

I concur with this review memo. I Wu 8/24/20

**FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch**

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DATE REVIEW COMPLETED:	24-AUG-2020
PRODUCT:	RYONCIL™ (remestemcel-L)
APPLICANT:	Mesoblast, Inc.
PROPOSED INDICATION:	For the treatment of steroid-refractory acute graft versus host disease (SR-aGvHD) in pediatric patients.
PHARM/TOX REVIEWER:	Danielle Brooks, PhD
PHARM/TOX TEAM LEADER:	Allen Wensky, PhD
PHARM/TOX BRANCH CHIEF:	Iwen Wu, PhD
PRODUCT (CMC) REVIEWERS:	Matthew Klinker, PhD (Chair) Alyssa Kitchel, PhD Elizabeth Lessey-Morillon, PhD Bao Nguyen, PhD Steven Bauer, PhD Heba Degheidy, PhD
CLINICAL REVIEWERS:	Kristin Baird, MD
PROJECT MANAGER:	Adriane Fisher
DIVISION DIRECTOR:	Tejashri Purohit-Sheth, MD, FACAAI, CQIA
OFFICE DIRECTOR:	Wilson Bryan, MD

EXECUTIVE SUMMARY:

Remestemcel-L is a cryopreserved cell suspension of ex-vivo culture-expanded adult human mesenchymal stromal cells (ceMSC) derived from allogeneic bone marrow and is intended for intravenous (IV) administration in pediatric patients with steroid-refractory acute graft versus host disease.

Nonclinical studies were conducted using human bone marrow-derived ceMSC and surrogate animal-derived ceMSC produced using an early manufacturing process. In vitro studies characterizing the effects of bone marrow-derived ceMSC in co-culture with allogeneic immune cells demonstrated that ceMSC suppressed alloreactive T cell proliferation and can modify the cytokine secretion profile of various immune cells in culture.

A GLP safety pharmacology study evaluating pulmonary function in (b) (4) rats was conducted with single IV administration of (b) (4) rat-derived ceMSC at dose levels of 8×10^6 , 16×10^6 , and 25×10^6 cells/kg via femoral vein catheter at rates of 0.02 - 1.6 mL/min. A high mortality rate was observed at dose levels of 16×10^6 cells/kg and 25×10^6 cells/kg at all infusion rates tested and occurred shortly after cell administration. The specific cause of death was undetermined based on the lack of histopathology findings, but possible cell aggregation was noted. Increases in respiratory rates and decreases in tidal volume were observed in surviving animals at all dose levels, but changes were considered within normal values. A cardiac safety pharmacology study in pigs was conducted with single IV infusion of 1×10^6 and 10×10^6 allogeneic pig ceMSC/kg using a (b) (4) catheter at a constant rate of 2-3 mL/min. No product-related effects on cardiac function were observed.

A cell distribution study was performed with administration of 10×10^6 (b) (4) labeled (b) (4) rat ceMSC/kg in (b) (4) rats. Cells were initially detected in the lung within the first hour following cell administration, followed by distribution to the liver, kidney and spleen within the first 24 hours, and continued to be detected at 240 hours following cell administration.

A GLP toxicity study in (b) (4) rats evaluated 3×10^6 , 8×10^6 , 15×10^6 , 20×10^6 and 37.5×10^6 (b) (4) rat ceMSC/kg in a dosing volume of 5 mL/kg by single bolus IV administration over 30 seconds. Animals were sacrificed on Day 7 or Day 14 post-administration. Dose levels of 15×10^6 cells/kg and higher resulted in increased mortality shortly after dosing, associated with shallow breathing, impaired righting reflex, and pale discolored skin. Animals administered 8×10^6 cells/kg had similar adverse clinical reactions including impaired righting reflex and red urine/discharge following dose administration but recovered within several days and survived to scheduled necropsy. Clinical pathology findings included decreased hemoglobin and red cell mass, and increased bilirubin, AST and ALT. The cause of death was undetermined for most animals that died prematurely, however low cell viability and possible aggregation were noted as potential contributing factors.

A second GLP single dose toxicity study in (b) (4) rats evaluated dose levels ranging from 10×10^6 to 75×10^6 cells/kg administered intravenously via femoral or jugular catheter at a controlled rate of 0.8 or 1.6 mL/min and dosing volume of 5 mL/kg. Animals were sacrificed on Day 7 or 14 post-administration. There were no deaths related to administration of ceMSC. Animals administered 65×10^6 or 75×10^6 cells/kg had adverse clinical signs including difficulty breathing and red urine/discharge. Clinical pathology and postmortem analysis were performed for groups administered 10×10^6 , 40×10^6 , and 65×10^6 cells/kg. There was a mild increase in hematuria in males administered 65×10^6 cells/kg at Day 7 that was not observed at Day 14. Increased spleen weight was observed at 40×10^6 and 65×10^6 cells/kg and increased adrenal gland

weight was observed at 65×10^6 cells/kg for male animals, however there were no histologic correlates.

In a GLP repeat dose toxicity study, (b) (4) rats received 2×10^6 , 10×10^6 or 20×10^6 (b) (4) rat ceMSC/kg administered in a 5mL/kg dosing volume by tail vein injection over a two-minute period twice per week for 4 weeks and weekly thereafter for a total of 13 dose administrations. Animals were sacrificed at Day 30 for interim analysis and Day 90 for terminal analysis. Several unscheduled deaths were observed in the groups administered 10×10^6 and 20×10^6 cells/kg on repeat administration days beginning on Day 15 (after the fifth administration). Clinical findings for the two higher dose level groups included red urine/discharge, salivation, hunched posture, decreased activity, pale skin, impaired righting reflex, and prostration. Increased spleen and adrenal gland weights were observed in ceMSC groups at the interim time point only and had no histologic correlates. Microscopic findings in the lungs included minimal to mild pulmonary thrombi and cellular emboli in all ceMSC-administered groups at the interim analysis, and minimal to moderate chronic inflammation at the terminal analysis. Additionally, minimal to moderate spermatocyte/spermatid degeneration was observed in males in the 10×10^6 and 20×10^6 cells/kg groups at the interim time point only. At the injection site, minimal to mild hemorrhage, mononuclear cell infiltrate, and thrombus were also noted at both the interim and terminal sacrifice time points.

A six-month GLP study in baboons was performed using (b) (4) labeled allogeneic baboon ceMSC to evaluate the safety and immunogenicity following IV administration of 5×10^6 cells/kg on Day 1 (b) (4). The IV administration was performed by injection into the saphenous vein with a dosing volume of 20 mL (b) (4). No toxicities were observed in the study and there were no significant effects of ceMSC on immune cell subsets. Low level alloantibody formation in ceMSC-administered animals was observed.

In a tumorigenicity study in athymic mice, (b) (4) administration of human ceMSC from five separate donors was evaluated at dose levels up to 50×10^6 cells/kg. There was no evidence of neoplastic tissue formation attributable to ceMSC over the six-week duration of the study.

Genotoxicity, carcinogenicity and reproductive and developmental toxicity studies were not performed for remestemcel-L. These studies are not warranted based on the product characteristics and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

Based on review of the nonclinical data in this BLA submission (STN #125706), there are no nonclinical deficiencies identified in the pharmacology/toxicology studies. There are no requests for further nonclinical testing of remestemcel-L at this time. The nonclinical data provided in this BLA submission support the approval of the licensure application.

Formulation and Chemistry:

Remestemcel-L is a cryopreserved cell suspension of ex-vivo culture-expanded adult human mesenchymal stromal cells (ceMSC) derived from allogeneic bone marrow formulated in 3.8 mL of cryo-medium composed of Plasma-Lyte A, dimethyl sulfoxide (DMSO), and human serum albumin. Remestemcel-L is administered intravenously. Dosing is based on the patient's body weight. The recommended dose level of remestemcel-L is 2×10^6 ceMSC/kg.

Abbreviations

(b) (4)	rat
aGVHD	Acute graft versus host disease
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
AST	Aspartate aminotransferase
ceMSC	Culture-expanded mesenchymal stromal cells
CK	Creatine kinase
cMSC	Canine mesenchymal stromal cells
CR	Complete response
(b) (4)	
(b) (4)	
(b) (4)	
DMSO	Dimethyl sulfoxide
ECG	Electrocardiogram
eGFP	Green fluorescent protein
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescent activated cell sorting
G-CSF	Granulocyte-colony stimulating factor
G-PBSC	G-CSF-mobilized peripheral blood stem cells
GLP	Good laboratory practice
Hb	Hemoglobin
HSA	Human serum albumin
HSC	Hematopoietic stem cell
IL	Interleukin
(b) (4)	
INF γ	Interferon gamma
IV	Intravenous
(b) (4)	
MHC	Major histocompatibility class
MSC	Mesenchymal stem cells
NOAEL	No observed adverse effect level
PBMC	Peripheral blood stem cell
(b) (4)	
PGE2	Prostaglandin E2

pMSC	Porcine mesenchymal stem cells
(b) (4)	
rMSC	Rat mesenchymal stem cells
ROA	Route of administration
TBI	Total body irradiation
TnI	Troponin I
TNF α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Related File(s)

IND #7939: Mesoblast, Inc.; Allogeneic Mesenchymal Stem Cells, Cultured ex vivo, (b) (4)
 Allogeneic Bone Marrow (Remestemcel-L,
 Mesoblast, Ltd.); ACTIVE

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INTRODUCTION

Acute graft versus host disease (aGVHD) is a syndrome that occurs following allogeneic hematopoietic stem cell (HSC) transplantation due to human leukocyte antigen (HLA) mismatch between the HSC donor and the recipient, resulting in an immune-mediated attack on the host tissue by the donor cells. It is estimated that approximately 50% of patients receiving allogeneic HSC transplant present with aGVHD [1], and of the those who develop aGVHD each year, 20% are pediatric [2]. Acute GVHD typically occurs within 100 days of HSC transplant, whereas chronic GVHD generally manifests later (>100 days). Acute GVHD is associated with morbidity and mortality, and is characterized by immune activation with systemic inflammation, cytotoxicity and tissue damage. Tissue damage leads to increased secretion of proinflammatory cytokines leading to further inflammation and end organ damage. The standard of care for aGVHD is immunosuppression with systemic corticosteroids, with response rates ranging between 40-50% [3]. For patients who are steroid refractory, the treatment options typically involve off-label use of immunosuppressant drugs.

Remestemcel-L is comprised of expanded allogeneic human bone marrow-derived mesenchymal stromal cells. These cells are population of plastic-adherent multipotent cells that can be differentiated toward osteogenic, adipogenic, and chondrogenic lineages in vitro. The recommended dose level of remestemcel-L in pediatric patients with steroid refractory aGVHD (SR-aGVHD) is 2×10^6 ceMSC/kg body weight. Patients will be administered remestemcel-L twice per week for 4 consecutive weeks with infusions administered at least 3 days apart. The product may be administered once a week for an additional 4 weeks if the patient's symptoms have not completely resolved. If symptoms recur after a complete response (CR), the treatment protocol may be repeated.

NONCLINICAL STUDIES

The nonclinical program utilized multiple surrogate animal-derived ceMSC manufactured similarly to the clinical product. This approach was taken to model administration of allogeneic ceMSCs in humans and evaluate the interactions between the cell product and recipient immune system. Each animal-derived ceMSC was manufactured, formulated and characterized using the same methods for human remestemcel-L as feasible. However, some modifications were necessary such as smaller scale manufacturing to accommodate species-specific culture requirements, and the final formulation was adjusted for DMSO content and use of species-specific serum. Characterization included immunophenotyping, trilineage differentiation assays, cell size and immunomodulatory bioactivity. However, the extent of characterization for each product was sometimes constrained by the availability and/or cross-reactivity of reagents and sub-optimal assay conditions for the species to be tested. Table 1 provides an overview of ceMSC from the different species used in the nonclinical studies compared to remestemcel-L.

Table 1. Summary of Preclinical ceMSC Characteristics

	Remestemcel-L	Rat	Dog	Baboon	Pig
Source	Bone marrow aspirate, single donor per donor cell bank	Bone marrow aspirate from (b) (4) strain rats, pooled from multiple donors	Bone marrow aspirate from humeral condyle	Bone marrow aspirate from iliac crest	Bone marrow aspirate from iliac crest
Cryomedium	Plasma-Lyte A (b) (4), DMSO (10%), HSA (5%)	Plasma-Lyte A (b) (4), DMSO (10%), rat serum (b) (4)	N/A – Used fresh from culture	Plasma-Lyte A (b) (4), DMSO (10%), autologous baboon serum or HSA (5%)	Plasma-Lyte A (b) (4), DMSO (b) (4), HSA (b) (4)
Cell Surface Phenotype	CD105 ⁺ , CD166 ⁺ , CD45 ⁻	CD73 ⁺ , CD90 ⁺ , CD45 ⁻	No data	CD105 ⁺ (SH-2), CD73 ⁺ (SH-4), SH-3 ⁺ , CD34 ⁺ , CD45 ⁻	CD44 ⁺ , CD29 ⁺ , CD45 ⁻
Multipotentiality	Osteogenic, adipogenic, chondrogenic	Osteogenic, adipogenic, chondrogenic	Osteogenic	Osteogenic, adipogenic	Osteogenic, adipogenic, chondrogenic
Immunomodulatory Activity	Inhibit activated T cell IL-2R α expression	Inhibit IL-2R α expression on Con-A stimulated splenocytes	No data	No data	No data
Release Testing	Sterility, mycoplasma, endotoxin, viral testing, viability	Sterility, mycoplasma, endotoxin, viral testing, viability	No data	No data	No data

Reviewer Comment:

- ❖ *Based on data provided by the applicant, the animal ceMSC are similar to remestemcel-L in phenotype and in vitro activity and are acceptable for use in the respective animal species. It should also be noted that although remestemcel-L is referred to by the applicant as mesenchymal stromal cells, the nonclinical studies refer to the test articles as mesenchymal stem cells.*

PHARMACOLOGY STUDIES**Summary List of Pharmacology Studies**

The following pharmacology studies were conducted to support the rationale for the administration of remestemcel-L for the treatment of aGVHD in pediatric subjects.

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance and suppression	Klyushnenkova et al., 2005 [4]
2	Human mesenchymal stem cells modulate allogeneic immune cell responses	Aggarwal et al., 2005 [5]

*In Vivo Studies****In Vivo Studies in Healthy Animals***

Study Number	Study Title / Publication Citation	Report Number
3	Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo	Bartholomew et al., 2002 [6]

Note: Study Nos. 1-2 are briefly summarized in this review memo under ‘Overview of Pharmacology Studies.’ Study No. 3 is not summarized in this review memo because the data is not directly applicable to the intended clinical use of remestemcel-L for aGVHD.

Overview of Pharmacology Studies*Overview of In Vitro Studies*

In vitro studies were performed to evaluate ceMSC immunophenotype, effects on allogeneic immune cell subsets, and mechanisms of immunomodulatory activities. The ceMSC used in these studies were obtained from multiple healthy human donors.

Key findings from Study 1 and Study 2 demonstrated that:

- ceMSC are positive for major histocompatibility complex (MHC) class I, weakly positive for MHC class II and negative for co-stimulatory molecules CD80, CD86 and CD40.
- ceMSC do not elicit a proliferative response from allogeneic T cells.
- ceMSC suppress mitogen- or alloantigen-induced T cell proliferation in a dose-dependent manner.
- The suppressive effects of ceMSC are mediated through the secretion of soluble factors including IL-6, IL-8, VEGF and PGE2.
- In ceMSC and immune cell co-cultures, levels of INF γ and IL-10 were increased while TNF α levels were decreased compared to control cultures of peripheral blood mononuclear cells.

SAFETY PHARMACOLOGY STUDIES

Summary List of Safety Pharmacology Studies

Distribution studies performed in rats demonstrated rat ceMSC accumulation in the lung and heart. Therefore, the following safety pharmacology studies were conducted to evaluate the effects of IV administration of rat ceMSC on pulmonary function in rats and pig ceMSC on pulmonary and cardiac function in pigs.

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
4	Pulmonary effects of (b) (4) strain rat ceMSC (rMSC) administered intravenously through the femoral vein in rats	R-040-05
5	Safety evaluation of IV MSC infusion in swine	R-S-086-02-R
6	Safety of allogeneic MSC (b) (4) in normal swine myocardium	R-S-080-02

Note: Study Nos. 4-5 are briefly summarized in this review memo under ‘Overview of Safety Pharmacology Studies.’ Study No. 6 is not summarized in this review memo because it contains data obtained using a different route of administration than the intended clinical route of administration for remestemcel-L.

Overview of Safety Pharmacology Studies

Overview of In Vivo Studies

Study #4

Report Number		p-040-05
Date Report Signed		27 December 2005
Title		Pulmonary effects of (b) (4) rMSC administered intravenously through the femoral vein in rats
GLP Status		Yes Deviation: Documentation of the purity and composition for each lot of vehicle and test article were not provided.
Testing Facility		(b) (4)
Objective(s)		To evaluate the potential effects of (b) (4) rat strain bone marrow-derived mesenchymal stem cells on pulmonary function, the potential change in pulmonary function as a function of the total number of stem cells infused (number of cells/kg), and to assess the potential change in pulmonary function as a function of the rate of cell infusion (mL/min).
Study Animals	Strain/Breed	(b) (4)
	Species	Rat
	Age	8.5 weeks
	Body Weight	175 – 196 g
	#/sex/group	3-5 male rats/group
Total #		56
Test Article(s)		(b) (4) strain rat ceMSC
Control Article(s)		Plasma-Lyte A-(b) (4)/DMSO-10%/Rat Serum (b) (4)

Route of Administration	Intravenous via femoral catheter			
Description of the Administration Procedure	The test article or vehicle was administered to all groups as a single dose through the femoral vein by intravenous catheter, at a dose volume 5 mL/kg. A syringe drive pump was used for test article administration for Groups 1, 3, 5, and 7. Groups 2, 4, and 6 were administered the test article via slow bolus injection. An extension set was connected to the syringe and to the swivel on the infusion jacket. Prior to jacketing the animal, the entire system was primed with saline. After the system was primed, the animal was connected to the jacket and placed in a plethysmograph chamber. The chamber was closed and the entire line was flushed with saline or Plasma-Lyte A solution to ensure patency prior to dosing. Once all animals were acclimated to the chamber for at least 30 minutes, the 1-hour predose monitoring period began. At the end of the predose monitoring period, a syringe with the calculated number of prepared cells was placed in a syringe or a syringe drive pump. The test article was administered until the syringe was empty. When the syringe with the prepared cells was empty, it was removed and a syringe with 0.6 mL of saline or 0.6 mL of Plasma-Lyte A was used to flush the system.			
Study Groups and Dose Levels	Group Number	Dose Level (cells/kg)	Infusion Rate (mL/min)	No. of Animals
	1	0	0.16	8
	2	8x10 ⁶	0.16	8
	3a	25x10 ⁶	0.16	4
	3b	16x10 ⁶	0.16	4
	4a	0	1.6	3
	4b	0	0.8	5
	5	8x10 ⁶	0.8	8
	6a	25x10 ⁶	1.6	5
	6b	16x10 ⁶	0.8	3
	7	25x10 ⁶	0.02	8
Dosing Regimen	Single administration			
Randomization	Yes			
Description of Masking	None			
Scheduled Sacrifice Time Points	4 hours post-test article or vehicle administration			

Key Evaluations and Assessments:

- Cageside observations, including morbidity, mortality, injury and availability of food were performed twice daily throughout the duration of the study.
- Detailed clinical observations were performed prior to dosing and at approximately 1- and 4-hours post-dose.
- Monitoring of pulmonary function began 1 hour prior to dosing using a plethysmograph chamber. Monitoring was continued for 4 hours following the start of test article or vehicle infusion.
- Microscopic evaluation of fixed hematoxylin and eosin stained paraffin sections was performed on tissues harvested at necropsy.

Key Results:

Mortality: There were a total of 11 unscheduled deaths following test article administration: 3 animals in Group 6b, 2 animals in Group 3b, 1 animal in Group 7, 1 animal in Group 3a, and 4 animals in Group 6a. All deaths occurred during or shortly following the saline flushing of the catheter lines. Per the study report, no visible signs of cell aggregation were noted during

delivery of the cell lines to the plethysmograph chamber catheter systems, however it is noted that possible precipitation may have occurred within the catheter system, either at the swivel located at the top of the chamber or in the catheter length located inside the animal. According to the study report, given the total length of the catheter involved in the dosing procedure, the bulk of the cell line delivery occurred during the saline flush of the catheter lines and it is possible that cell clumping may have occurred within the drug ex vivo delivery system or in vivo during the interval of cell delivery and completion of the saline flush, contributing to the mortalities observed in the study.

Clinical findings: For the unscheduled deaths, clinical findings included arched postures, open gaping mouths, seizures and collapse. No other clinical findings were observed.

Pulmonary evaluations:

Respiratory rate: No physiologically-relevant respiratory depression was observed at the test article dose levels administered. Respiratory rates increased in all animals immediately following administration. Larger increases were observed in animals administered rat ceMSC compared to control animals that did not receive cells. Rates of respiration were affected by both flow rate as well as cell concentration. The flow rate of 0.16 mL/min produced increased respirations from approximately 175 breaths-per-minute (control animal values) to approximately 225 breaths-per-minute following delivery of 8×10^6 cells/kg and approximately 275 breaths-per-minute following delivery of 16×10^6 cells/kg. In animals administered 8×10^6 cells/kg at a follow rate of 0.8 mL/min, breaths-per-minutes increase to 250 (compared to 225 at 0.16 mL/min). Similar changes were observed for all cell dose levels. When compared to published data of respiratory rates for rats, all values were within the normal range (upper limit of 320 breaths per minute).

Tidal volume: Control animals demonstrated group mean tidal volumes within the normal range of values. A decrease in tidal volume was observed following test article administration. Similar results were observed at 8 and 16×10^6 cells/kg at all administration rates. At 25×10^6 cells/kg, further decreases were observed with increasing infusion rates. However, all changes remained within the normal range of values.

Minute Volume: No clear dose-level or infusion rate changes in minute volume were observed.

Microscopy: There were no test article related microscopic findings.

Reviewer Comments:

- ❖ *Only male animals were used in this study. There is no rationale provided as to why female animals were not included.*
- ❖ *There was a high rate of unexpected animal deaths in the study that could not be definitively linked to dose levels administered or the rate of infusion. However, the deaths indicate that the presence of cells was a key contributing factor (i.e., no deaths reported for Groups 1, 4a, and 4b).*
- ❖ *There were no significant changes in histopathological findings related to the test article, including in animals that died early/suddenly before the 4-hour timepoint.*

- ❖ *The pathology report suggests that the lack of postmortem findings supports the sponsor's proposed cause of death as cell clumping prior to administration.*
- ❖ *Respiratory rates and tidal volumes were affected by administration of the test article, but all changes were within normal values for the strain of rats used.*
- ❖ *The dose levels administered in this study are 4- to 12.5-fold higher than the clinical dose level*

Study #5

Report Number		R-S-086-02-R		
Date Report Signed		26 August 2003		
Title		Safety evaluation of IV MSC infusion in swine		
GLP Status		No		
Testing Facility		(b) (4)		
Objective(s)		To evaluate the safety of intravenous ceMSC administration in healthy domestic swine. Both acute infusion toxicity, and long-term histologic safety were examined.		
Study Animals	Strain/Breed	(b) (4) swine		
	Species	Pig		
	Age	5 – 5.75 months		
	Body Weight	32 – 53 kg		
	#/sex/group	3 females/group		
Total #		18		
Test Article(s)		Allogeneic porcine culture expanded mesenchymal stem cells (pig ceMSC)		
Control Article(s)		(b) (4) Plasma-Lyte A, (b) (4) DMSO, (b) (4) porcine serum		
Route of Administration		Intravenous injection via central venous line		
Description of the Administration Procedure		Test article and vehicle solution were administered intravenously via the (b) (4) catheter at a constant rate of 2-3 mL/minute. Dosing of an animal took 10 to 30 minutes, depending on the dose level. Animals in Groups A and B received 60 mL total volume. Animals in Group C received 30 mL total volume. Dosing occurred in three swine per day.		
Study Groups and Dose Levels		Group	Dose Level (cells/kg)	Sacrifice Time Point
		A	10x10 ⁶	6 weeks
		A	10x10 ⁶	24 weeks
		B	0	6 weeks
		B	0	24 weeks
		C	1x10 ⁶	6 weeks
Dosing Regimen		Single administration		
Randomization		Yes		
Description of Masking		Throughout the study the three groups were referred to only as Groups “A”, “B” and “C”. Only the operator recording the cell thawing and release criteria information knew the treatment for each letter designation. With the exception of the principal investigator, who performed the randomization, all individuals associated with data acquisition and analysis were blinded to the treatment any individual animal received.		

Scheduled Sacrifice Time Points	6 weeks and 24 weeks post-infusion
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Key Evaluations and Assessments:

- Animal body weight, appetite and clinical observations were noted throughout the study (intervals not specified).
- Electrocardiograms (ECGs) were obtained continuously from baseline through 1-hour posttest article or vehicle administration. Follow-up recordings were made at 24 hours and 2, 6, 12, and 24 weeks.
- Hemoglobin (Hb) saturation readings were continuously monitored from baseline through 1-hour posttest article or vehicle administration and 24 hours post administration.
- Heart rate and respiration rate measurements were made at baseline through 1-hour post-test article or vehicle administration.
- Hematology and clinical chemistry samples were obtained at baseline, 24 hours post test article or vehicle administration and 1, 2, 6, 12 and 24 weeks.
- Cardiac enzymes, Troponin 1 (Tn1) and creatine phosphokinase MB fraction (CK-MB) were determined from serum samples obtained at baseline and 24 hours post test article or vehicle administration.
- Necropsies were performed at the scheduled sacrifice date. Macroscopic observations were recorded and tissues/organs were collected for microscopic evaluation.

Key Results:

Mortality: Two animals had to be euthanized prior to their scheduled sacrifice date. One animal in Group A on day 52 and one animal in Group C on day 73. The cause of death for both animals was determined to be endocarditis secondary to catheter associated infection.

Clinical observations: No test-article related observations were noted.

ECG: A small number of animals experienced tachycardia following test article or vehicle administration. There was no correlation to the test article or test article dose level as it was observed in all study groups.

Hemodynamic parameters: There were no differences observed between test article and control groups for any parameter at any time point.

Clinical pathology: Elevations in creatine kinase (CK) were observed at baseline, 24 hours, week 6, week 12 and week 24 which were attributed to skeletal muscle injury as a result of the restraint procedure needed to collect samples. Findings were present in all groups and were not considered to be related to test article administration.

Pathology: No findings related to test article administration.

Reviewer Comments:

- ❖ *The administration of vehicle, low or high dose pig ceMSC was generally well tolerated by the animals in this study.*
- ❖ *Electrocardiographic studies showed very few occurrences of arrhythmias following infusion which did not appear to be related to the test article as it occurred in all test*

groups, including the control. Therefore, risk of cardiac events following administration of the test article at the clinical dose level appears low.

PHARMACOKINETIC STUDIES (Cell Distribution)

Summary List of Pharmacokinetics Studies

The following cell distribution studies were conducted.

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
Primary Studies		
7	Pilot biodistribution study of (b) (4) labeled allogeneic mesenchymal stem cells following intravenous delivery in rats	R-055-05
Supporting Studies		
8	Safety and efficacy of dose escalation of cMSC infused into lethally-irradiated canines receiving bone marrow hematopoietic support	TRD-001
9	Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome	Chapel et al., 2003 [7]
10	Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion	Devine et al., 2001 [8]
11	Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates	Devine et al., 2003 [9]
12	Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep	Liechty et al., 2000 [10]
13	Allogeneic MSC "homing" to myocardial infarction following intravenous infusion	R-S-081-02
14	A study of intravenous mesenchymal stem cell treatment in porcine model of myocardial ischemia/reperfusion injury	R-089-06
15	Mesenchymal stem cells as vehicles for gene delivery	Mosca et al., 2000[11]

Note: Study No. 7 is briefly summarized in this review memo under 'Overview of Pharmacokinetic Studies.' Study Nos. 8-15 are not summarized in this review memo because they contain data not directly applicable to the intended clinical use of remestemcel-L for aGVHD. Study Nos. 8-11 evaluated animal-derived ceMSC in models of total body irradiation (TBI) followed by autologous bone marrow transplant, however several study design parameters limit the relevance of this data, including not using a model of GVHD, use of autologous ceMSC, and timing of ceMSC administration prior to or concurrently with bone marrow transplant. Study Nos. 12-15 utilize in utero transplantation, models of myocardial injury, or gene delivery.

Overview of Pharmacokinetic Studies

Study #7

Report Number	R-055-05
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Date Report Signed		14 March 2006
Title		Pilot biodistribution study of (b) (4) labeled allogeneic mesenchymal stem cells following intravenous delivery in rats
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To provide preliminary data to characterize the biodistribution of rat ceMSC labeled with (b) (4) following IV infusion into rats.
Study Animals	Strain/Breed	(b) (4)
	Species	Rat
	Age	8 weeks
	Body Weight	178-190 g
	#/sex/group	12 males/group
Total #		24
Test Article(s)		(b) (4) labeled (b) (4) strain rat ceMSC
Control Article(s)		(b) (4) labeled rat ceMSC
Route of Administration		IV
Description of the Administration Procedure		The test and control articles were administered once on Day 1 via a femoral catheter by slow bolus injection over 21 to 25 seconds. Following each dose, approximately 0.5 mL of Sodium Chloride for Injection, USP, was administered as a rinse.
Study Groups and Dose Levels		Group 1 received the test article: 2.5×10^6 cells/mL at a dose volume of 4 mL/kg per animal of (b) (4) labeled rat ceMSC Group 2 received the control article: 2.5×10^6 cells/mL at a dose volume of 4 mL/kg per animal of (b) (4) labeled rat ceMSC
Dosing Regimen		Single infusion
Randomization		No
Description of Masking		N/A
Scheduled Sacrifice Time Points		Four rats from each group were sacrificed at 1, 24 or 240 hours post infusion.

Key Evaluations and Assessments:

- Observations for mortality, morbidity, injury and the availability of food and water were conducted twice daily for all animals.
- Detailed clinical examinations were performed pre-test, immediately post-administration and prior to scheduled necropsy.
- Tissues and organs were collected from each group as follows:
 - 1 hour: blood, lungs, and liver
 - 24 hours: blood, brain, heart, lungs, liver, spleen, kidneys, testes, lymph nodes, bone marrow, carcass, urine, and feces
 - 240 hours: blood, brain, heart, lungs, liver, spleen, kidneys, testes, lymph nodes, bone marrow, carcass, urine, and feces

Samples obtained from Group 1 animals were processed for (b) (4) content analysis. Samples from Group 2 animals were processed for histology.

Key Results:

There were no unscheduled deaths in this study; all animals survived to their scheduled necropsy date.

Four animals (one in Group 1, and three in Group 2) experienced adverse clinical reactions within 5 minutes of dose administration. The reactions included cold skin, decreased activity, shallow breathing, and loss of righting reflex. By one-hour post dose, three animals had red material around their noses, and one still had decreased activity and cold skin. One animal in Group 2 had red discharge from its penis. There were no abnormalities detected at twenty-four hours post-dose and thereafter.

Within the first hour of IV administration, (b) (4)-labelled rat allogeneic ceMSC were cleared from the circulation and were detected in the lungs, cell distribution to the liver, kidneys and spleen was observed by 24 hours, and cells continued to be detected through 240 hours post-administration (**Figure 1**).

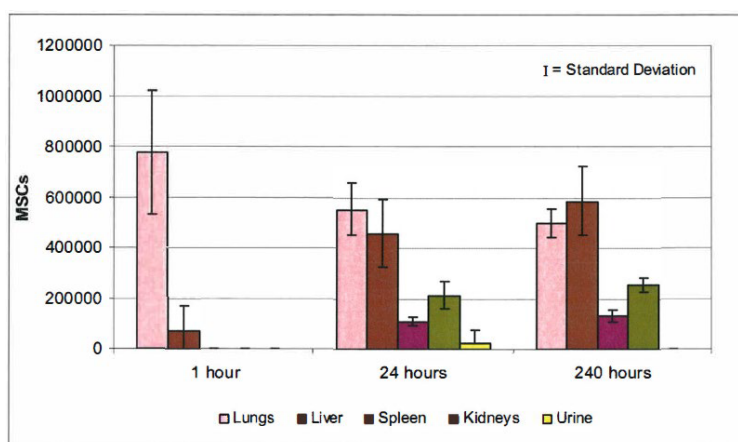


Figure 1. (b) (4) labeled rat ceMSC distribution by organ at different timepoints. Y-axis represents the number of ceMSC detected. X-axis represents the time point following ceMSC administration. The error bars depict the standard deviation of the valued obtained for the four organs analyzed at each time point.

Source: Module 4.2.2.3, Preclinical study report R-005-05, pg 5/101.

Reviewer Comments:

- ❖ *Clinical observations were seen immediately following administration, but all animals appeared to recover within 24 hours.*
- ❖ *As expected with IV administration, the cells were mainly found within the lungs and liver, and (b) (4) was still detected at 10 days post administration, suggesting the cells are not immediately cleared from the system.*
- ❖ *Per the study report, distribution of cells to the lungs and liver was also observed by fluorescence microscopy of (b) (4) labelled cells and was not observed in the spleen or kidneys. However, only a single representative image for each organ was provided.*

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of remestemcel-L following administration in various animal species.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
Primary Studies		
16	Acute toxicity study of rat bone marrow mesenchymal cells in (b) (4) rats	P-024-04
17	Acute toxicity study of (b) (4) rat bone marrow-derived mesenchymal cells in (b) (4) rats	P-054-05
18	13-week subchronic study of (b) (4) rat strain mesenchymal stem cells administered via intravenous infusion in (b) (4) rats	P-115-06
19	Six-month safety and immunology study in baboons of allogeneic baboon mesenchymal stem cells labeled with (b) (4)	(b) (4) No. 366
Supporting Studies		
20	Preliminary intravenous dose range finding study of human mesenchymal cells in (b) (4) rats	P-014-04
21	Preliminary intravenous dose range finding study of human mesenchymal cells in jugular catheterized rats	R-016-04
22	Pilot dose range finding study of (b) (4) rMSCs with femoral catheterized (b) (4) rats	P-027-04
23	Safety and efficacy of dose escalation of cMSCs infused into lethally irradiated canines receiving bone marrow hematopoietic support	TRD-002
24	Safety and effectiveness of (b) (4)	R-S-085-02
25	2-week pilot multiple dose range finding study of (b) (4) rat strain mesenchymal stem cells administered via intravenous infusion in (b) (4) female rats	P-091-06

Note : Study Nos. 16-19 are summarized in this review memo under ‘Overview of Toxicology Studies.’ Study Nos. 20-25 are not summarized in this memo as they are pilot studies or because they contain data obtained from a different route of administration than the intended clinical use of remestemcel-L.

Developmental and Reproductive Toxicology Studies:

No DART studies were conducted.

Genotoxicity Studies:

No genotoxicity studies were conducted.

Carcinogenicity/Tumorigenicity Studies:

These data are summarized at the end of the “Overview of Toxicology Studies”.

Study Number	Study Title / Publication Citation	Report Number
26	Tumorigenicity of human mesenchymal stem cells: nude mouse model	RR-086-06

Other Safety/Toxicology Studies

No other toxicology studies were conducted.

Overview of Toxicology Studies**Study #16**

Report Number	P-024-04			
Date Report Signed	7 November 2005			
Title	Acute Toxicity Study of Rat Bone Marrow Mesenchymal Cells in (b) (4) Rats			
GLP Status	Yes Deviations: No documentation of the strength, purity and composition of the Plasma-Lyte A used on study.			
Testing Facility	(b) (4)			
Objective(s)	The purpose of this study was to evaluate and characterize the acute toxicity of the test article, rat ceMSC.			
Study Animals	Strain/Breed	(b) (4)		
	Species	Rats		
	Age	6-8 weeks		
	Body Weight	Male: 167-167 g; Female: 133-150 g		
	#/sex/group	50 rats/sex		
	Total #	100 rats		
Test Article(s)	(b) (4) strain rat ceMSC			
Control Article(s)	Saline control Plasma-Lyte vehicle control: Plasma-Lyte A (b) (4) supplemented with DMSO (10%) and (b) (4) rat serum			
Route of Administration	Intravenous injection via an externalized catheter or the tail vein			
Description of the Administration Procedure	<p>Healthy, catheterized animals were used for this study. The test and control articles were administered within 4 hours of preparation via an externalized catheter or the tail vein by a slow bolus injection over 30 seconds.</p> <p>Immediately prior to test article administration via the externalized catheter, an attempt was made to remove the heparin/glycerol plug from the cannula. This was followed by a flush of approximately 0.5 mL of 0.9% Sodium Chloride for Injection, USP. If the flush was successful, the animal was administered the test article via the cannula. If the saline flush was not successful, the test article was administered via the tail vein. Administration of the test article or Plasma-Lyte vehicle was followed by a Plasma-Lyte flush. A saline flush was administered following administration of saline.</p>			
Study Groups and Dose Levels	<i>Group</i>	<i>Dose level (cells/kg)</i>	<i>Dose Volume (mL/kg)</i>	<i>No. of Animals</i>
	1	Saline	5	10/sex
	2	Plasma-Lyte	5	10/sex
	3	3x10 ⁶ cells/kg	5	10/sex
	4	20x10 ⁶ cells/kg	5	5/sex
	5	37.5x10 ⁶ cells/kg	5	5 female
	6	15x10 ⁶ cells/kg	5	6 male, 5 female
	7	8x10 ⁶ cells/kg	5	9 male, 5 female
Dosing Regimen	Single administration			
Randomization	Yes			

Description of Masking	Not described
Scheduled Sacrifice Time Points	Day 7 and Day 14 post test article administration

Key Assessments:

- Cage side observations were performed twice daily for morbidity, mortality, injury and availability of food and water.
- Detailed clinical observations were performed at receipt, prior to randomization, 5 minutes post dose, 1 and 2 hours post dose and daily thereafter.
- Body weights were taken at receipt, prior to randomization, and daily for the duration of the study.
- Clinical pathology evaluations were conducted pretest and prior to the interim and terminal necropsies.
- Macroscopic examinations were performed at scheduled necropsy for external abnormalities, and abdominal, thoracic and cranial cavity abnormalities. A full complement of tissues and organs was collected.
- Organ weights were obtained at scheduled necropsy.
- Microscopic examination of fixed and stained paraffin sections was performed on samples obtained at scheduled necropsy.

Key Results:

Mortality: Mortality was observed in test groups administered $\geq 15 \times 10^6$ cells/kg. One female and one male in Group 6 (15×10^6 cells/kg) died within one hour after dosing. The cause of death was undetermined for these animals. Four females and four males in Group 4 (20×10^6 cells/kg) died within two days after dosing. The cause of death was determined to be cardiomyopathy for one female while the remaining animals had an undetermined cause of death. Four females in Group 5 (37.5×10^6 cells/kg) died within five minutes after dosing (males were not dosed in this group due to the immediate deaths observed in females). Disseminated intravascular coagulopathy, indicated by severe renal thrombosis microscopically, was considered to be the cause of death in one female. For the remaining three females cause of death was undetermined. All remaining animals survived until the scheduled sacrifice.

Clinical findings: Significant clinical findings were mostly observed in the high dose level groups. Two animals administered 8×10^6 cells/kg had a loss of righting reflex or decreased activity within five minutes of dose administration. Three animals in this same group also had red discharge that correlated with red material under the cage presumed to be discolored urine. The remaining animals that received 15×10^6 cells/kg all had impaired righting reflex, red discharge and red material under the cages which persisted for several days after administration. Animals that received 20×10^6 cells/kg and 37.5×10^6 cells/kg showed shallow breathing, loss of righting reflex and pale discolored skin within five minutes of dose administration. Most of the animals in these two groups died within two days following test article administration.

Body weights: There were no test-article related effects on body weight for animals that survived to termination.

Clinical pathology:

Hematology: There were no test-article related effects in animals receiving dose levels of 3×10^6 cells/kg and 8×10^6 cells/kg. In animals receiving 15×10^6 cells/kg, erythrocytes, hemoglobin, hematocrit and MCHC were decreased in females compared to saline and Plasma-Lyte controls at the interim interval (Day 7). Samples were not collected at the terminal analysis for this group.

Clinical chemistry: There were no test-article related effects in animals receiving dose levels 3×10^6 cells/kg and 8×10^6 cells/kg. In animals receiving 15×10^6 cells/kg, total bilirubin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was elevated in females compared to saline and Plasma-Lyte controls at Day 7. Samples were not collected at the terminal analysis for this group.

Macroscopic evaluations: No test article related macroscopic changes were observed in animals of either sex that were alive at the time of scheduled necropsy.

Of the animals that died after dosing, the cause of death could not be determined for most animals. Mild cardiomyopathy was observed in one animal and disseminated intravascular coagulopathy was observed in another animal.

Organ weights: There were no test-article related effects on organ weights.

Microscopic evaluations: There was an increase in incidence and severity of cardiomyopathy observed in females at all dose levels compared to the controls, but there was no clear dose-related response for severity. This finding was more pronounced in animals who died early during the study. Two of the male animals receiving 20×10^6 cells/kg that died early in the study demonstrated focal necrosis, consistent with infarction. However, the significance of the necrosis was not clear. In both male and female animals receiving 20×10^6 cells/kg that died early on the study, there was also evidence of inflammation in the liver. This finding was not observed in all animals and relation to the test article was not determined.

No other test article related microscopic changes were observed.

Reviewer Comments:

- ❖ *Although this study was performed under GLP, masking was not done, which could confound interpretation of the results.*
- ❖ *Justification for the selection of sacrifice timepoints was not provided.*
- ❖ *The study report states that administration occurred over 30 seconds, but infusion rate or total volume infused are not provided.*
- ❖ *Animals administered 3×10^6 cells/kg survived to their scheduled necropsy times and demonstrated no relevant changes in their clinical pathology or histopathology.*
- ❖ *Animals administered 8×10^6 cells/kg demonstrated adverse clinical reactions shortly following dose administration but recovered and survived to scheduled necropsy.*
- ❖ *Dose levels of rat ceMSC 15, 20 and 37.5×10^6 cells/kg were toxic when administered intravenously, with numerous adverse clinical findings and changes in tissue histopathology. For one animal mild cardiomyopathy was determined to be cause of death, while in another disseminated intravascular coagulopathy was the cause of death.*

Microscopic evaluations indicate cardiomyopathy and infarction may have contributed to other deaths, though no clear cause of death was determined for most animals.

- ❖ *It should be noted that the test article was prepared into individual syringes for each animal for dosing and kept either (b) (4) for up to 4 hours. The study report states that homogeneity and stability of the product were not analyzed and there is no discussion in the study protocol of (b) (4) prior to IV administration. Additionally, post administration cell viability analysis ranged from 24% to 46%. As most deaths occurred at higher dose levels, it is plausible that the poor viability, high cell density and poor homogeneity contributed to the clinical signs observed and early mortalities.*
- ❖ *Based on these findings the no observable adverse effect level (NOAEL) is 3×10^6 cells/kg for this study. However, given the caveats of the quality of the rat ceMSC test article and unspecified infusion rate, conclusions regarding the safety of the clinical ceMSC product cannot be made.*

Study #17

Report Number		P-054-05
Date Report Signed		20 October 2006
Title		Acute toxicity study of (b) (4) rat bone marrow-derived mesenchymal cells in (b) (4) rats
GLP Status		Yes Deviation: No documentation of the strength, purity, composition and stability of each lot of test article used on study.
Testing Facility		(b) (4)
Objective(s)		The purpose of this study was to evaluate and characterize the acute toxicity of the test articles.
Study Animals	Strain/Breed	(b) (4)
	Species	Rat
	Age	8-12 weeks
	Body Weight	131-201 g
	#/sex/group	5-10/sex/group
Total #		110
Test Article(s)		(b) (4) strain rat ceMSC
Control Article(s)		Vehicle: Plasma-Lyte A (b) (4) supplemented with 10% DMSO and (b) (4) FBS
Route of Administration		Intravenous injection via femoral or jugular catheter

Description of the Administration Procedure	Healthy animals were used for this study. Animals were femorally or jugularly catheterized to allow central administration of the cellular test article. Animals received the test article at 5 mL/kg dose volume at varying rates of infusion. A syringe filled with 0.9% Sodium Chloride for Injection, USP, was attached to the catheter with a blunt needle and gently aspirated to ensure patency of the catheter. The syringe was disconnected and a syringe containing the vehicle or test article was connected to the catheter with an extension line. The vehicle or test article syringe was inserted into an infusion pump and the pump was set to the specified rate and volume. Following completion of the dose the syringe was disconnected from the extension line. A syringe containing at least 1.0 mL of vehicle diluted with 0.9% Sodium Chloride for Injection, USP, was connected to the extension line, inserted into the infusion pump, and administered at the same rate as the dose. The extension line was disconnected from the catheter.				
Study Groups and Dose Levels	Group	Dose Level (cells/kg)	Dose Rate (mL/min)	Catheter Site	No. of Animals
	1	25x10 ⁶	1.6	Femoral	5 males
	2	25x10 ⁶	1.6	Femoral	5 males
	3	50x10 ⁶	1.6	Femoral	5 males
	4	75x10 ⁶	1.6	Femoral	5 males
	5	75x10 ⁶	0.8	Femoral	5 males
	6	75x10 ⁶	0.8	Jugular	5 males
	7	0 (vehicle)	0.8	Jugular	10/sex
	8	10x10 ⁶	0.8	Jugular	10/sex
	9	40x10 ⁶	0.8	Jugular	10/sex
	10	65x10 ⁶	0.8	Jugular	10/sex
Dosing Regimen	Single administration				
Randomization	Yes				
Description of Masking	Not described				
Scheduled Sacrifice Time Points	Day 7 and Day 14 post test article administration				

Key Assessments:

- Cageside observations for morbidity, mortality, injury and availability of food and water were performed twice daily for the duration of the study.
- Clinical observations were performed prior to study initiation, at 1 and 2 hours post dose administration and daily thereafter.
- Body weights were recorded daily
- Clinical pathology samples were collected from animals in groups 7-10 at scheduled sacrifice on Day 7 and Day 14.
- Necropsy was performed on all animals in groups 7-10 at scheduled sacrifice date. Animals were examined for external, abdominal, thoracic and cranial cavity abnormalities. Organs were removed, weighed and tissues processed for microscopic evaluation.
- Microscopic examination was performed on hematoxylin and eosin stained paraffin sections by a veterinary pathologist.

Key Results:

Mortality: No animals in Groups 1-6 died during the study. Several female animals in Groups 7-10 died on study due to complications related to the jugular catheter. This may have occurred

only in females and not in males due to the smaller size of the females and the increased difficulties with surgical placement in the smaller animals. One female animal in Group 9 and one female in Group 10 were found dead on Day 1 prior to the 1-hour post-dose observation. These animals had observations of inflammation, edema, or hemorrhage at the catheter site and the deaths were related to problems with the catheters and not considered test article-related. Two female animals in Group 7 (control group) died on study on Days 5 and 8, respectively, and these deaths were related to cardiac inflammation, necrosis, or bacteremia secondary to problems related to the catheter placement and not considered test article-related. One female animal in Group 9 was found dead on day 10. This animal had observations of swelling at the catheter site one day prior to dose, 1- and 2-hours post-dose, and continuing through Day 9. Additionally, observations of thin appearance were noted starting on Day 4 and decreased activity and impaired righting reflex were noted on Days 8 and 9. The animal lost 28% body weight by Day 8. Per the study report, these clinical observations and body weight loss are considered to be a result of catheter complications and this death was not considered test article-related.

Clinical observations: Several animals that received 75.0×10^6 cells/kg at both rates of 1.6 and 0.8 mL/min had difficulties breathing or red material noted in the pan under the cage. One male animal receiving 65×10^6 cells/kg had red discharge from the penis at 1 and 2 hours post administration of the test article. All but one female animal receiving 65×10^6 cells/kg had observations of either yellow or red discoloration of the anogenital region or vulva, red urine, or red material in the cage pan at 1 or 2 hours post administration of the test article. These clinical observations were considered to be test article-related, although a discussion on the relationship to the test article was not provided.

Body weights: No test article related changes were observed.

Clinical pathology: There were no test article related changes in hematology parameters, coagulation parameters or clinical chemistry parameters.

Macroscopic evaluations: There were no test-article related findings.

Organ weights: A 14% increase in absolute splenic weight and a 12% increase in relative spleen to body weight were observed in the males receiving 65×10^6 cells/kg at day 14. In addition, increased relative spleen to body weight and spleen to brain weight were observed in receiving 40×10^6 cells/kg (10% and 15%, respectively). These changes were interpreted to be reflective of biological variation as there were no correlative changes observed histologically or on macroscopic organ evaluation between the findings and dose.

There was a 49% increase in absolute adrenal gland weights and a 56% increase in relative adrenal gland to body weights in male animals receiving 65×10^6 cells/kg sacrificed on day 14. Toxicologic significance was not definitively determined as no histologic changes were observed.

Microscopic evaluations: There were no test-article related findings.

Reviewer Comments:

- ❖ Documentation regarding the strength, purity, composition and stability of each lot of test article used on the study was not provided by the sponsor. Results of sterility, mycoplasma, and endotoxin evaluations of the test article were evaluated in Supporting Study #27 which includes full characterization of rat ceMSC. As part of the study protocol, cell viability and homogeneity were determined pre- and post-dose administration. Average cell viability was 88% and 80% pre and post-dose, respectively. Small cell clumps were observed in one of five samples analyzed (75×10^6 cells/kg dose).
- ❖ Rationale for the sacrifice time points was not provided.
- ❖ Cause of death for the female animals with early mortality was attributed to complications with the jugular catheter, however no clinical pathology or histopathology was performed to confirm this.
- ❖ Clinical findings were observed at dose levels of 65×10^6 and 75×10^6 cells/kg which included difficulty breathing and red discharge from the anogenital region. The study report attributed these findings to the administration of the test article. Whether it was due to the high cell concentration, rate of infusion or other causes was not discussed.
- ❖ There were fewer unscheduled deaths and adverse clinical findings in this study than the previous study (Study #16), despite administration of higher dose levels. The applicant speculated that this is due to a clear lack of cell clumping prior to dose administration in the current study and the use of a consistent rate of administration, suggesting cell clumping contributed to the findings in the prior study. This rationale seems plausible as the current study added additional protocol steps to ensure cell homogeneity and cell viability prior to dosing, and ensured consistent infusion rates with the use of an infusion pump.
- ❖ Based on the study results, the NOAEL is 40×10^6 cells/kg for this study using rat ceMSC at an infusion rate of 0.8 mL/min. This is the only toxicology study that used an infusion pump to ensure consistent and continual rates of infusion. However, the study only evaluated a single infusion while remestemcel-L is administered twice per week for four weeks with the option of once per week for four additional weeks at 2×10^6 cells/kg infused at 4-6 mL/min in subjects ≥ 35 kg.

Study #18

Report Number	P-115-06
Date Report Signed	31 January 2008; Amended 5 May 2016
Title	13-Week Subchronic Study of (b) (4) Rat Strain Mesenchymal Stem Cells Administered Via Intravenous Infusion in (b) (4) Rats
GLP Status	Yes Deviation: No documentation of the strength, purity, composition and stability of each lot of test article used on study.
Testing Facility	(b) (4)
Objective(s)	The purpose of this study was to evaluate the potential medium-term chronic effects of multiple, sequential doses of test article when administered via intravenous infusion to (b) (4) rats.
Study Animals	Strain/Breed (b) (4)

	Species	Rats			
	Age	8 weeks			
	Body Weight	137-203 g			
	#/sex/group	20/sex/group			
	Total #	380			
Test Article(s)		(b) (4) strain rat ceMSC			
Control Article(s)		Vehicle: Plasma-Lyte A (b) (4) supplemented with DMSO (10%) and rat serum (b) (4)			
Route of Administration		Intravenous injection via the tail vein			
Description of the Administration Procedure		<p>Vehicle or test article were administered to all animals via slow injection at 5 mL/kg/dose into the tail vein on scheduled administration days. Animals were dosed using a 26G 3/8 inch needle attached to an appropriately sized syringe. The needle was placed into the tail vein and each animal was administered the vehicle or test article by slow bolus injection for approximately 2 minutes (± 10 seconds) duration. A stop clock was used to monitor the actual time of the injection. If the needle had to be removed and reinserted, the clock was stopped and restarted when the injection resumed. The time monitored on the clock was recorded at the start of dosing and at the completion of the injection. A (b) (4)</p>			
Study Groups and Dose Levels			<i>Group</i>	<i>Dose Level (cells/kg)</i>	<i>Number of Animals</i>
		<i>Main Study</i>	1	Vehicle	20/sex
			2	0.2×10^7	20/sex
			7	1.0×10^7	20/sex
			3	2.0×10^7	20/sex
		(b) (4)	(b) (4)	(b) (4)	(b) (4)
Dosing Regimen		<p>The vehicle and test article were administered to main study animals on Days 1, 4, 8, 11, 15, 18, 22, 25, 29, 36, 43, 50, and 57 for a total of 13 doses.</p>			
Randomization		Yes			
Description of Masking		Not described			
Scheduled Sacrifice Time Points		Day 30 (interim) and Day 92 or 95 (terminal)			

Key Assessments:

- Cageside observations for morbidity, mortality, injury and the availability of food and water were performed twice daily.
- Clinical examinations were performed once weekly.
- Body weights were recorded at randomization and weekly after test article dosing initiation.
- Food consumption was recorded weekly for main study animals.
- Ophthalmoscopic examinations prior to administration and at scheduled necropsy.

- Samples for clinical pathology (blood and urine) were collected from designated main study animals at the interim and terminal sacrifice time points.
- Blood samples for immunotoxicology analysis were collected from main study animals on Day 30.
- (b) (4)
- At Day 30 sacrifice, spleens were collected from main study animals for evaluation of splenocyte proliferation.
- Necropsy examinations were performed on all surviving animals at scheduled sacrifice. Organ weights were recorded and tissues were processed for microscopic evaluation.

Key Results:

Mortality: Five animals (male and female) administered 2×10^7 cells/kg died on the day of test article administration on Days 15, 18 (two animals), 22, and 29. There were no unusual clinical observations and cause of death could not be determined. One additional male animal administered 2.0×10^7 cells/kg was found dead on Day 30 (the day following test article administration), likely attributed to test article administration. Prior to death this animal exhibited adverse clinical signs, including prostration, loss of righting reflex, pale skin and decreased activity. Two males administered 1.0×10^7 cells/kg died on Day 29 of the study. A cause of death for these animals could not be determined.

Clinical findings: Test article related findings were observed in animals administered 2.0×10^7 cells/kg and included red material in the anogenital area or in the cage pan, salivation, hunched posture, decreased activity, pale skin, impaired righting reflex, prostration and death. Findings were initially recorded on Day 15 and again on Days 22 and 29. Test article related findings were also observed in animals administered 1.0×10^7 cells/kg and included red material in the anogenital area on Day 22, red material from the penis on Day 29, decreased activity, impaired righting reflex and death on Day 29. No test article related findings were observed in animals administered 0.2×10^7 cells/kg.

Body weights: There were no test article related changes in body weight.

Food consumption: There were no test article related changes in food consumption.

Ophthalmoscopic examinations: There were no test article related findings noted during ophthalmoscopic examinations.

Clinical pathology:

Hematology: There were no test article related effects on hematology parameters compared to control.

Clinical chemistry: Male and female animals administered 1.0×10^7 cells/kg demonstrated a slight decrease in albumin and albumin/globulin ratios (A/G) combined with increases in globulin at both the interim and terminal analyses. Male animals administered 2.0×10^7 cells/kg also demonstrated alterations in albumin, A/G and globulin. Though changes were mild they were attributed to the test article.

Urinalysis: There were no test article related changes in urinalysis parameters.

Immunotoxicology analyses:

Immunophenotyping: A statistically significant increase in the absolute mean number of natural killer (NK) cells and percentage of NK cells relative to total lymphocyte count in male animals administered 0.2×10^7 and 1.0×10^7 cells/kg compared to control animals was observed. No increase was observed in male animals administered 2.0×10^7 cells/kg. No NK cell number increase was observed in any female animal.

The mean number of total lymphocytes, T cells and CD4+ T cells were significantly increased in female animals administered 1.0×10^7 cells/kg compared to control animals. In this same group of animals, the mean number of B cells was significantly lower than control animals.

While significant, all levels were reported to be within the normal acceptable variation range. Reference ranges for this species/strain were not included in the study report for confirmation.

(b) (4)

Induced splenocyte proliferation: Isolated splenocytes were cultured in the absence or presence of Concanavalin-A (Con A) and the level of proliferation measured. Splenocytes isolated from animals receiving the test article were slightly more proliferative than those isolated from vehicle control animals in the absence of Con A, but the change was considered to be biologically insignificant. Proliferation increased upon Con A stimulation and was similar in all groups, including vehicle control.

Anti-allogeneic antibody formation: Administration of the test article at dose levels of 1.0×10^7 and 2.0×10^7 cells/kg triggered the formation of alloantibodies at low titers. However, there was no corresponding findings by histopathological analysis, therefore the levels of alloantibody formation were considered biologically insignificant.

Macroscopic evaluations: There were no test article related macroscopic findings.

Organ weights: In male animals administered 1.0×10^7 and 2.0×10^7 cells/kg there was a significant test article related increase in absolute and relative spleen weight and adrenal gland relative to body weight and brain weight. In female animals at all dose levels there was a significant test article related increase in absolute and relative spleen weight.

Microscopic evaluations: In male and female animals at all dose levels, there were test article related changes consisting of minimal to mild cellular emboli present in the lungs at the interim analysis (Day 30). At the terminal analysis (Day 92 or 95), the incidence of cellular emboli was much lower and considered reversible. A few males and females had minimal to moderate chronic inflammation in the lungs at the terminal necropsy with the incidence being higher in the 1.0×10^7 cells/kg group compared to the 2.0×10^7 cells/kg group. Therefore, no clear dose-dependence was observed.

At the interim analysis, hemorrhage, mononuclear cell infiltrate, and thrombus were observed at the injection site in animals administered the test article. Several changes were continued to be more pronounced in animals administered the test article at the terminal analysis compared to controls. These findings were consistent with mild irritation at the injection site.

Minimal to moderate spermatocyte/spermatid degeneration was observed in four out of 11 males at the 1.0×10^7 cells/kg dose level and four out of nine males at the 2.0×10^7 cells/kg dose level at the interim analysis. The change was unilateral or bilateral and was completely reversible at the terminal analysis.

Reviewer Comments:

- ❖ *Documentation regarding the strength, purity, composition and stability of each lot of test article used on the study was not provided by the sponsor. Results of sterility, mycoplasma, and endotoxin evaluations of the test article were evaluated in Supporting Study #27. As part of the study protocol, cell viability and homogeneity were determined pre and post-dose administration. Average cell viability was 80% and 59% pre and post-dose, respectively.*
- ❖ *Rationale for the sacrifice time points was not provided.*
- ❖ *No test article related findings were observed at the 0.2×10^7 cells/kg dose level.*
- ❖ *There were no significant effects of the test article on the host immune cell quantities or host immune system functionality.*
- ❖ *Although multiple administrations of the test article triggered low levels of anti-allogeneic MSC antibody formation, these were considered biologically insignificant.*
- ❖ *Test-article related microscopic finding in the lungs and injection site of male and female animals and testes in male animals observed at the interim analysis were reversible based on the terminal analysis.*
- ❖ *No infusion pump was used in this study, all injections were performed manually. Lack of a consistent injection rate could have attributed to animal death at the higher cell concentrations.*
- ❖ *Based on the high rate of mortality and clinical observations at the 1×10^7 and 2×10^7 cells/kg dose levels, the NOAEL for this study is 2×10^6 cells/kg for up to 13 administrations. Although this is the same as the recommended clinical dose level, no infusion pump or consistent rate of injection was used in this study, therefore applicability of the study results to the clinical dose level and rate of infusion are unclear.*

Study #19

Report Number	(b) (4) 366
Date Report Signed	5 April 2002
Title	Six-month safety and immunology study in baboons of allogeneic baboon mesenchymal stem cells labeled with (b) (4)
GLP Status	Yes

Testing Facility		(b) (4)	
Objective(s)		To determine the safety and immunologic consequences of administering baboon allogeneic mesenchymal cells by routes of clinical significance.	
Study Animals	Strain/Breed	(b) (4) (baboon)	
	Species	Nonhuman primate	
	Age	11-14 years	
	Body Weight	13.5 – 25.3 kg	
	#/sex/group	3 females/group	
	Total #	9	
Test Article(s)		(b) (4) -labeled baboon ceMSC; (b) (4) -labeled baboon ceMSC	
Control Article(s)		Vehicle: (b) (4) Plasma-Lyte A, (b) (4)	
Route of Administration		IV for Day 1 administration, (b) (4)	
Description of the Administration Procedure		<p>MSCs from male donors were labeled with (b) (4) for tracking purposes and injected into female baboons as follows: Group 1 control animals received vehicle; Group 2 received (b) (4) -labeled baboon ceMSC intravenously (IV) (b) (4)</p> <p>(b) (4) Group 3 was treated similarly to Group 2 except that the (b) (4). Intravenous injections of the test article or vehicle were in a dose volume of 20 mL (b) (4)</p> <p>(b) (4) No details regarding the injection procedure were provided.</p>	
Study Groups and Dose Levels		<i>Group</i>	<i>Day 1 Administration (IV)</i>
		1	Vehicle
		2	5x10 ⁶ cells/kg (b) (4) labeled ceMSC
		3	5x10 ⁶ cells/kg (b) (4) labeled ceMSC
Dosing Regimen		Animals received an initial administration of cells on Day 1, (b) (4)	
Randomization		No, animals were selected for groups based on their MHC typing	
Description of Masking		None	
Scheduled Sacrifice Time Points		Week 27 post initial IV administration	

Key Assessments:

- Clinical observations were performed daily beginning in week -1.
- Body weights were recorded in week -1, day 1 and weeks 4, 5, 6, 7, 8, 9, 11, 13, 17, 21 and 27.
- Food consumption was recorded beginning in week -1 and weekly thereafter.
- Blood samples were collected in weeks -1, 5, 11 and 27. The following evaluations were performed:
 - Hematology parameters
 - Clinical chemistry parameters
 - FACS analysis
 - Antinuclear antibodies and Ig panel

- All surviving animals were sacrificed and necropsied at week 27. Organs were collected and weighed. Tissues were processed for histological examination.

Key Results:

Clinical signs: No test article related clinical signs were observed in any group.

Body weights: There were no test article related effects on body weight.

Food consumption: There were no test article related effects on body weight.

Clinical pathology:

Hematology: There were no test article related changes in hematology parameters.

Clinical chemistry: No differences in clinical chemistry parameters were observed.

FACS analysis: The CD3⁺CD4⁺/CD3⁺CD8⁺ ratio was maintained throughout the study and the total number of lymphocytes was unchanged.

Antinuclear antibodies and Ig panel: There were no test article related changes in antinuclear antibodies (ANA) and Ig panel (IgG and IgM).

Sedimentation rate: No test article related differences in sedimentation rate were observed.

Organ weights: There were no test article related effects on organ weights.

Histopathology: Per the study pathologist, administration of baboon ceMSC did not result in any clinically significant histopathology findings that could be directly linked to the test article.

Three neoplastic findings were observed in this study, 1) a benign lipoma was observed in the thoracic cavity of a Group 2 female, 2) a benign carotid body adenoma was observed in thyroid gland from a Group 2 female and 3) a benign pheochromocytoma was observed in the adrenal gland from a Group 3 female. The group size was too small to permit assessment of oncogenic effects of test article. According to the pathology report, some incidental tumors would be expected in animals that were approximately 11-14 years old.

Central nervous system (CNS) lesions (focal gliosis in brain and spinal cord, perivascular and/or meningeal inflammation in brain and/or spinal cord) were observed in one Group 2 animal and mild axonal degeneration of peripheral nerve was observed in another Group 2 animal. These were interpreted as typical in 11-14-year-old baboons. The CNS inflammation of perivascular regions in the brain or in meninges observed was consistent with viral infection.

Reviewer Comments:

- ❖ *Administration of allogeneic baboon ceMSC from both second- and third-party donors did not appear to result in any immunogenicity findings in healthy, non-irradiated, non-ablated baboons up to 27 weeks post administration.*
- ❖ *The applicant has proposed that since ceMSC are non-immunogenic, the use of allogeneic ceMSC in the patients will not result in an immunogenic response. However, there are key differences between the NHPs in this study and the intended patient*

population and applicability of the findings from this study to the clinical situation may be limited.

- ❖ Also, it should be noted that although tissues were collected in order to examine for the presence of ceMSC by both (b) (4) and histology, the applicant was unable to supply the actual data and therefore no conclusions can be made as to the relationship between the presence of allogeneic ceMSC and immunogenicity.
- ❖ The baboon ceMSC used in this study were labeled with (b) (4) and it is unclear whether the label has any effects on ceMSC persistence or its safety profile.

Study #26

Report Number		RR-086-06		
Date Report Signed		26 September 2008		
Title		Tumorigenicity of human mesenchymal stem cells: nude mouse model		
GLP Status		No		
Testing Facility		(b) (4)		
Objective(s)		To test for the tumorigenic potential of ex vivo cultured human mesenchymal stem cells (ceMSC).		
Study Animals	Strain/Breed	(b) (4)		
	Species	Mice		
	Age	8-20 weeks		
	Body Weight	Not provided		
	#/sex/group	5-10/sex/group		
Total #		120		
Test Article(s)		Human bone marrow derived, culture expanded mesenchymal stem cells (ceMSC) from 5 independent donors		
Control Article(s)		Negative Control: Culture media Positive Controls: NIH 3T3 cells		
Route of Administration		(b) (4)		
Description of Administration Procedure		Animals were randomized and injected with either test article, negative control or vehicle control (b) (4) into the fold at the left flank between the femur and the caudal abdomen. Mice were monitored for 6 weeks for tumor formation.		
Study Groups and Dose Levels		<i>Group</i>	<i>Cell Dose (cells/kg)</i>	<i>Treatment</i>
		A	50x10 ⁶ , donor #1	ceMSC
		B	50x10 ⁶ , donor #2	ceMSC
		C	12.5x10 ⁶ , donor #3	ceMSC
		D	50x10 ⁶ , donor #4	ceMSC
		E	50x10 ⁶ , donor #5	ceMSC
		F	0	Culture media
		G	50x10 ⁶	NIH 3T3
Dosing Regimen		Single administration		
Randomization		Yes		
Description of Masking		None		
Scheduled Sacrifice Time Points		Day 42		

Key Assessments:

- Cage side observations for general wellbeing and for the presence of a tumor were performed at least daily.
- Manual palpation for the presence of tumors began 1 week after cell inoculation and occurred 3 times weekly for the duration of the study.
- In the presence of tumors reaching 1 cm or if animals began to regress prior to the scheduled sacrifice time point, they were sacrificed and necropsy was performed.
- All tumor masses or gross abnormalities observed upon necropsy were collected for histological analysis.

Key Results:

There was no evidence of tumors or other lesions attributable to the test article in any animal in Groups A-E. Additionally, no evidence of tumor was noted in animals receiving the negative control. In contrast, 7 out of 10 animals administered the positive control, NIH 3T3 cells, had detectable tumor by Day 42.

Reviewer Comments:

- ❖ *There are several limitations to this study that affect interpretation of the data:*
 - *The test article was administered (b) (4) instead of by the intended clinical IV route of administration.*
 - *The limited duration of the study.*
 - *Only 7 out of 10 positive control animals developed tumors and it is unclear whether this was an issue of selection of the positive control, test system, or loss of cells during the administration procedure.*

ADDITIONAL SUPPORTING STUDIES

Summary List of Additional Supporting Studies

The following product characterization and analytical studies were conducted to support the nonclinical studies summarized in this review memo. The methodologies used were acceptable and relevant findings were incorporated in the reviews of the respective studies above.

Study Number	Study Title / Publication Citation	Report Number
27	Characterization of Rat Mesenchymal Stem Cells (rMSCs)	RR-125-07
28	Characterization of Porcine MSCs	RR-069-00
29	Characterization of porcine mesenchymal stem cells: Assessment of cell surface protein phenotype and differentiation potential	RR-020-04
30	Qualification of mesenchymal stem cell labeling and detection utilizing (b) (4)	R-036-05

APPLICANT'S PROPOSED LABEL

Subsections 8.1-8.3 of Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14).

Section 13.1 ('Carcinogenesis, Mutagenesis, Impairment of Fertility') should be revised to accurately reflect the available nonclinical data.

Section 13.2 ('Animal Toxicology and/or Pharmacology') should be revised to include only information from the nonclinical studies necessary for the safe and effective use of the product such.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns for the clinical dosing, route, and indications for use. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

RYONCIL, remestemcel-L, culture expanded mesenchymal stem cells, graft versus host disease, toxicity, immunogenicity

References:

1. Lee, S.E., et al., *Risk and prognostic factors for acute GVHD based on NIH consensus criteria*. Bone Marrow Transplant, 2013. **48**(4): p. 587-92.
2. Faraci, M., et al., *Etanercept as Treatment of Steroid-Refractory Acute Graft-versus-Host Disease in Pediatric Patients*. Biol Blood Marrow Transplant, 2019. **25**(4): p. 743-748.
3. Bacigalupo, A., *Management of acute graft-versus-host disease*. Br J Haematol, 2007. **137**(2): p. 87-98.
4. Klyushnenkova, E., et al., *T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression*. J Biomed Sci, 2005. **12**(1): p. 47-57.
5. Aggarwal, S. and M.F. Pittenger, *Human mesenchymal stem cells modulate allogeneic immune cell responses*. Blood, 2005. **105**(4): p. 1815-22.
6. Bartholomew, A., et al., *Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo*. Exp Hematol, 2002. **30**(1): p. 42-8.
7. Chapel, A., et al., *Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome*. J Gene Med, 2003. **5**(12): p. 1028-38.
8. Devine, S.M., et al., *Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion*. Exp Hematol, 2001. **29**(2): p. 244-55.
9. Devine, S.M., et al., *Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates*. Blood, 2003. **101**(8): p. 2999-3001.
10. Liechty, K.W., et al., *Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep*. Nat Med, 2000. **6**(11): p. 1282-6.
11. Mosca, J.D., et al., *Mesenchymal stem cells as vehicles for gene delivery*. Clin Orthop Relat Res, 2000(379 Suppl): p. S71-90.