GVHD, and who have an adequate understanding of the efficacy and safety of Temcell.
- Since Temcell is associated with the risk of hepatic dysfunction etc., and the number of patients treated with Temcell is very limited, the use of other therapies should also be considered carefully before the initiation of treatment with Temcell.

The above conclusions by PMDA will be discussed at the Expert Discussion.

8.5.5. Dosage and administration or method of use

8.5.5.1. Dosage and administration

The proposed dosage and administration statement for Temcell was as shown below:
The usual dosage of Temcell is 2×10^6 human mesenchymal stem cells/kg body weight administered as a slow intravenous infusion. Temcell should be diluted with physiological saline prior to infusion. As a rule, Temcell should be administered twice weekly for 4 weeks for a total of 8 infusions (at least 3 days apart). Continued therapy with 4 once-weekly infusions is allowed for additional 4 weeks, depending on the degree of symptoms.

The applicant’s explanation on the appropriateness of the proposed dosage and administration:
The same dosage regimen as that used in Study 280 (Prochymal at a dose of 2.0×10^6 cells/kg was given twice

weekly for 4 weeks [infusions were administered at least 3 days apart for a total of 8 infusions]) was chosen for Study JR-031-301. The dosage regimen for Study 280 was selected based on the findings obtained from the two clinical studies (Study 260 [*Biol. Blood Marrow Transplant* 2009; 15: 804-11] and Study 270 [Osiris clinical study report]) which compared 2 doses of Prochymal, 2.0×10^6 and 8.0×10^6 cells/kg, in adult patients with acute GVHD (the patients received a total of 2 doses). The findings were as follows: (1) those studies did not detect any clinical difference in efficacy or safety between the two dose groups; (2) there were subjects who achieved a CR following treatment with Prochymal and then experienced flares of their acute GVHD, suggesting that the efficacy of two doses of Prochymal was limited (Study 260); and (3) a total of 8 infusions of 8.0×10^6 cells/kg of Prochymal (infusions were administered at least 72 hours apart) were tolerated (Study 270).

In Study JR-031-301, Temcell was given at a dose of 2×10^6 cells/kg twice weekly for 4 weeks for a total of 8 infusions (at least 3 days apart). Subjects who had a PR or MR at 4 weeks after the first infusion were eligible for continued therapy with an additional 4 weekly infusions. Subjects who achieved a CR after the first infusion and who had a subsequent flare of Grades II-IV GVHD by Week 10 were allowed to be retreated with Temcell twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions). Since (1) Study JR-031-301 demonstrated the efficacy of Temcell; (2) 9 of 15 subjects who received continued therapy achieved a CR of ≥28 days duration; and (3) 1 subject with a flare of acute GVHD after achieving a CR was retreated with Temcell and then achieved a CR of ≥28 days duration, the applicant considered it appropriate to establish the dosage and administratin of Temcell based on the above dosage and dose regimen used in Study JR-031-301.

PMDA's view:
No exploratory, dose-finding study of Temcell has been conducted, which means that the optimal dosage and dose regimen has not been determined. Meanwhile, since a certain level of efficacy has been demonstrated for the dosage and administration of Temcell used in Study JR-031-301, the proposed dosage and adminstration of Temcell is acceptable. However, the Precautions of Dosage and Administration section should state that only patients who achieved a PR or MR at Week 4 are eligible for continued therapy. It is also necessary to collect post-marketing information on the safety of continued therapy through post-marketing surveillance etc., and appropriately provide the information to healthcare professionals.

8.5.5.2. Infusion rate of Temcell

The proposed infusion rate of Temcell was as follows:
A dose of Temcell prepared for administration should be given as a slow intravenous infusion at a controlled rate of 4 mL/min (≤6 mL/min) and should not be co-infused with other agents. For patients weighing ≤50 kg, it should be infused slowly over at least 10 minutes.

The applicant's explanation on the appropriateness of the proposed infusion rate:
Prochymal was infused at a rate of 4 to 6 mL/min in the clinical studies including Study 280, which was

tolerable, and the same infusion rate was therefore used also in Studies JR-031-201 and JR-031-301. As a result, no events considered related to the infusion rate were reported. Stability studies on infusion solutions showed that [redacted] at 22.4°C to 26.7°C was ≥[redacted]% up to [redacted] hours after preparation. As long as the diluted product is kept at room temperature and infused within 3 hours, there should be no quality problems even if the infusion rate is <4 mL/min. However, since slow infusion is important in ensuring safety, the maximum infusion rate needs to be specified. Thus, the following instruction was decided: "Temcell should be given as a slow intravenous infusion at a controlled rate of 4 mL/min (≤6 mL/min)".

PMDA's view:
The applicant's explanation about the "method of use" and the proposed infusion rate of Temcell are acceptable because Studies JR-031-201 and JR-031-301 have demonstrated the efficacy and safety of JR-031 to a certain extent.

8.5.5.3. Precautions regarding concomitant use with other drugs

The applicant's explanation on concomitant use with other drugs:
In Studies JR-031-201 and JR-031-301, concomitant use of therapies for acute GVHD (second-line therapies) other than symptomatic treatment was prohibited. If CSP, TAC, ATG, MMF, methotrexate, or corticosteroids (including those administered as standard-of-care treatment) that had been used as GVHD prophylactic agents were continued, no dose increase was allowed during treatment with Temcell (however, dose increases to maintain optimal trough blood concentrations of CSP or TAC, switching between CSP and TAC [Study JR-031-301 only], and dose increases [up to the dose at the time of the first infusion of Temcell] due to exacerbation during steroid taper were allowed). Therefore, the effects of concomitant second-line agents cannot be assessed. In view of the results of clinical studies of Prochymal where concomitant use of second-line agents was permitted, there is little need to restrict the concomitant use of second-line agents or advise any caution.

PMDA's view:
Since the tolerability of Temcell administered concomitantly with drugs that are positioned as second-line treatment by the JSHCT guideline has been demonstrated, the applicant's explanation is acceptable. Temcell may be used concomitantly with these agents as long as Temcell is used at a medical institution with adequate facilities for the treatment of emergencies under the supervision of a physician with adequate knowledge and experience in hematopoietic stem cell transplantation. However, the following information should be provided through information materials: results of Study 280 that evaluated the efficacy of Prochymal added to other drugs that are normally used as second-line treatment; and the rules for concomitant use with other drugs in Studies JR-031-201 and JR-031-301.

8.5.5.4. Criteria for interruption, discontinuation, and resumption

The applicant's explanation on the criteria for interruption, discontinuation, and resumption of Temcell:
The protocols for Studies JR-031-201 and JR-031-301 stated that treatment with Temcell (infusion) should be discontinued "if worsening of respiratory state or changes in vital signs that require treatment, low

percutaneous oxygen saturation (i.e., a state in which percutaneous oxygen saturation of <90% persists for ≥3 minutes), etc., are detected during infusion and if the event is assessed by the investigator or sub-investigator as likely to be related to Temcell." No criteria for treatment resumption were provided in either study. None of the subjects in Studies JR-031-201 and JR-031-301 discontinued treatment with Temcell as per the above protocols.

On the other hand, the protocol for Study 280 stated that treatment with Prochymal should be discontinued "if the subject have symptoms or signs of respiratory disorder, e.g., tachypnea, cyanosis, and shortness of breath, regardless of oximetry readings" or "if low percutaneous oxygen saturation (<85%) persists for 3 to 5 minutes, regardless of whether the subject has symptoms of dyspnoea." No criteria for treatment resumption were provided. In Study 280, 3 subjects experienced hypoxia, oxygen saturation decreased, and chest pain (1 subject each), which led to interruption or discontinuation of Prochymal. Since the symptoms resolved after administration of oxygen in the subject with oxygen saturation decreased and the symptoms resolved in about 3 minutes in the subject with chest pain, both subjects resumed and completed treatment.

Based on the above, the criteria for treatment discontinuation in Studies JR-031-201 and JR-031-301 focused on changes in respiratory state, vital signs, and percutaneous oxygen saturation during infusion and was considered appropriate. The same criteria should be used after the market launch. Criteria for treatment resumption are unlikely to be necessary because treatment will be resumed appropriately upon discretion of the physician.

PMDA's view:
Although no subjects discontinued treatment based on any of respiratory state, vital signs, and percutaneous oxygen saturation during infusion of Temcell as per the protocol in Studies JR-031-201 or JR-031-301, physicians should closely monitor the patient's condition as in the case of Studies JR-031-201 and JR-031-301 and promptly take actions such as discontinuation, as needed, in order to ensure safety. No particular criteria for treatment resumption are necessary because treatment resumption should be determined based on each individual patient's condition, after assessment of the cause of the event occurring during infusion and its relationship with Temcell.

8.5.5.5. Retreatment

The applicant explanation on retreatment:
In Study JR-031-301, subjects who achieved a CR after the first infusion and who had a subsequent flare of Grades II-IV GVHD by Week 10 were allowed to be retreated with Temcell twice weekly for 4 weeks (infusions were administered at least 3 days apart for a total of 8 infusions). In Study JR-031-301, 1 subject had a flare of acute GVHD at 1 week after achieving a CR and was retreated with Temcell. The subject achieved a CR of ≥28 days duration. Thus, the applicant considered it appropriate to establish criteria for retreatment.

PMDA's view:

In Study JR-031-301, 1 patient who had had a flare of acute GVHD was retreated with Temcell as per the protocol and achieved a CR. On the basis of the finding, retreatment with Temcell is acceptable. Therefore, it is necessary to appropriately provide information on the criteria for retreatment with the dosage regimen as specified in Study JR-031-301 and the retreated subject in Study JR-031-301 to healthcare professionals. Post-marketing information on the efficacy and safety of retreatment in patients with flares of acute GVHD should be collected through post-marketing surveillance etc., and the information should be appropriately provided to healthcare professionals.

8.5.6. Pediatric patients

The applicant's explanation on the efficacy and safety of Temcell in pediatric patients:

Three pediatric patients (aged <15 years; namely, 4, 5, and 6 years of age) were enrolled into Studies JR-031-201 and JR-031-301. Efficacy results are as shown in Table 8.23. One subject achieved a CR of ≥28 days duration.

Table 8.23. Assessment of efficacy by age group

Study identifier	Grade of GVHD	Age	CR of ≥28 days duration	Survival at 24 weeks post first infusion
JR-031-201	All subjects (II-IV)	<15 years	100 (1/1)	100 (1/1)
		≥15 years	53.8 (7/13)	76.9 (10/13)
	III or IV	<15 years	100 (1/1)	100 (1/1)
		≥15 years	25.0 (1/4)	75.0 (3/4)
JR-031-301	All subjects (III or IV)	<15 years	0 (0/2)	100 (2/2)
		≥15 years	52.2 (12/23)	56.5 (13/23)

Proportion of subjects achieving endpoint (%) (No. of subjects achieving endpoint/No. of evaluable subjects)

Safety results are as shown in Table 8.24. Pediatric patients were not at an increased risk of any adverse event.

Table 8.24. Incidences of adverse events by age group

Adverse event	Study JR-031-201/202 Study JR-031-301		Study 280			
			Prochymal		Placebo	
Age	<15 years	≥15 years	<15 years	≥15 years	<15 years	≥15 years
	N = 3	N = 36	N = 14	N = 149	N = 10	N = 71
Infections and infestations	3 (100)	33 (91.7)	11 (78.6)	135 (90.6)	7 (70.0)	60 (84.5)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0 (0)	3 (8.3)	1 (7.1)	20 (13.4)	0 (0)	12 (16.9)
Blood and lymphatic system disorders	3 (100)	20 (55.6)	1 (7.1)	75 (50.3)	1 (10.0)	27 (38.0)
Immune system disorders	3 (100)	15 (41.7)	3 (21.4)	56 (37.6)	3 (30.0)	23 (32.4)
Endocrine disorders	0 (0)	2 (5.6)	3 (21.4)	19 (12.8)	1 (10.0)	9 (12.7)
Metabolism and nutrition disorders	1 (33.3)	16 (44.4)	4 (28.6)	110 (73.8)	2 (20.0)	37 (52.1)
Psychiatric disorders	0 (0)	8 (22.2)	2 (14.3)	89 (59.7)	1 (10.0)	30 (42.3)
Nervous system disorders	2 (66.7)	16 (44.4)	3 (21.4)	64 (43.0)	4 (40.0)	34 (47.9)
Eye disorders	1 (33.3)	8 (22.2)	3 (21.4)	41 (27.5)	1 (10.0)	20 (28.2)
Ear and labyrinth disorders	0 (0)	2 (5.6)	0 (0)	4 (2.7)	1 (10.0)	4 (5.6)
Cardiac disorders	0 (0)	5 (13.9)	2 (14.3)	54 (36.2)	1 (10.0)	19 (26.8)
Vascular disorders	3 (100)	5 (13.9)	4 (28.6)	59 (39.6)	1 (10.0)	31 (43.7)
Respiratory, thoracic and mediastinal disorders	0 (0)	12 (33.3)	8 (57.1)	95 (63.8)	5 (50.0)	44 (62.0)
Gastrointestinal disorders	2 (66.7)	24 (66.7)	9 (64.3)	113 (75.8)	5 (50.0)	58 (81.7)
Hepatobiliary disorders	2 (66.7)	20 (55.6)	1 (7.1)	43 (28.9)	3 (30.0)	19 (26.8)
Skin and subcutaneous tissue disorders	2 (66.7)	22 (61.1)	5 (35.7)	67 (45.0)	2 (20.0)	28 (39.4)
Musculoskeletal and connective tissue disorders	0 (0)	11 (30.6)	2 (14.3)	71 (47.7)	3 (30.0)	34 (47.9)
Renal and urinary disorders	1 (33.3)	15 (41.7)	3 (21.4)	64 (43.0)	1 (10.0)	32 (45.1)
Reproductive system and breast disorders	0 (0)	0 (0)	0 (0)	3 (2.0)	0 (0)	3 (4.2)
Congenital, familial and genetic disorders	0 (0)	0 (0)	0 (0)	2 (1.3)	0 (0)	0 (0)
General disorders and administration site conditions	1 (33.3)	23 (63.9)	2 (14.3)	113 (75.8)	3 (30.0)	52 (73.2)
Investigations	3 (100)	33 (91.7)	3 (21.4)	80 (53.7)	1 (10.0)	36 (50.7)
Injury, poisoning and procedural complications	0 (0)	15 (41.7)	1 (7.1)	28 (18.8)	1 (10.0)	16 (22.5)
Surgical and medical procedures	0 (0)	0 (0)	0 (0)	0 (0)	1 (10.0)	0 (0)

n (%)
MedDRA/J ver. 16.1

PMDA's view:

Although there is very limited clinical experience with Temcell in pediatric patients with acute GVHD, Temcell may be indicated for pediatric patients as well, provided that Temcell is used by or under the supervision of a physician with adequate knowledge and experience in hematopoietic stem cell transplantation at a medical institution with adequate facilities for the treatment of emergencies. PMDA concluded that Temcell may be used in pediatric patients with the same dosage regimen as in adult patients, but that post-marketing information should be collected through post-marketing surveillance etc. and the information should be appropriately provided to healthcare professionals. The above PMDA's decision is built on the following:

- There is no established treatment also for pediatric patients with corticosteroid-refractory acute GVHD following hematopoietic stem cell transplantation and those patients have a poor prognosis.
- There was a subject who achieved a CR in Study JR-031-201.
- The tolerability of Temcell in pediatric patients was demonstrated in Study JR-031-301.

9. Risk Analysis

The applicant's explanation on the post-marketing use-results survey plan:

In order to evaluate the post-marketing safety and efficacy of Temcell in routine clinical settings, a post-marketing use-results survey of acute GVHD patients treated with Temcell using the central registration system (target sample size of 600 patients; observation period, 52 weeks) is planned. Given that the frequency of the least common adverse event was 0.6% in Study 280, 500 patients need to be included in the survey study designed to have a 95% power to detect at least one adverse event occurring at an incidence of 0.6%. Thus, a target sample size of 600 was chosen to allow possible exclusion of patients from the analysis. An observation period of 52 weeks was chosen since safety findings reported beyond Week 52 in Study JR-031-202 included no new events for which a causal relationship to Temcell could not be ruled out.

The information to be collected is as follows: patient information, the details of hematopoietic stem cell transplantation, the date of onset of acute GVHD, complications, the use of Temcell, clinical laboratory tests, acute GVHD assessment, adverse reactions and infections, and concomitant medications/therapies (prophylaxis and treatment of GVHD).

PMDA's view on the post-marketing use-results survey plan:

As explained by the applicant, safety findings reported beyond Week 52 in Study JR-031-202 included no events for which a causal relationship to Temcell could not be ruled out. Thus, the observation period proposed by the applicant is acceptable. On the other hand, since safety information on Temcell is very limited, all patients treated with Temcell should be included in the survey during the re-examination period. The information to be collected should also include the following: hepatic dysfunction and infections, which were reported as fatal adverse events in Studies JR-031-201/202 and JR-031-301; and relapse of the underlying disease; the risk of promoting growth of malignant tumors other than the underlying disease; the tumorigenic and carcinogenic risk; the risks associated with intravenous infusion of allogeneic cells (events possibly associated with circulatory disorder due to cellular embolism and thrombogenesis, events possibly associated with intravascular hemolysis, events possibly associated with an immune response); gastrointestinal haemorrhage; skin disorder; and renal impairment. Since safety information on Temcell is very limited, the frequencies of adverse reactions and infections should be analzed at regular intervals based on the data from the post-marketing use-results survey on Temcell. The obtained safety information should be provided to healthcare professionals, while appropriate actions should be taken immediately, such as implementing additional safety measures as needed.

The details of the post-marketing use-results survey will be finalized, based on the comments on Temcell safety assessments at the Expert Discussion.

10. Results of Compliance Assessment Concerning the Data Submitted in the New Regenerative Medical Product Application and Conclusion by PMDA

10.1. PMDA's conclusion on the results of document-based GLP/GCP inspection and data integrity assessment

The assessment is currently ongoing and its results and PMDA's conclusion will be reported in the Review Report (2).

10.2. PMDA's conclusion on the results of GCP on-site inspection

The inspection is currently ongoing and its results and PMDA's conclusion will be reported in the Review Report (2).

11. Overall evaluation at the time of preparing Review Report (1)

Based on the submitted data, PMDA has concluded that a certain level of efficacy of Temcell in the treatment of acute GVHD following hematopoietic stem cell transplantation has been demonstrated and its safety is clinically acceptable. Since no second-line treatment has been established for patients with acute GVHD who have failed to respond to corticosteroids, Temcell with a different mechanism of action from currently available second-line therapies has clinical significance as a therapeutic option for acute GVHD.

Temcell may be approved if it can be concluded based on the comments from the Expert Discussion that there are no particular problems.

The current Indication or Performance section is presented below.

[Indication or performance]
Acute graft versus host disease following hematopoietic stem cell transplantation

Precautions for indication or performance

- Temcell should be used in patients who have failed to respond adequately to corticosteroids.
- Patients for use of Temcell should be selected by physicians with a full knowledge of the content of the Clinical Studies section, e.g., the severity of acute GVHD, and an adequate understanding of the efficacy and safety of Temcell.
- Since Temcell is associated with the risk of hepatic dysfunction etc., and the number of patients treated with Temcell is very limited, the use of other therapies should also be considered carefully before the initiation of treatment with Temcell.

Review Report (2)

August 6, 2015

1. Product Submitted for Registration

[Brand name] Temcell HS Inj.
[Non-prporietary name] Human (allogeneic) bone marrow-derived mesenchymal stem cells
[Applicant] JCR Pharmaceuticals Co., Ltd.
[Date of application] September 26, 2014

2. Content of the Review

The comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined in the following sections. The expert advisors for the Expert Discussion were nominated based on their declarations etc., concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions etc., by Pharmaceuticals and Medical Devices Agency" (PMDA Administrative Rule No. 8/2008 dated December 25, 2008).

2.1. Efficacy

On the basis of the results from a Japanese phase II/III study (JR-031-301) in patients with Grade III or IV acute graft versus host disease (GVHD) secondary to allogeneic hematopoietic stem cell transplantation (bone marrow transplantation, peripheral blood stem cell transplantation, cord blood transplantation) who have failed to respond to systemic corticosteroid therapy, PMDA comprehensively evaluated the efficacy of Temcell, considering the seriousness of acute GVHD and the lack of established second-line treatment for patients with acute GVHD who have failed to respond to corticosteroids. Consequently, PMDA has concluded that a certain degree of efficacy of Temcell in patients with acute GVHD has been demonstrated [see Section 8.5.2. of Review Report (1)].

The above conclusion by PMDA was supported by the expert advisors at the Expert Discussion.

2.2. Safety

PMDA's conclusion on the safety of Temcell:

Attention should be paid to the development of the following events during the treatment with Temcell: hepatic dysfunction, infections, relapse of the underlying disease, the risk of promoting growth of malignant tumors other than the underlying disease, the tumorigenic and carcinogenic risk, the risks associated with intravenous infusion of allogeneic cells (events possibly associated with circulatory disorder due to cellular embolism and thrombogenesis, events possibly associated with intravascular hemolysis, events possibly associated with an immune response), gastrointestinal haemorrhage, skin disorder, electrolyte abnormality, hyperglycaemia, hypertension, and renal impairment. Therefore, Temcell should be tolerable if it is used by or under the supervision of a physician with adequate knowledge and experience in hematopoietic stem cell transplantation

at a medical institution with adequate facilities for the treatment of emergencies and in a setting where appropriate measures such as laboratory monitoring and management are taken [see Section 8.5.3. of Review Report (1)].

The above conclusions by PMDA were supported by the expert advisors at the Expert Discussion.

2.3. Indication or performance

PMDA's conclusion:

Temcell is a therapeutic option for patients with acute GVHD who have failed to respond to corticosteroids and "Indication or performance" should be as shown below. "Precautions for indication or performance" should include precautionary statements about the patient population eligible for Temcell [see Section 8.5.4. of Review Report (1)].

[Indication or performance]

Acute graft versus host disease following hematopoietic stem cell transplantation

Precautions for indication or performance

1. Temcell should be used in patients who have failed to respond adequately to steroid therapy.
2. Patients for use of Temcell should be selected by physicians with a full knowledge of the content of the Clinical Studies section, e.g., the severity of acute GVHD, and an adequate understanding of the efficacy and safety of Temcell.

Since the above conclusions drawn by PMDA were supported by the expert advisors at the Expert Discussion, PMDA instructed the applicant to modify "Precautions for indication or performance" as shown above. The applicant agreed to do so, and PMDA accepted it.

2.4. Dosage and administration or method of use

PMDA concluded that "Dosage and administration or method of use" for Temcell should be as shown below, based on the dosage regimen used in Study JR-031-301 [see Section 8.5.5. of Review Report (1)].

[Dosage and administration or method of use]

The usual dosage of Temcell is 2×10^6 human mesenchymal stem cells/kg body weight administered as a slow intravenous infusion at a controlled rate of 4 mL/min. One bag of Temcell should be diluted with 18 mL of physiological saline. Temcell should be administered twice weekly for 4 weeks with infusions at least 3 days apart. The dose of 2×10^6 human mesenchymal stem cells/kg can be futher given once weekly for additional 4 weeks, depending on the degree of symptoms.

The above conclusion drawn by PMDA was supported by the expert advisors at the Expert Discussion. PMDA instructed the applicant to modify "Dosage and administration or method of use" as shown above. The applicant

agreed to do so and PMDA accepted it.

2.5. Post-marketing use-results survey plan (draft)

PMDA's conclusion:

Because of very limited safety data on Temcell safety and other reasons, a use-results survey intended for evaluation of the post-marketing safety etc., of Temcell in routine clinical settings should be planned and conducted, in consideration of the following points [see Section 9. of Review Report (1)].

- All patients treated with Temcell should be included in the survey during the re-examination period.
- The priority items to be investigated should include hepatic dysfunction, infections, gastrointestinal haemorrhage, rash, renal impairment, concomitant medications for GVHD treatment, relapse of the underlying disease, development and relapse of malignant tumors other than the underlying disease, malignancies derived from Temcell, and adverse events associated with intravenous infusion of allogeneic cells (events possibly associated with circulatory disorder due to cellular embolism and thrombogenesis [dyspnoea, hypoxia, oxygen saturation decreased, arrhythmia, thrombosis], events possibly associated with intravascular hemolysis, events possibly associated with an immune response [allergic reactions, oedema]).
- Information on donor sources for hematopoietic stem cell transplantation should also be included in the items to be investigated.
- The frequencies of product-related adverse events and infections and other information should be analyzed at regular intervals. The obtained safety information should be provided to healthcare professionals through information materials etc., while appropriate actions should be taken immediately, such as implementing additional safety measures as needed.

At the Expert Discussion, the above conclusions drawn by PMDA were supported by the expert advisors and the following comments were made by the expert advisors.

- The Japanese phase II/III study (JR-031-301) was conducted in a small number of subjects and the resulting efficacy data on Temcell is limited. Since the therapeutic effect of Temcell may differ according to the underlying disease, donor source for hematopoietic stem cell transplantation, previous therapy, etc., the duration of response to Temcell should be analyzed in a post-marketing use-results survey. Moreover. since a variety of items are investigated, a useful data entry procedure needs to be devised.

Taking account of the comments from the Expert Discussion, PMDA instructed the applicant to re-examine the post-marketing use-results survey plan. The applicant presented the outline of the amended use-results survey plan (draft) as shown in Table 9 and explained that the results of efficacy assessments by stage of organ dysfunction will be collected and the duration of response to Temcell will also be analyzed.

PMDA accepted the applicant's response above.

Table 9. Outline of the post-marketing use-results survey plan (draft)

Objectives	To evaluate the safety and efficacy of Temcell in routine clinical settings.
Survey method	All-case surveillance
Patients to be surveyed	Acute GVHD patients
Observation period	52 weeks
Target sample size	All patients treated with Temcell during the re-examination period
Main information to be collected	The priority items to be investigated are as follows: ・Hepatic dysfunction ・Infections ・Gastrointestinal haemorrhage ・Rash ・Renal impairment ・Relapse of the underlying disease ・Development and relapse of malignant tumors other than the underlying disease ・Malignancies derived from Temcell ・Adverse events associated with intravenous infusion of allogeneic cells (events possibly associated with circulatory disorder due to cellular embolism and thrombogenesis [dyspnoea, hypoxia, oxygen saturation decreased, arrhythmia, thrombosis], events possibly associated with intravascular hemolysis, events possibly associated with an immune response [allergic reactions, oedema]) ・Concomitant medications for GVHD treatment Other main items are as follows: ・Patient information ・Details of hematopoietic stem cell transplantation (including donor sources) ・Date of onset of acute GVHD ・Concurrent/past illness ・Use of Temcell ・Acute GVHD assessment ・Product-related adverse event ・Concomitant medications/therapies (prophylaxis of GVHD)

2.6. Viral safety of Heparin Sodium Injection used in collecting human bone marrow aspirates

Heparin Sodium Injection is used in collecting a human bone marrow aspirate from a donor. PMDA asked the applicant to explain the conformity of Heparin Sodium Injection to the Standard for Biological Ingredients.

The applicant's explanation:

Heparin Sodium Injection is manufactured with heparin sodium derived from porcine small intestine (collected from healthy animals) as a raw material and its manufacturing process includes the steps of viral inactivation/removal by heat-treatment and chemical treatment with oxidizing agents. The applicant confirmed that Heparin Sodium Injection conforms to the Standard.

PMDA accepted the applicant's response and concluded that the quality of Temcell is adequately controlled.

2.7. Human bone marrow donor screening (serology and PCR tests)

The applicant's response:

In consideration of product safety etc., human bone marrow donor screening tests will include serology and PCR tests for cytomegalovirus and Epstein-Barr virus (Table 10). Serology and PCR tests are performed at least [redacted] days prior to collection of bone marrow aspirate.

Table 10. Donor screening (Serology and PCR tests)

Tests	Test results
Human immunodeficiency virus	Negative
Hepatitis C virus	
Surface antigen of hepatitis B virus	
Anti-hepatitis B virus antibody	
Human T-cell leukemia virus	
Treponema pallidum	
Cytomegalovirus	
Epstein-Barr virus	
Hepatitis B virus DNA	
Hepatitis C virus RNA	
West Nile fever virus	
Human parvovirus B19	
Chagas' disease	

PMDA accepted the applicant's response.

2.8. Results of compliance assessment concerning the data submitted in the application and conclusion by PMDA

2.8.1. PMDA's conclusion on the results of document-based GLP/GCP inspection and data integrity assessment

Document-based inspection and data integrity assessment were conducted in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics for the data submitted in the application. The inspection and assessment revealed no particular problems. PMDA thus concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

2.8.2. PMDA's conclusion on the results of GCP on-site inspection

GCP[18] on-site inspection took place in accordance with the provisions of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics for the data submitted in the application (5.3.5.2.1, 5.3.5.2.2, 5.3.5.2.3).

PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

2.9. Regenerative medical product designation

In accordance with the "Concept of biological product, specified biological product, and regenerative medical product designation" (the notification issued jointly by the Director of the Director of Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW [Notification No.1105-1] and the Counselor for Medical Device and Regenerative Medicine Product Evaluation, Minister's Secretariat, MHLW [Notification No.1105-2], dated November 5, 2014), Temcell should be granted regenerative medical product designation

[18] Although the marketing application for Temcell was submitted as a regenerative medical product, the Ministerial Ordinance on GCP for drug trials was applied to clinical trials of Temcell because these clinical trials were conducted before the enforcement of the Ministerial Ordinance on GCP for regenerative medical product trials.

because Temcell is a regenerative medical product manufactured with allogeneic bone marrow-derived mesenchymal stem cells as its raw materials and it is difficult to inactivate or remove pathogenic agents in its manufacturing process.

3. Overall Evaluation

As a result of the above review, PMDA has concluded that Temcell may be approved with the following conditions, after modifying "Indication or performance" and "Dosage and administration or method of use" as shown below, if precautionary statements are included in the package insert and information on proper use is provided appropriately after the market launch. Since Temcell is an orphan regenerative medical product, the re-examination period should be 10 years. The product is classified as a designated regenerative medical product.

[Indication or performance]
Acute graft versus host disease following hematopoietic stem cell transplantation

[Dosage and administration or method of use]
The usual dosage of Temcell is 2×10^6 human mesenchymal stem cells/kg body weight administered as a slow intravenous infusion at a controlled rate of 4 mL/min. One bag of Temcell should be diluted with 18 mL of physiological saline. Temcell should be administered twice weekly for 4 weeks with infusions at least 3 days apart. The dose of 2×10^6 human mesenchymal stem cells/kg can be further given once weekly for additional 4 weeks, depending on the degree of symptoms.

[Conditions for approval]

1. The applicant is required to ensure that the product is used by or under the supervision of a physician with adequate knowledge of and experience with hematopoietic stem cell transplantation at a medical institution with adequate facilities for the treatment of emergencies and in a setting where appropriate measures are taken, such as laboratory monitoring.
2. The applicant is required to ensure that a use-results survey that covers all patients treated with the product is conducted during the re-examination period and that appropriate actions are taken as needed.