

Clinical Pharmacology BLA Review

Division of Clinical Evaluation and Pharmacology/Toxicology (DCEPT),

Office of Tissues and Advanced Therapy (OTAT)

Submission Number: 125706/00

Product Name: Remestemcel-L Ex Vivo Cultured Adult Human Mesenchymal Stem Cells (MSCs)

Proposed Indication: Treatment of acute Graft versus Host Disease (aGvHD) in pediatric patients when aGvHD has failed to respond to treatment with systemic corticosteroids.

Applicant: Mesoblast, Inc.

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RPM: Adriane Fisher

Reviewer: Million Tegenge, PhD

Clinical Pharmacology Reviewer, General Medicine Branch 2, DCEPT, OTAT

Through: Lei Xu, MD, PhD

Chief, General Medicine Branch 2, DCEPT, OTAT

Through: Tejashri Purohit-Sheth, MD

Director, DCEPT, OTAT

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Study#1- A Single-arm, Prospective Study of Remestemcel-L, *Ex-vivo* Culture Expanded Adult Human Mesenchymal Stromal Cells, for the Treatment of Pediatric Patients who Have Failed to Respond to Steroid Treatment for Acute GVHD (#MSB-GVHD001/002)

Executive Summary

Acute graft-versus-host disease (aGvHD) is the major life-threatening complication of allogeneic hematopoietic cell transplantation. In this Biologics License Application (BLA) submission, remestemcel-L is proposed for the treatment of aGvHD in pediatric patients when aGvHD has failed to respond to treatment with systemic corticosteroids. The active ingredient in remestemcel-L is human culture-expanded mesenchymal stromal cells (ceMSC) isolated from the bone marrow of healthy adult donors. Conventional clinical pharmacology studies such as dose escalation/dose finding, absorption, distribution, metabolism, excretion, drug-drug interaction and special population studies were not performed due to the cellular nature of remestemcel-L, and therefore were not warranted. Pharmacodynamic (PD) studies were conducted to characterize the immunomodulatory effects of ceMSC and their underlying mechanism of action (MOA). Human clinical data from biomarker characterization in the later phase study, MSBGVHD001/002, was employed as a primary source to generate evidence on the immunomodulatory PD bioactivity of remestemcel-L in pediatric subjects with steroid-refractory aGvHD (n=40, age range 0.6 to 17 years). At Baseline, elevated levels of the following PD biomarkers were noted in blood consistent with an inflammatory state characteristic of aGvHD: hepatocyte growth factor (HGF), interleukin-8 (IL-8), interleukin-2 receptor α (sIL-2R α), tumor necrosis factor receptor type I (TNFR1), regenerating islet-derived protein 3 α (Reg3 α), and suppressor of tumorigenicity 2 (ST2). Treatment with remestemcel-L reduced TNFR1 and ST2 levels by 76 % and 72 % at Day 180 as compared to the baseline levels, respectively. Both TNFR1 and ST2 have been shown to be released by activated T lymphocytes and their decrease upon treatment with remestemcel-L demonstrates PD activity consistent with a reduced inflammatory state. Further, the circulating levels of CD3+CD4+CD25+HLA-DR+ T cells, which represent activated T cells, were reduced by 54 % at Day 180 following treatment with remestemcel-L as compared to the baseline values. Overall the reduction in levels of secreted factors (TNFR1 and ST2) and activated T cells provided clinical evidence of the immunomodulatory pharmacodynamic effects of remestemcel-L in pediatric subjects with steroid-refractory aGvHD.

Recommendations

This BLA is acceptable for approval from the clinical pharmacology perspective. The Applicant should incorporate the clinical pharmacology labeling comments.

Background

Graft-versus-host disease (GVHD) is the major life-threatening complication of allogeneic hematopoietic cell transplantation (allo-HCT). GVHD occurs when immune competent T cells in the donated tissue (the graft) recognize the recipient (the host) as foreign. The resulting immune response activates donor T cells to gain cytolytic capacity then attack the recipient to eliminate foreign antigen (s)- bearing cells. The two main clinical presentations are acute GVHD (aGVHD) and chronic GVHD. The diagnosis of aGVHD relies on clinical, laboratory, and biopsy assessment of target organs. The severity of aGVHD is graded clinically based on involvement of the three main target organs: skin (the most frequent and often the earliest clinical manifestation of aGVHD), gastrointestinal tract (second most common), and liver¹. Amongst all allogeneic hematopoietic cell transplant patients, 30-50% develop aGVHD (grade I-IV) and 14% experience severe aGVHD (grade III-IV)¹. The standard front-line treatment for aGVHD generally consists of corticosteroids, which produce a response in about half of all patients. A variety of immunosuppressive agents are used as second-line therapy, but efficacy against steroid-refractory aGVHD remains an unmet medical need.

In this BLA submission, remestemcel-L is proposed for the treatment of steroid refractory aGVHD. It is administered by intravenous (IV) infusion at a dose of 2×10^6 cells/kg twice a week for 4 consecutive weeks. The active ingredient in remestemcel-L is human culture-expanded mesenchymal stromal cells (ceMSC) isolated from the bone marrow of healthy adult donors. The rationale for development of remestemcel-L as a treatment for aGVHD is noted by the Applicant to be attributed to the following characteristics:

- immunomodulatory pharmacodynamic bioactivity, which may reduce immune activation and inflammation, and limit tissue damage in aGVHD.

¹ Zeiser and Blazar. Acute Graft-versus-Host Disease Biology, Prevention and Therapy. N Engl J Med. 2017 November 30; 377(22): 2167–2179

- hypo-immunogenic phenotype, which allows for the product to be administered without HLA matching between the MSC donor and recipient.
- capacity for *ex vivo* proliferation, enabling the manufacture of therapeutically-relevant numbers of cells.

Summary of Clinical Pharmacology Findings

The data supporting clinical pharmacology of remestemcel-L were gathered during the development and characterization of ceMSC. Conventional clinical pharmacology studies such as dose escalation/dose finding, absorption, distribution, metabolism, excretion, drug-drug interaction and special population studies were not performed due to the cellular nature of remestemcel-L, and therefore were not warranted.

1. Justification of Dosing Regimen

No formal clinical dose finding, or dose regimen optimization studies have been performed during the development of remestemcel-L for treatment of aGvHD in pediatrics. In early studies, human mesenchymal stromal cells were evaluated to treat steroid-refractory, severe aGvHD with a median dose of 1×10^6 cells/kg for 1-3 infusions. Furthermore, the initial expanded access programs (EAP) included 10 pediatric subjects aged 2-15 years who were infused with allogeneic ceMSC at a dose of 2×10^6 cells/kg twice a week for 4 weeks. In Study MSBGVHD001/002, treatment with remestemcel-L at a dose of 2×10^6 cells/kg, administered by IV infusion twice a week for 4 weeks was generally safe and well-tolerated through day 180.

2. Mechanism of Action and Pharmacodynamics

Pharmacodynamic (PD) studies were performed to characterize the immunomodulatory effects of ceMSC and their underlying mechanism of action (MOA) based on *in vitro*, nonclinical and clinical studies. Human clinical data from biomarker characterization in Study MSBGVHD001/002 provided evidence of the immunomodulatory PD bioactivity of remestemcel-L in pediatric subjects with steroid-refractory aGvHD. A summary of the major PD characteristics of ceMSC from *in vitro* and nonclinical studies are provided below:

- ceMSC were shown to inhibit alloantigen- and mitogen-stimulated T cell proliferation in a dose-dependent manner and reduced the secretion of the pro-inflammatory cytokine, tumor necrosis factor α (TNF α).
- ceMSC were shown to have immunomodulatory pharmacodynamic bioactivity on a range of immune cell subsets, including naïve and effector T cells, DC and NK cells, inducing shifts towards anti-inflammatory phenotypes as evidenced by alterations to their cytokine secretion profiles.
- *In vitro* studies showed that the inhibitory effects of ceMSC on activated T cells are mediated, at least in part, by PGE2.
- Knock-down of TNF receptor type I (TNFR1) expression attenuated the immunosuppressive activity of ceMSC, providing evidence that the immunomodulatory effects of ceMSC are partly dependent on signaling through TNFR1.

Human data from biomarker characterization in Study MSBGVHD001/002 provided evidence of the immunomodulatory PD bioactivity of remestemcel-L in pediatric subjects with steroid-refractory aGvHD. A total of 40 pediatric subjects (age range 0.6- 17 years) out the 55 subjects enrolled in study MSBGVHD001/002 provided blood samples for PD biomarker analysis. A

summary of the major PD characteristics of ceMSC from pediatric aGvHD studies are listed below:

- At Baseline, elevated levels of the following PD biomarkers were noted: hepatocyte growth factor (HGF), interleukin-8 (IL-8), interleukin-2 receptor α (sIL-2R α), TNFR1, regenerating islet-derived protein 3 α (Reg3 α), and suppressor of tumorigenicity 2 (ST2). This observation is consistent with an inflammatory state characteristic of aGvHD.
- The levels of TNFR1 & ST2 progressively decreased up to 180 days following treatment with remestemcel-L. Day 180 levels as compared to baseline levels of TNFR1 & ST2 were reduced by 76% and 72%, respectively. Both TNFR1 and ST2 have been shown to be released from activated T lymphocytes and their decrease upon treatment with remestemcel-L demonstrate PD activity presumably resulting in attenuation of aGvHD.
- The circulating levels of CD3+CD4+CD25+HLA-DR+ T cells declined by Day 28 and progressively declined up to Day 180 by 54 % as compared to the baseline values. CD25 is the alpha chain of the trimeric IL-2 receptor and is upregulated on T cells relatively early following stimulation of the T cell receptor (TCR)/CD3 complex, while HLA-DR appears later and is a late stage marker of activated T cells. The CD3+CD4+ T-cells expressing both CD25 and HLA-DR are representing fully activated T cells at Baseline, and which progressively decreased following treatment with remestemcel-L.
- Overall, the reduction in levels of secreted factors (TNFR1 and ST2) and activated T cells provide clinical evidence of the immunomodulatory pharmacodynamic effects of remestemcel-L in pediatric subjects with steroid-refractory aGvHD.

3. Pharmacokinetics

Pharmacokinetic (PK) studies of remestemcel-L have not been performed in humans. Because of the cellular nature of remestemcel-L, conventional methods cannot be applied for PK monitoring.

Thus, PK findings were largely based on nonclinical studies and published exploratory human studies:

- Following IV administration in healthy rats, allogeneic ceMSC are rapidly cleared from the bloodstream, initially accumulating in the lungs before re-distributing to other organs, including the liver, kidney and spleen in the first 24 hours after cell injection.
- In canine and non-human primate models of total body irradiation (TBI) and hematopoietic stem cell transplantation (HCT), the presence of injury and inflammation appeared to influence the sub-acute and longer-term distribution of ceMSC, with evidence of greater numbers of ceMSC detected in injured tissues compared to non-injured tissues.
- In non-human primate models of TBI and HCT, ceMSC were observed to persist long term (~9 months) at low levels in various tissues (bone marrow, gastrointestinal tract, liver, kidney, spleen, lymph nodes, lung and skin).
- These nonclinical findings are in line with published data from exploratory clinical studies in aGvHD, which indicate that intravenously administered MSC are rapidly cleared from the circulation and, in some subjects can be detected at low levels in lymph nodes, lung and gastrointestinal tissues^{2,3,4}.

² Ringdén et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. *Transplantation*. 2006 May 27;81(10):1390-7.

³ Koç et al. Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy.

J Clin Oncol. 2000 Jan;18(2):307-16.

⁴ Von Bahr et al. Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. *Stem Cells*. 2012 Jul;30(7):1575-8.

4. Immunogenicity Risk Evaluation

Since ceMSC is an allogenic product, there is a potential for development of anti-drug (donor) antibodies (ADA) or anti-HLA antibodies. Humoral immune response was not characterized in pediatric patients with aGvHD in Study MSBGVHD001. Previously, the Applicant characterized the humoral immune response (ADA and anti-HLA antibodies) in two clinical studies in patients with Crohn's disease and type 1 diabetes. For Crohn's disease, 1 out of 25 patients (4 %) tested positive for anti-HLA antibodies, but no patient tested positive for ADA up to Day 56 following remestemcel-L treatment. For type 1 diabetes, 13 out of 42 (31%) for remestemcel-L treated and 5 out of 21 (24 %) subjects in the placebo group had at least one positive test for anti-HLA antibodies at any time point. Six out of 42 (14%) remestemcel-L treated subjects and 0 out of 21 (0 %) subjects in the placebo group tested positive for ADA during the 1 year follow-up period, respectively. The clinical significance of ADA or anti-HLA antibodies following treatment with remestemcel-L is not fully understood.

Clinical Pharmacology Labeling Comments

The following are the recommended revisions to the Clinical Pharmacology section of the USPI.

12.1 Mechanism of Action

The mechanism of action for remestemcel-L is unknown but may be related to immunomodulatory effects. Acute GVHD occurs when alloreactive donor-derived T cells within the donated tissue (graft) trigger an immunological response, and alloreactive donor-derived T cells play a role in mediating the systemic inflammation, cytotoxicity and potential end organ damage associated with acute GVHD. Data from *in vitro* studies demonstrate that ceMSCs inhibit T cell activation as measured by proliferation and secretion of pro-inflammatory cytokines.

Acute GVHD is a serious and the major life-threatening complication of allogeneic hematopoietic stem cell transplantation (HSCT) that occurs when alloreactive donor T cells within the donated tissue (HSC graft) recognizes the recipient's tissues (host) as foreign and triggers an immunological response characterized by systemic inflammation, cytotoxicity and potential end organ damage. In this complex pathobiology, alloreactive HSC donor derived T cells play a pivotal role in effecting mediating cytotoxicity and tissue damage, with involvement of other activated immune cells, including natural killer (NK) cells, macrophages and neutrophils. Inflammatory cytokines released by injured tissue sustain the activity of these immune effector cells and perpetuate further tissue and end organ damage, Data from *in vitro* and clinical studies demonstrate that MSC have immunomodulatory effects on T lymphocytes, including inhibition of activated T cell proliferation and decreasing pro-inflammatory cytokine secretion by TH1 cells and increasing anti-inflammatory cytokine secretion by TH2 cells. MSC were also shown to have immunomodulatory activities on other immune cell subtypes, including dendritic cells and natural killer cells. These immunomodulatory activities may reduce immune activation and inflammation and limit tissue damage in acute GVHD. and thereby lead to improved clinical outcomes

12.2 Pharmacodynamics

In a nonclinical proof of concept study in a baboon model of allograft rejection, ceMSC were shown to have immunomodulatory activity in an alloreactive T cell driven immune response. A single intravenous dose of allogeneic ceMSC prolonged allograft survival to 11 days compared with 7 days in control treated animals.

In vitro studies show that human ceMSC have immunomodulatory effects on various immune cell types. ceMSC suppress mitogen activated and alloantigen activated T cell proliferation and reduced the . In cultures of T cells, dendritic cells and natural killer cells, MSCs decrease levels of pro-inflammatory cytokines. e.g. tumor necrosis factor α (TNF α) and interferon γ (IFN γ), and increase levels of anti-inflammatory mediators e.g. interleukin 10 (IL-10), interleukin 4 (IL-4) and prostaglandin E2 (PGE2).

Human ceMSC are characterized by a hypo-immunogenic phenotype. ceMSC express low levels of major histocompatibility complex (MHC) class I and minimal levels of human leukocyte antigen (HLA) DR, CD40, CD80, and CD86, which are essential for immune recognition. Functionally, ceMSC do not elicit a proliferative response in allogeneic lymphocytes *in vitro*.

Human pharmacodynamic data were obtained from analysis of blood samples in pediatric subjects with steroid-refractory aGVHD (n=40; age range 0.6-17 years) following treatment with remestemcel-L at a dose of 2×10^6 cells/kg. At Baseline, elevated levels of tumor necrosis factor receptor type I (TNFR1) and suppressor of tumorigenicity 2 (ST2) were observed consistent with the inflammatory state of aGVHD. Treatment with remestemcel-L reduced the levels of TNFR1 and ST2 by 76% and 72%, respectively, at Day 180 as compared to baseline values. Further, the circulating levels of CD3+CD4+CD25+HLA-DR+ T cells, which represent activated T cells, were reduced by 54% at Day 180 following treatment with remestemcel-L as compared to the baseline values.

Comment to Applicant:

Please provide and confirm the % change at Day 28 and 180 as compared to Baseline values for levels of TNFR1, ST2 and circulating levels of CD3+CD4+CD25+HLA-DR+ T cells.

12.3 Pharmacokinetics

~~Pharmacokinetic studies have not been performed in humans. Absorption, metabolism and excretion studies do not apply to cell therapies. The distribution profile of RYONCIL following intravenous administration was modeled in nonclinical animal studies.~~

~~Data from studies in healthy rats show that following intravenous infusion, MSC are rapidly cleared from the circulation initially accumulating in the lungs before redistributing to other organs. At 24 h after infusion, cells were primarily detected in the lungs, liver, kidney and spleen.~~

~~Long term studies following intravenous infusion of MSC in animal models of total body irradiation and HSCT in dogs, baboons and macaques show that MSC distribute widely, and may preferentially distribute to sites of inflammation and tissue damage in this model, such as the bone marrow, GI tract, and skin. Allogeneic MSC were detected at low levels in various tissues up to 9 months following infusion in a baboon model of TBI and HSCT.~~

Comment to Applicant:

You have not provided human PK data. Several intrinsic and extrinsic factors are expected to influence the clearance and tissue distribution of MSC, and it is difficult to extrapolate the nonclinical results to human.

Appendix

Study#1- A Single-arm, Prospective Study of Remestemcel-L, *Ex-vivo* Culture Expanded Adult Human Mesenchymal Stromal Cells, for the Treatment of Pediatric Patients who Have Failed to Respond to Steroid Treatment for Acute GVHD (#MSB-GVHD001/002)

Primary Objectives:

- To evaluate the efficacy of remestemcel-L in pediatric subjects with Grades B-D aGvHD who have failed to respond to steroid treatment post allogeneic HSCT
- To gather additional information on the safety of remestemcel-L in pediatric subjects with Grades B-D aGvHD who have failed to respond to steroid treatment post allogeneic HSCT

Secondary Objectives:

- To determine the correlation between response to remestemcel-L at Day 28 and survival at Day 100
- To obtain quality-of-life (QOL) data on remestemcel-L-treated subjects via the Pediatric Quality of Life Inventory™ (PedsQL™) and the pediatric global health-related quality of life (HRQOL) Parent Proxy Report
- To measure the functional status of remestemcel-L-treated subjects using the Karnofsky/Lansky scale

Exploratory Objective:

- To capture and analyze biomarker expression by remestemcel-L-treated subjects

Methodology

Subjects were treated with intravenous (IV) remestemcel-L at a dose of 2×10^6 MSCs/kg actual body weight at screening, twice per week, for each of 4 consecutive weeks (Initial Therapy). Eligible subjects were permitted to receive Continued Therapy, an additional 4 once-weekly infusions of remestemcel-L at the Initial Therapy dose of 2×10^6 MSCs/kg actual body weight at screening. Subjects participating in Studies MSB-GVHD001 and MSB-GVHD002 had the option of participating in an exploratory biomarker sub-study. Blood samples for biomarker assessments were collected at Baseline (prior to treatment with remestemcel-L) and at Days 28, 100, 160 and 180. Frozen samples were sent to a central lab for batched analysis. Plasma levels of

interleukin-2 receptor α (IL-2R α), TNFR1, interleukin-8 (IL-8), hepatocyte growth factor (HGF), elafin and regenerating islet-derived protein 3 α (REG3 α) were measured using a (b) (4) immunoassay, while suppressor of tumorigenicity 2 (ST2) levels were measured by (b) (4). Immune cell subsets (T cells, B cells and NK cells, regulatory T cells and activated T cells) were analyzed by (b) (4). Table 1 provides a summary of the assays used for biomarker testing in Studies MSBGVHD001 and MSB-GVHD002.

The MAGIC algorithm probability (MAP, also referred to as the MAGIC biomarker score (MBS)) is a biomarker algorithm that estimates the probability of 6-month non-relapse mortality (NRM) for individual patients during HSCT and in early stages of aGvHD. The MBS has been referred to as a “liquid biopsy” for lower GI injury in aGvHD, which is difficult to treat and is major cause of death in patients with GVHD as it incorporates two key biomarkers of GI crypt damage, Reg3 α and ST2. The MBS was determined using ST2 and Reg3 α results in the algorithm below:

$$\log [-\log (1 - \text{MAP})] = -11.263 + 1.844(\log_{10}\text{ST2}) + 0.577(\log_{10}\text{REG3}\alpha)$$

Longitudinal biomarker analyses were performed using the restricted maximum likelihood (REML) approach to estimate random and fixed effects in a repeated measures linear mixed effects model. The model allowed accommodation of between-subject and within-subject variation and for *post hoc* tests to be performed to provide comparisons of biomarker levels between study time points (baseline and days 28, 100, 160 and 180 post-first MSC infusion). Study subjects were assumed as a random effect and the comparisons between study timepoints to be the fixed effect. The predicted value of each biomarker at each time point was calculated as Least Squares Means (LSM). Correlations between continuous subject demographics and characteristics, baseline biomarker levels and responder and survivor probabilities were examined using the Pearson correlation method.

Table 1: Summary of Assays for Exploratory Biomarker Testing in MSB-GVHD001 and MSB-GVHD002

Biomarker	Test Method	Assay	Assay Manufacturer	Sample Type	Validation Parameters	Accuracy and Sensitivity Performance
Elafin, HGF, Reg3 α , TNFR1, IL-8, IL-2R α						
ST2						
T cells, B cells, NK cells						
Activated T cell (CD25 $^{+}$, HLA-DR $^{+}$)						
Treg (CD25 $^{+}$ /CD127 $^{lo-}$)						

(a) Accuracy was determined in validation of the (b) (4) assay for serum (EDTA) samples. BCT: blood collection tube. (b) (4) assay. HGF: hepatocyte growth factor. HLA-DR: Human leukocyte antigen-DR. IL-2R α : interleukin-2 receptor- α . IL-8: interleukin-8. LLOQ: lower limit of quantification. NK cells: natural killer cells. QC: quality control. Reg3 α : regenerating islet-derived protein 3 α . ST2: suppressor of tumorigenicity-2. TNFR1: tumor necrosis factor receptor type I. ULOQ: upper limit of quantification.

Source: Applicant's Table 2 from MSB-GVHD001/002 Biomarkers Final Analysis Report

Results of Pharmacodynamic Biomarker Analysis

Demographics of Subjects in Biomarker Analysis

In total, 55 subjects were enrolled into Study MSB-GVHD001/MSB-GVHD002 (full analysis set (FAS)). Fifty-four (54) subjects were treated with at least one dose of remestemcel-L (safety population), and 40 of these subjects further participated in the exploratory biomarker sub-study in Studies MSB-GVHD001 and/or MSB-GVHD002 (Table 2). The results displayed in Table 3 demonstrate that the demographics of the subjects in the PD biomarker analysis are representative of the overall study subjects enrolled in Studies MSB-GVHD001/MSB-GVHD002.

Table 2: Summary of Biomarker Samples Collected in MSB-GVHD001/MSB-GVHD002

	MSB-GVHD001 (N=36)						MSB-GVHD002 (N=21)			
Subjects (N)	36						21			
Samples (N)	Baseline		Day 28		Day 100		Day 160		Day 180	
	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood	Plasma	Whole Blood
	30	29	34	33	26	24	17	14	20	19

Source: Applicant's Table 9 from MSB-GVHD001/002 Biomarkers Final Analysis Report

Table 3: Demographics of Subjects in the PD Biomarker Analysis

	FAS	Safety Population ^(a)	Exploratory Biomarker Subgroup
N	55	54	40
Demographics			
Age			
Mean±SD	7.4±5.4	7.5±5.4	8.5±5.1
Median	7.0	7.0	10
Min-Max	0.6-17.0	0.6-17.0	0.6-17.0
Sex; N (%)			
Male	35 (63.6)	35 (64.8)	28 (70)
Female	20 (36.4)	19 (35.2)	12 (30)
Race; N(%)			
American Indian/Alaska Native	3 (5.5)	3 (5.6)	2 (5)
Asian	3 (5.5)	3 (5.6)	2 (5)
Black/African American	8 (14.5)	8 (14.8)	5 (12.5)
Native Hawaiian/Pacific Islander	0 (0.0)	0 (0.0)	0 (0.0)
White	31 (56.3)	30 (55.5)	23 (57.5)
Other	10 (18.2)	10 (18.5)	8 (20)
Weight (kg)			
Mean±SD	28.8 (18.9)	29.2±19.0	31.0±17.5
Median	25.5	25.8	30.4
Range	4.6-90.1	4.6-90.1	4.6-73.0

Source: Applicant's Table 7 from MSB-GVHD001/002 Biomarkers Final Analysis Report

Baseline Biomarker Profile

The median levels of soluble biomarkers HGF, IL-8, sIL-2R α , TNFR1, Reg3 α , and ST2 were increased in study subjects compared to levels observed in healthy adults, consistent with an inflammatory state characteristic of aGvHD. However, the level of Elafin was within the range observed in healthy adults (Table 4). The median MBS was 0.369, with 60% of subjects (N=18/29) having a baseline MBS \geq 0.291, suggesting that most subjects at baseline were Ann Arbor 3 and at high risk for 6 month NRM (Table 4).

Table 4: Descriptive Analysis of Soluble Biomarkers at Baseline

	N	Mean	SD	Median	Min	Max	Reference Range (a, b)
Soluble Biomarkers(c)							
Elafin	30	3.86	0.54	3.82	3.09	5.03	3.45-4.18
HGF	30	2.64	0.34	2.62	1.80	3.42	1.10-2.45
IL-8	30	1.55	0.42	1.53	1.07	2.53	0.46-1.33(d)
Reg3 α	30	4.03	0.50	3.99	2.97	5.67	2.84-3.94
sIL-2r α	30	3.24	0.26	3.19	2.70	3.79	2.34-3.02
TNF-R1	29	3.95	0.31	3.91	3.61	5.31	3.10-3.91
ST2	29	2.33	0.24	2.36	1.82	2.60	1.29-1.72
MAGIC Biomarker Score (MBS)							
MBS	29	0.372	0.163	0.371	0.149	0.762	High Risk: \geq 0.291 Low risk: $<$ 0.291

(a) Reference ranges for soluble biomarkers were determined during assay validation. For elafin, HGF, IL-8, Reg3 α , sIL-2R α and TNFR1, data were generated from N=50 healthy adult donors (N=25 males, N=25 females). (b) For ST2, data were generated from N=25 healthy donors (N=13 males; N=12 females). (c) Test results for soluble markers were log₁₀ transformed. For elafin, HGF, IL-8, Reg3 α , sIL-2R α and TNFR1, original units = pg/ml. For ST2, original units = ng/ml. (d) Lower level estimated as 0.5xLLOQ; log₁₀(2.905) = 0.46

Source: Applicant's Table 10 from MSB-GVHD001/002 Biomarkers Final Analysis Report

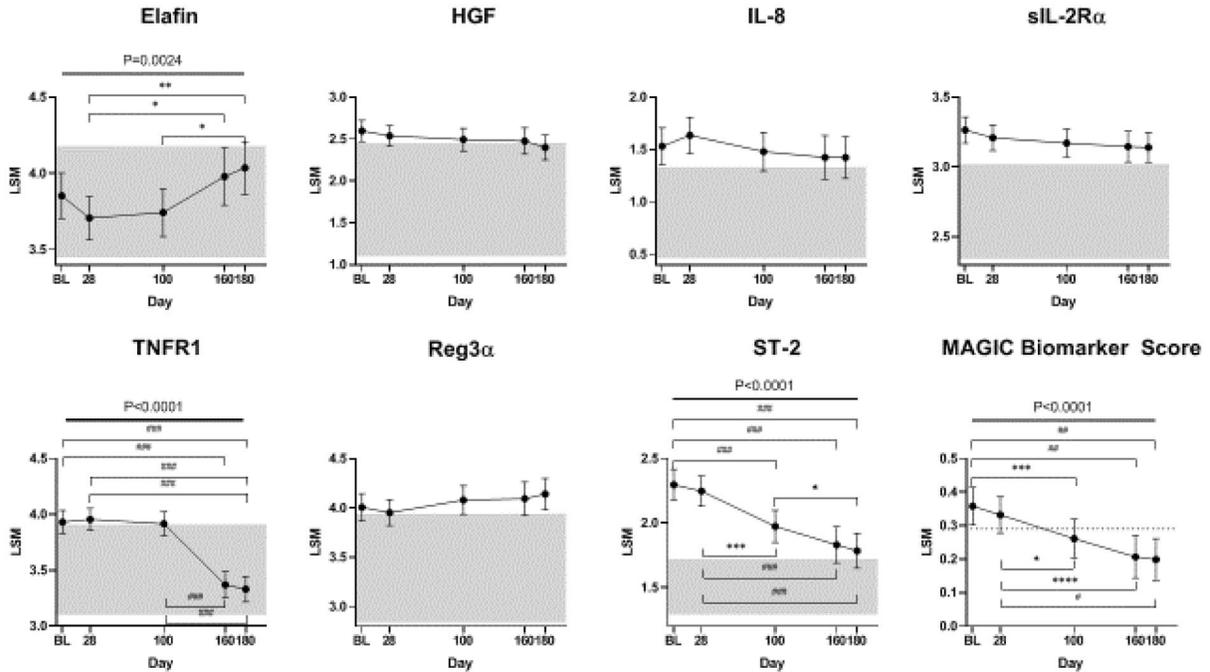
Longitudinal Biomarker Analysis:

Figure 1 displays the longitudinal change of soluble PD biomarker levels over the course of MSB-GVHD001 (baseline and days 28 and 100) and MSB-GVHD002 (days 160 and 180). There were no significant differences between study timepoints in estimated levels of HGF, IL-8 and sIL-2R α . The levels of the following PD biomarker changes from Baseline were reported as statistically significant:

- Elafin levels increased at Days 160 and 180 as compared to Baseline

- TNFR1 levels decreased at Days 160 and 180 as compared to Baseline
- ST2 levels steadily and significantly decreased from baseline to Day 180

Figure 1: Analysis of Soluble Biomarker Levels at Baseline, Days 28, 100, 160 and 180.



Source: Applicant's Figure 1 from MSB-GVHD001/002 Biomarkers Final Analysis Report

The circulating levels of activated T cells declined at Day 28 versus baseline for subjects in study MSB-GVHD001 (Table 4). Specifically, the percentage of activated T cells defined by their composite expression of CD3+CD4+HLA-DR+ and CD3+CD4+CD25+HLA-DR+ were significantly reduced at Day 28 relative to levels measured at baseline (%CD3+CD4+HLA-DR+, Day 0 (N=27): 54.55±23.96 vs Day 28 (N=32): 37.38±18.97, P<0.0001; %CD3+CD4+CD25+HLA-DR+, Day 0 (N=28): 27.45±13.31 vs Day 28 (N=31): 18.42±11.5, P=0.0084). CD25 is the alpha chain of the trimeric IL-2 receptor and is upregulated on T cells early following stimulation of the TCR/CD3 complex, while HLA-DR appears later and is considered to be a late stage marker of activated T cells. Figure 2 displays the estimated frequencies of CD3+CD4+ and CD3+CD8+ T cells expressing the activation markers CD25 and HLA-DR at baseline and days 28, 100, 160 and 180. The proportion of CD3+CD4+CD25+ HLA-DR+ T cells significantly and progressively declined up to Day 180.

The longitudinal changes of activated T cells by Day 28 in responder group are displayed in Figure 3.

Levels of CD3+CD4+CD25+HLA-DR+ decreased in all groups from baseline to Day 28, then continued to decrease over time through Day 180 in all responder groups. In complete responders, the downward trend resulted in significant differences from Baseline (LSM = 21.22, 96=5% CI=13.56-28.89) at Day 100 (LSM = 8.70, 95% CI=1.07-16.33, P=0.0150) and Day 180 (LSM = 9.04, 95%CI=1.45-16.63, P=0.0485). However, the levels of CD3+CD4+CD25+HLA-DR+ were highly variable in the Day 28 non-responder group.

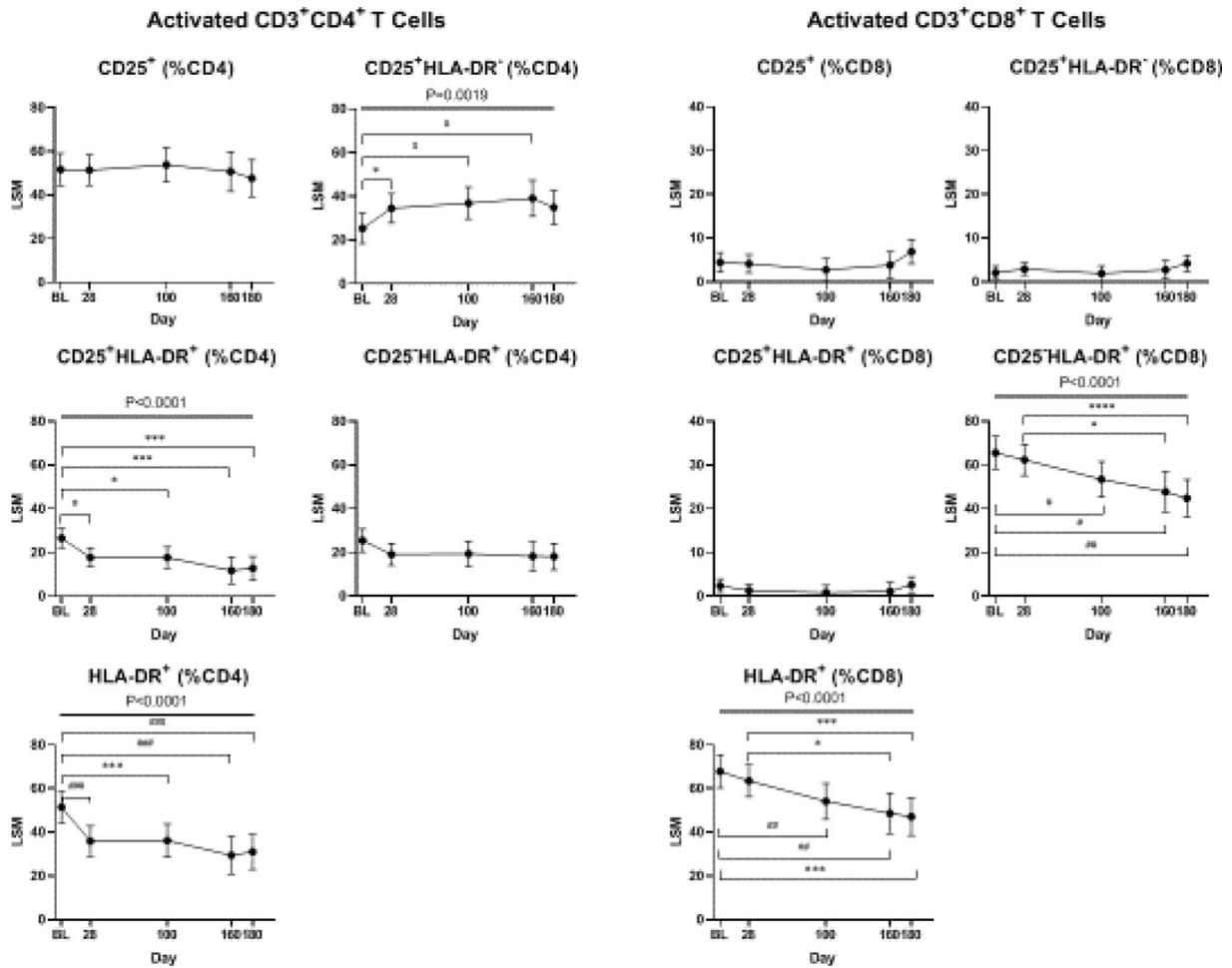
Table 4: % Activated T Cells at Screening and Day 28 in the Biomarker Subgroup

Activated T Cell Phenotype			All Subjects (Biomarker Subgroup) (N=33)	Day 28 Overall Responders (N=22)	Day 28 Non- Responders (N=11)
% (CD4+)	CD3+CD4+CD25+	Screening	53.6±16.2	49.4+16.7	54.9+15.7
		Day 28	51.9+20.5	43.1+16.7	55.3+20.2
	CD3+CD4+HLA-DR+	Screening	54.5+23.5	55.8+27.4	53.8+22.6
		Day 28	37.4+19.0‡	38.0+27.4‡	37.1+19.9*
	CD3+CD4+CD25+HLA-DR+	Screening	28.0+13.4	24.7+14.9	29.0+12.5
		Day 28	18.4+11.5†	14.5+14.9**	20.0+12.7
% (CD8+)	CD3+CD8+CD25+	Screening	4.3+5.0	5.0+4.9	4.1+5.3
		Day 28	4.2+4.0	3.6+4.9	4.5+4.2
	CD3+CD8+HLA-DR+	Screening	71.3+22.6	74.9+25.9	67.9+20.8
		Day 28	64.8+21.0	74.0+25.9	61.1+20.1
	CD3+CD8+CD25+HLA-DR+	Screening	68.9+22.0	2.9+4.5	2.2+4.1
		Day 28	63.5+20.9	1.2+4.5	1.3+1.6

N shown indicates the number of subjects in the biomarker sub-study for whom there are results from the activated T cell ^{(b) (4)} panel. Data represented as Mean ± SD. For the overall biomarker group and each responder sub-group, the mean % of each phenotype at screening and day 28 were compared using Tukey's HSD test. *p<0.02; **p<0.01; †p<0.001; ‡p<0.0001. Source: Applicant's Table 29 from Module 3.2.S.3.1

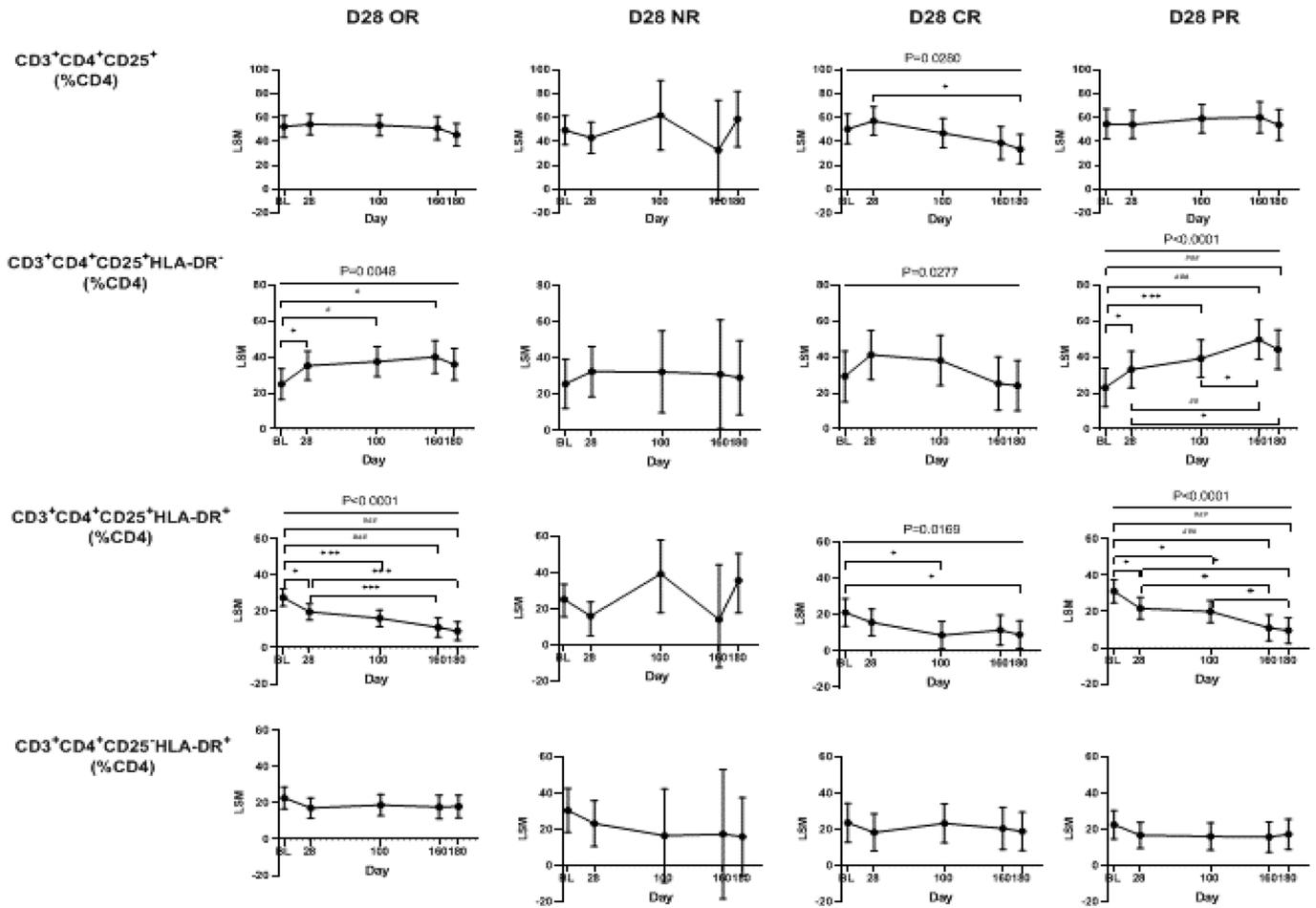
The percentage and absolute counts of CD4+CD25+CD127-/Lo regulatory T cells are displayed in Figure 4. Like overall trend in the CD3+CD4+ population, the concentration of Tregs tended to decrease from Baseline to Day 28 then increased from Day 28 through Day 180. There were no significant differences in Treg numbers between OR vs NR groups at Baseline. At Day 28, the percentage of CD4+CD25+CD127-/lo Tregs was significantly increased in OR (15.37 ± 10.99) compared to NR (8.69 ± 6.28).

Figure 2: Analysis of Activated T cells at Baseline, Days 28, 100, 160 and 180 following ceMSC infusion.



Source: Applicant's Figure 3 from MSB-GVHD001/002 Biomarkers Final Analysis Report

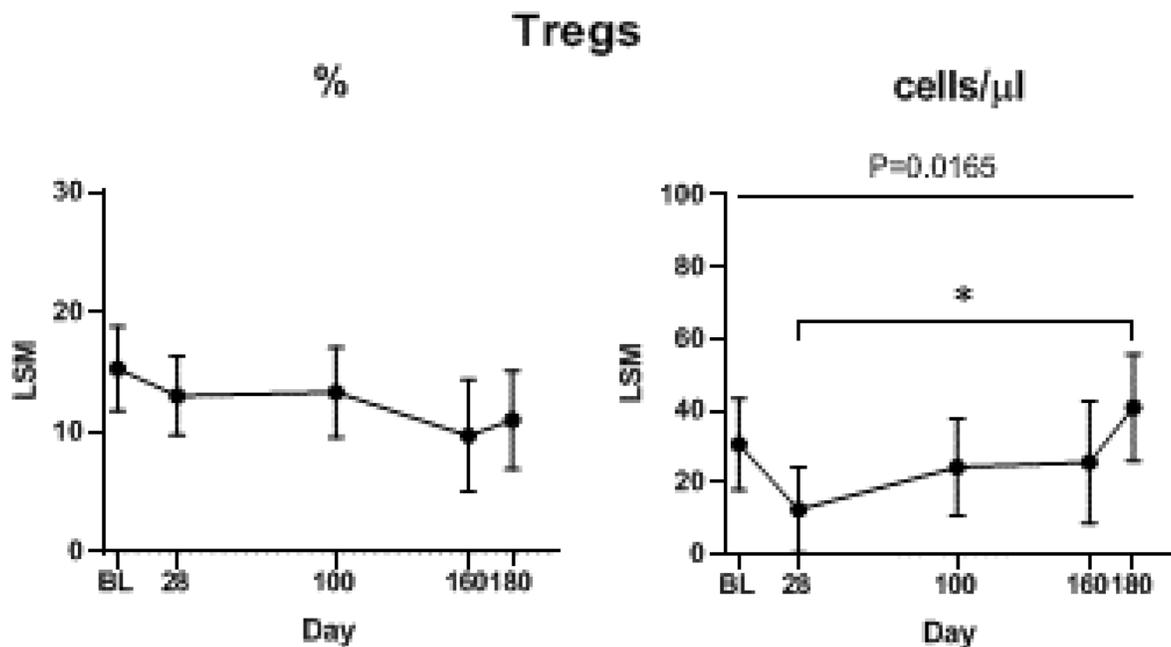
Figure 3: Longitudinal Sub-group Analysis of Activated T cells Based on Day 28 Response



OR-overall responder; NR-non responder, CR-complete response, PR-partial response

Source: Applicant's Figure 20 from MSB-GVHD001/002 Biomarkers Final Analysis Report

Figure 4: CD4+CD25+CD127-/Lo regulatory T cell levels at baseline and at days 28, 100, 160 and 180 days following first MSC infusion in all subjects.



Source: Applicant's Figure 4 from MSB-GVHD001/002 Biomarkers Final Analysis Report

Reviewer Comments:

1. Pharmacodynamics:

During the review of this BLA submission, we requested the following information requests supporting MOA and PD characterization:

- Longitudinal quantitative description of all biomarkers relevant for defining mechanism of action (MOA) and pharmacodynamic (PD) of remestemcel-L.
- Descriptive statistical analysis of biomarker levels at baseline versus levels following post-drug administration (e.g. Day 28 and Day 100).
- Correlation analysis of biomarker levels and clinical outcomes including sub-group analysis of biomarker levels in patients (e.g. demographics and clinical response as complete/partial and non-responder).

The Applicant addressed the clinical pharmacology comments by submitting biomarker analysis report. At Day 28 following treatment with remestemcel-L, the levels of TNFR1 was not changed but levels of ST2 declined by 17% from the baseline values. Treatment with

remestemcel-L reduced TNFR1 and ST2 levels by 76% and 72 % at day 180 as compared to the baseline levels, respectively. Both TNFR1 and ST2 have been shown to be released by activated T lymphocytes and their steady decrease upon treatment with remestemcel-L demonstrate PD activity resulting in reduced inflammatory state. Further, the circulating levels of CD3+CD4+CD25+HLA-DR+ T cells, which represent fully activated T cells, were reduced by 54% at Day 180 following treatment with remestemcel-L as compared to the baseline values. Overall the reduction in levels of secreted factors (TNFR1 and ST2) and activated T cells provide clinical evidence of the immunomodulatory pharmacodynamic effects of remestemcel-L in pediatric subjects with steroid-refractory aGvHD. These results are in part further justified by *in vitro* PD characterization. However, the sample size was too small to fully understand how the levels vary between responder and non-responders. Future studies are required to further characterize the PD biomarkers to employ for therapeutic dosing decision or predicting efficacy.

2. Pharmacokinetics:

Since remestemcel-L is administered via IV route clearance and distribution are the most relevant PK parameters. The Applicant indicated major methodological hurdle as current methods for assessing the distribution of cell-based therapies require either modification of cells to introduce a label and/or *in vivo* tissue sampling that is practically limited in humans. The Applicant provided data on the distribution characteristics of ceMSC based on nonclinical animal studies. We conducted preliminary qualitative matching analysis of the Applicant submitted nonclinical versus published exploratory clinical studies on the biodistribution of MSC following IV infusion (Table 5). The qualitative matching analysis indicate rapid clearance from the circulation and potential distribution to mechanistically relevant organ (e.g. GIT, lymph nodes). Several intrinsic and extrinsic factors are expected to influence the clearance and distribution of MSC, and it is difficult to extrapolate the nonclinical results to human with the existing data. Future studies are needed to address the knowledge gaps in elucidating the *in vivo* fate of infused MSC in human to better characterize the mechanism of action, efficacy and safety. The results of the available published exploratory biodistribution clinical studies indicate the feasibility of developing and optimizing methods (e.g. PCR or whole-body imaging) for human PK assessments.

Table 5: Qualitative Matching of None-human Primate Model versus Human Studies on Biodistribution of MSC following Intravenous Infusion

	Intravenous MSC dose (x 10 ⁶ cells/kg)	Sampling time after MSC infusion	MSC Quantification Method	Tissue with detectable level of MSC
Applicant non-human primate model; TBI & HCT	18.5	9 months	PCR	Bone marrow, GIT, Liver, Kidney, Spleen, Lymph nodes, Lung, Skin
Applicant pivotal clinical trial; aGvHD	2 and twice per week	ND	ND	ND
Human clinical (Ringden et al. 2006); aGvHD	1.3	9 days	PCR	GIT, Lymph nodes
Human clinical (von Bahr et al. 2012); aGvHD	1.9	7 days	PCR	Bone marrow, GIT, Liver, Kidney, Spleen, Lymph nodes, Lung
Human clinical (von Bahr et al. 2012); aGvHD	0.7 and 1.4	24 days	PCR	GIT, Lymph nodes
Human clinical (Gholamrezanezhad et al. 2011); liver cirrhosis	3.5-6	Serial imaging at 2, 4, 6, 24 hours and at 2, 7 and 10 days	Planar whole-body acquisitions	Lung (~ 33.5% at 2hour vs ~2% at day 10), Spleen (~30-40% at day 10), Liver (~13-17% at day 10)

TBI-total body irradiation; HST- hematopoietic cell transplantation; MSC-mesenchymal stem cells; ND-not determined

Source: Prepared by Reviewer