INVESTIGATOR'S BROCHURE

MultiStem®

PERIOD COVERED BY THIS REPORT:

26-November-2018 through 25-November-2019

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INVESTIGATOR'S BROCHURE RECEIPT

Herewith I acknowledge the receipt of the Investigator's Brochure for MultiStem®.

Investigator's Signature	Date

Investigator's Name

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
ALL	Acute Lymphocytic Leukemia
AMI	Acute Myocardial Infarction
AML	Acute Myelocytic Leukemia
ARDS	Acute Respiratory Distress Syndrome
ASA	Aminosalicylic Acid
ATP	Adenosine Triphosphate
AZA	Azathioprine
CD	Cluster of Differentiation
CHF	Chronic Heart Failure
CFR	Code of Federal Regulations
CHMP	Committee for Medicinal Products for Human Use
CLL	Chronic Lymphocytic Leukemia
CML	Chronic Myelocytic Leukemia
CNI	Calcineurin Inhibitor
CNS	Central Nervous System
DMSO	Dimethyl Sulfoxide
EMA	European Medicines Agency
EU	European Union
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
FDA	Food and Drug Administration
FPI	First Patient In
GFP	Green Fluorescent Protein
GI	Gastrointestinal
GLP	Good Laboratory Practice
GvHD	Graft versus Host Disease
HCT/Ps	Human Cells, Tissues, and Cellular and Tissue Based Products
HLA	Human Leukocyte Antigen
HSA	Human Serum Albumin
HSCT	Hematopoietic Stem Cell Transplantation
HTS	HypoThermosol
IB	Investigator Brochure
IFN	Interferon
IHC	Immunohistochemistry
IL	Interleukin
IND	Investigational New Drug
IV	Intravenous
KC	Keratinocyte-Derived Cytokine
LAD	Left Anterior Descending Artery
LPO	Last Patient Out
LPS	Lipopolysaccharide
LV	Left Ventricular
LVEF	Left Ventricular Ejection Fraction
MAPC	Multipotent Adult Progenitor Cells
MASTERS	MultiStem Administration for Stroke Treatment and Enhanced Recovery Study
MCAL	Middle Cerebral Artery Ligation
MCAO	Middle Cerebral Artery Occlusion

Abbreviation	Definition
MDS	Myelodysplastic Syndrome
MHC	Major Histocompatibility Complex
MI	Myocardial Infarction
MIP	Macrophage Inflammatory Protein
MiSOT	Mesenchymal Stem Cells in Solid Organ Transplantation
MLR	Mixed Lymphocyte Reactions
6-MP	6-Mercaptopurine
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MSC	Mesenchymal Stem Cells
mTOR	Mammalian Target of Rapamycin
N/A	Not Applicable
NHLBI	National Heart, Lung and Blood Institute
NOAEL	No-Observable-Adverse-Effect Level
NOD/SCID	Non-Obese Diabetic / Severe Combined Immunodeficiency
NSTEMI	Non-ST Elevation Acute Myocardial Infarction
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCI	Percutaneous Coronary Intervention
PD	Population Doublings
PMDA	Pharmaceuticals and Medical Devices Agency
PO2	Partial Pressure of Oxygen
PT	Preferred Term
qPCR	Quantitative Polymerase Chain Reaction
RBC	Red Blood Cell
RMAT	Regenerative Medicine Advanced Therapy
SC	Subcutaneous
SCI	Spinal Cord Injury
SDF	Stromal Cell-Derived Factor
SNP	Single Nucleotide Polymorphism
SOC	System Organ Class
SOT	Solid Organ Transplant
STEMI	ST Elevation Myocardial Infarction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBD	To Be Determined
TNF	Tumor Necrosis Factor
tPA	Tissue Plasminogen Activator
TREASURE	Treatment Evaluation of Acute Stroke for Using in Regenerative Cell Elements
UC	Ulcerative Colitis
UK	United Kingdom
UKR	Universitätsklinikum Regensburg
US	United States
VEGF	Vascular Endothelial Growth Factor
WBC	White Blood Cell
WHO	World Health Organization
WHF	World Heart Federation

1 REGULATORY UPDATE

This harmonized Investigator Brochure (IB) is for the single active substance MultiStem®, covering the reporting period 26-November-2018 through 25-November-2019.

The Development International Birth Date for MultiStem is 26-November-2008.

This harmonized MultiStem IB contains relevant clinical development information for MultiStem in the following indications: prophylaxis and treatment of graft versus host disease (GvHD), IND # 13507; treatment of acute myocardial infarction (AMI), IND # 13554; treatment of ischemic stroke (Stroke), IND # 13852, treatment of moderate and severe acute respiratory distress syndrome (ARDS), IND # 16460 and treatment of acute spinal cord injury (SCI), expanded access, single patient emergency sponsored under Stroke IND # 13852.

In addition, this harmonized IB contains updated information regarding three additional completed or terminated MultiStem clinical trials conducted by sponsors other than Athersys [treatment of ulcerative colitis, IND # 14512 / EudraCT # 2010-022766-27, sponsor Pfizer; treatment of GvHD, expanded access, single patient emergency Investigator (Dr. Laura Newell) sponsored IND # 16128 and Mesenchymal stem cells in solid organ transplant, EudraCT # 2009-017795-25, sponsor Freistaat Bayern / Universitätsklinikum Regensburg (UKR)]. There is also one ongoing ARDS clinical trial being conducted in Japan by HEALIOS K.K.

Table 1-1 below summarizes the regulatory status of MultiStem ongoing and completed/terminated clinical studies, and the corresponding IND and/or EudraCT numbers.

Table 1-1. Regulatory Status of MultiStem Clinical Trials

	US IND #	Status of Clinical Study as of 25-November-2019	Clinical Study Disposition	Europe EudraCT #
Graft versus Host Disease	13507	Phase 1 – prophylaxis - completed in November 2011	Safe to proceed (10/2007)	Study was conducted in Belgium (EudraCT # 2010-018760-16)
		Phase 2/3 – prophylaxis – (registration) planned	SPA agreement granted 12/2015	TBD
Acute Myocardial Infarction	13554	Phase 1 – STEMI - completed in February 2012	Safe to proceed (12/2007)	N/A
		Phase 2 – NSTEMI – ongoing, subject follow-up with enrollment stopped	Protocol accepted (01/2015)	N/A
Ischemic Stroke	13852	MASTERS-1 Phase 2 – completed December 2016	Safe to proceed (12/2008)	Study is completed in the UK (EudraCT # 2012-005749-18)
		MASTERS-2 Phase 3 – treatment – (registration) - ongoing	SPA agreement granted 09/2016 Fast Track designation granted 05/2017 RMAT designation granted 08/2017	Study not initiated yet in EU (EudraCT # 2019-001680-69)
		TREASURE Phase 2/3 – treatment – ongoing	Safe to proceed (08/2016) PMDA	N/A (Study conducted in Japan)
Acute Respiratory Distress Syndrome	16460	Phase 1/2 – Moderate/Severe ARDS – completed July 2019	Safe to proceed (07/2015) Fast Track designation granted 04/2019	Study is completed in the UK (EudraCT# 2015-001586-96)
	Non-IND	ONE-BRIDGE Phase 2 – ARDS caused by pneumonia - ongoing	Safe to proceed (10/2018) PMDA	N/A (Study conducted in Japan)
***Ulcerative Colitis (Partner /Study Sponsor - Pfizer)**	14512	Phase 2 – completed follow-up visits November 2014	Safe to proceed (11/2011)	Study is completed in the EU (EudraCT # 2010-022766-27)
***Treatment of GvHD**	16128	Phase 1b – expanded access use, single patient emergency IND - terminated	Safe to proceed (08/2014)	N/A
Treatment of SCI	13852	Phase 1b – expanded access use, single patient emergency under Stroke IND - terminated	Safe to proceed (02/2016)	N/A
***MiSOT (Study Sponsor – UKR)**	Non-IND	Phase 1/2 – Liver transplant - terminated	Safe to proceed (11/2011)	Study was terminated in Germany (EudraCT # 2009-017795-25)

***** additional ongoing or completed MultiStem clinical trials conducted by sponsors other than Athersys
SPA : Special Protocol Assessment designation
RMAT: Regenerative Medicine Advanced Therapy Designation

As of 25-November-2019, MultiStem product is being used or has been used in clinical trials as listed above in Table 1-1.

- Five studies with MultiStem product have been completed:

1. AMI treatment Phase 1 dose-escalation, open-label, safety study in 25 patients including six registry patients after acute myocardial infarction.
2. GvHD prophylaxis Phase 1 dose-escalation, open-label, safety study in 36 patients undergoing hematopoietic stem cell transplant.

3. Ulcerative colitis: a blinded, placebo-controlled Phase 2 clinical trial sponsored by Athersys' partner, Pfizer. This study enrolled 105 patients.
4. Ischemic stroke: a blinded, placebo-controlled Phase 2 clinical trial in 134 subjects who suffered an acute ischemic stroke.
5. ARDS: a blinded, placebo controlled Phase 1/2 clinical trial sponsored sponsored by Athersys. This study enrolled 36 subjects.

- Four studies with MultiStem product are ongoing:

1. AMI: an ongoing Phase 2 – study sponsored by Athersys. The study has enrolled 34 subjects as of this document's cutoff date. The study is ongoing with subject follow-up although no further subjects will be enrolled due to poor enrollment.
2. Ischemic stroke: a blinded, placebo-controlled Phase 2/3 clinical trial in approximately 220 subjects who suffered an acute ischemic stroke in Japan. This study has enrolled 94 subjects as of this document's cutoff date.
3. Ischemic stroke: a blinded, placebo-controlled Phase 3 clinical trial in approximately 300 subjects who suffered an acute ischemic stroke in North America, Europe, and Asia Pacific. This study has enrolled 28 subjects in the US as of this document's cut-off.
4. ARDS: an open-label, standard treatment controlled Phase 2 clinical trial in approximately 30 subjects who suffered ARDS caused by pneumonia in Japan. This study has enrolled 8 subjects in Japan as of this document's cut-off.

- One study and two, single patient expanded access cases were terminated:

1. Treatment of GvHD: an expanded access use, single patient emergency Investigator-sponsored IND was opened in August-2014. The patient was steroid refractory GI and Liver Grade 4, received 4 of 6 MultiStem scheduled treatment doses over a period of 3 months (21-August-2014 through 24-October-14). The patient died 05-November-2014. Death not related to MultiStem treatment. The study was terminated.
2. Treatment of acute SCI: an expanded access use, single patient emergency sponsored under the Athersys Stroke IND was granted in February-2016. The patient had a C4/C5 fracture and dislocation from an ice hockey accident and received 1.2 billion cells of MultiStem on 23-February-2016, which was infused within 36 hours of initial injury. The patient is a quadrapelic and was released from the hospital to continue their recovery. The study was terminated.
3. MiSOT: a non-IND investigator-initiated trial for the indication of liver transplantation which was a Phase 1, open-label study, evaluating safety and feasibility of MultiStem for immunomodulation therapy after liver transplantation sponsored by Freistaat Bayern in Germany. Three subjects were enrolled and the study was terminated due to poor enrollment.

- MultiStem product (adult adherent bone marrow-derived multipotent stem cells) has been granted:
  - Orphan drug designation for the indication "prophylaxis of graft versus host disease" (15-September-2010) by the United States Food and Drug Administration (FDA)
  - Orphan drug designation for the prevention of GvHD by the European Commission (16-January-2014, EU/3/13/1233)
  - The GvHD program has also received "Fast Track" designation by the FDA (February 2015)
  - The registration pivotal Phase 2/3 adoptive, double blind study design for prevention of GvHD in adults and children older than or equal to 13 years of age following hematopoietic cell transplant for hematological malignancies has been approved by EMA and a positive opinion was adopted in February 2015 by CHMP
  - Similarly the FDA granted Athersys an agreement to Special Protocol Assessment (SPA) for the single pivotal Phase 2/3 registration GvHD study (December 2015)
  - FDA has also granted the pivotal Phase 3 Ischemic Stroke study MASTERS-2 the SPA designation to support registration (September 2016)
  - The registration pivotal Phase 3, double blind study design (MASTERS-2) for treatment of Ischemic Stroke in Adults within 36 hours of event, has been approved by EMA and a positive opinion was adopted in June 2017 by CHMP
  - FDA has granted Ischemic Stroke Fast Track designation (May 2017)
  - FDA has granted Ischemic Stroke Regenerative Medicine Advanced Therapy (RMAT) designation (August 2017)
  - FDA has granted Acute Respiratory Distress Syndrome Fast Track designation (April 2019)
- MultiStem has been well tolerated in approximately 324 subjects exposed to MultiStem as of this document's cut-off date across a range of acute and chronic disorders, doses, dose regimens and routes of delivery
- This product has not been commercially approved anywhere in the world

2 SUMMARY

MultiStem is an adult adherent cell product derived from the bone marrow of a non-related donor and expanded *ex vivo*. MultiStem cell therapy represents a mechanistically novel approach for numerous indications, such as treatment following AMI, prevention of GvHD, treatment of acute ischemic stroke, treatment of UC, support during and after SOT and treatment of ARDS.

The evaluation of MultiStem for clinical use is based on nonclinical pharmacological, biodistribution and safety studies in animal models that support the investigational use of MultiStem cells in these indications. *In vitro* pharmacology studies of rat and human MultiStem cells have shown that MultiStem can inhibit T-cell proliferation, indicating that the cells are immunosuppressive. This conclusion was further supported by *in vivo* pharmacology studies conducted in pig AMI models, mouse and rat GvHD and ischemic injury models, and a rat cardiac transplant model.

MultiStem cells initially localize to the lungs after intravenous (IV) injection. Pulmonary and cardiovascular safety pharmacology studies have demonstrated that after single and multiple dose IV infusions of rat or human MultiStem cells, there were no adverse effects on pulmonary function and no effects on cardiovascular safety measurements.

The nonclinical assessment of the pharmacokinetics of MultiStem cells focused on biodistribution and residual presence in tissue following dosing. The biodistribution of MultiStem following IV infusion of cells in a mouse model of GvHD has been evaluated through *in vivo* imaging of cells labeled with a luciferase reporter gene (RM-080714-01FR). Imaging of animals in this model indicated an initial accumulation of MultiStem cells in the lungs in the hours immediately following administration, followed by re-distribution to the gastrointestinal (GI) tract over the following 24 to 48 hours. In the majority of samples measured, the bioluminescence reporter signal was below the limit of detection at 10 days post infusion, suggesting that the majority of the administered cells were cleared by this time point.

Biodistribution and persistence of multi-potent adult progenitor cell (MAPC®)/MultiStem cells have also been evaluated in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice and in other rodent and pig disease models. There was no evidence of tumorigenicity in subcutaneous (SC) and IV nude mouse tumorigenicity studies or in any other nonclinical studies where tissues were evaluated. The majority of detectable allogeneic rat MAPC cells were cleared from tissues within a few days of administration in rat studies. In addition, 2- and 4-week biodistribution studies in the brains of stroke injured rats were conducted, and no detectable signal was found by quantitative Polymerase Chain Reaction (qPCR) for human cells in the animal brains at either time point.

Generation of an immune response against MultiStem cells, as measured by alloantibody or T-cell activation responses, has not been observed *in vitro* or *in vivo* in single- or multiple-dose cell administration rat studies.

Single-dose toxicity studies in mice and rats have been conducted with both human and rat MultiStem cells. The cells were well-tolerated up to 10 million cells/dose (500 million cells/kg; human MultiStem delivered SC in mice) and up to 40 million cells/dose (200 million cells/kg; rat MAPC delivered IV in rats). Allogeneic rat MultiStem cells have been administered to rats in an IV study up to 5 weeks in duration (once weekly dosing). The no-observed-adverse-effect level (NOAEL) in this study was 2.5 million cells/dose (12.5 million cells/kg). Two IV doses of 10 million allogeneic rat cells/dose (50 million cells/kg) have been given one week apart to rats with no adverse effects observed. Additional studies have demonstrated that direct delivery of 200 million MultiStem cells into the adventitia of the pig coronary artery was well tolerated.

The utility of MultiStem is being explored in a number of clinical trials, including 6 completed or terminated trials in patients following AMI and acute ishemic stroke, as a prophylactic treatment for GvHD in patients undergoing hematopoietic stem cell transplantation (HSCT), in patients with UC, ARDS, and following liver transplantation, and during four ongoing trials in patients following an acute ischemic stroke, ARDS, and AMI. These studies use different MultiStem formulations and concentrations, but identical cellular constituents. While MultiStem was delivered locally to the heart in the AMI trials, it was infused as single or multiple IV infusions in the completed GvHD, ischemic stroke, liver transplantation, and UC trials. As of 25-November-2019, approximately 324 patients have received MultiStem in these ongoing and completed or terminated trials. From completed or terminated trials, this included 19 AMI patients receiving transarterial injection of MultiStem; 36 GvHD patients receiving IV infusion of MultiStem through a central line; 84 UC patients, 71 acute ischemic stroke patients, 26 ARDS patients, and 1 SCI and 1 GvHD treatment expanded access use patients receiving an IV infusion of MultiStem through a peripheral line, and 3 liver transplant patients receiving MultiStem via the portal circulation and through a peripheral line. From ongoing trials, this included an estimated 61 ischemic stroke patients and 5 ARDS patients receiving an IV infusion of MultiStem through a peripheral line and an estimated 17 AMI patients receiving transarterial injection of MultiStem. As of 25-November-2019, there have been no infusional or allergic reactions reported per protocol definitions and adverse events have been consistent with the disease states being studied in completed or ongoing trials. The ARDS trial in Japan had a report of a serious adverse event of chills possibly related to MultiStem. In the UC trial, 2 separate serious adverse events of hypersensitivity and pancytopenia were reported and considered possibly related to MultiStem or the product used to dilute the stem cells.

3 INTRODUCTION

MultiStem is an allogeneic bone-marrow derived MAPC-based medicinal product. Accordingly, MultiStem is a proprietary subset of the allogeneic bone marrow-derived stem cells. MultiStem refers to the registered (or trademarked), clinical grade MAPC product. In scientific publications and in several instances in this document, the two nomenclatures, MultiStem and MAPC are used interchangeably. The product consists of expanded stem cells isolated from allogeneic bone marrow. Cells are expanded *ex vivo* from a single donor The potential benefit of MultiStem in various indications is summarized below.

3.1 Stem Cell Treatment and Potential Indications

3.1.1 Acute Myocardial Infarction

Cardiovascular disease is the leading cause of death globally. Despite important advances in the last twenty years, cardiovascular diseases killed an estimated 17.3 million people in 2008. Of these, an estimated 7.3 million were due to coronary artery disease and 6.2 million were due to stroke (WHO, 2013). Additionally, AMI frequently leads to congestive heart disease, which itself is often characterized by a significant decline in quality of life and high morbidity and mortality rates. Despite many advances over the last two decades, AMI and other diseases caused by ischemic heart disease continue to remain a major cause of morbidity and mortality.

The most frequent cause of AMI is a blockage of blood flow to the heart caused by rupture of an atherosclerotic plaque and formation of an occluding blood clot. Arrhythmia occurs in some form in > 90% of patients. Primary care is aimed at relieving distress, reversing ischemia, limiting the infarct size, reducing cardiac work, and preventing and treating complications (Merck, 2005). Triage is important in determining what further treatments are required for the individual patient.

Aspirin, if not contraindicated, should be used to treat patients with AMI and continued indefinitely to reduce vascular death, nonfatal myocardial infarction (MI), and nonfatal stroke. Oxygen and morphine are used as needed. Thrombolytic therapy is most effective in the first few minutes and hours of AMI. Drugs available in the US include streptokinase, streptokinase-urokinase, and tissue plasminogen activator (tPA). Additional medications, including aspirin, clopidogrel, and dipyridamole, can reduce the risk of AMI in patients with known risk factors such as acute ST-elevation (STEMI) or non-ST elevation (NSTEMI) MI, unstable angina, prior MI, ischemic stroke, and peripheral arterial disease (Aronow, 2007).

During AMI, ischemia causes myocardial cell death and progressive loss of contractile tissue. Subsequently, structural changes lead to left ventricular remodeling, finally resulting in the development of heart failure (Assmus et al., 2006). Cardiac cells possess a limited ability to regenerate. Therefore AMI also results in replacement of "perished"

cardiomyocytes by scar tissue. The consequence of this is lowering of myocardial contractile function and the development of heart failure.

The mortality rates associated with AMI have significantly decreased over the past 2 decades (Rogers et al., 2000; Rogers et al., 2007). Beginning first with thrombolytic therapy for AMI, and more recently with growing acceptance and availability of primary percutaneous coronary intervention (PCI) for ST-elevation AMI, the mortality rates of this devastating ischemic event have decreased from almost 15% in clinical trials in the late 1980s to < 5% in recent primary percutaneous coronary intervention trials (Montalescot et al., 2001; Grines et al., 2002; Stone et al., 2002). Accompanying this decrease in mortality has been a significant increase in patients with chronic heart failure (CHF). It is estimated that 22% of men and 46% of women who have experienced MI will be disabled with heart failure (Thom et al., 2006).

3.1.1.1 Stem Cell Treatment in Acute Myocardial Infarction

Cellular therapy for cardiac disease is a burgeoning field of clinical research, aimed at developing potential treatments for patients with ischemic heart disease and/or congestive heart failure. Cell therapeutics hold the promise of treating cardiovascular disease through the protection or support of ischemic tissue, thereby improving function and preventing or ameliorating cardiovascular remodeling and decline in function. Ischemic injury to the heart can result in significant myocyte cell death and impaired ventricular function. Scar formation and compensatory remodeling can lead to long term consequences associated with congestive heart failure. Pre-clinical and early clinical data suggests that cell therapy can provide therapeutic benefit through multiple potential biological mechanisms and paracrine effects including preventing apoptosis and limiting inflammatory damage, stimulating angiogenesis, and producing homing factors that may mobilize patient stem cells or progenitors thereby improving cardiac recovery.

The goal of cell therapy for patients with AMI is to improve cardiac function without increasing the risk of sudden cardiac death in an attempt to decrease the development and onset of CHF. MultiStem has the potential for achieving this goal by protecting cardiomyocytes from cell death and stimulating new angiogenesis. Nonclinical studies with small and large animals using both autologous and allogeneic MultiStem at the time of AMI demonstrated significant improvements in cardiac function. MultiStem can be delivered to the peri-infarct zone surrounding the ischemic scar via catheter-based delivery that leads to improved cardiac function. Trophic factors, such as vascular endothelial growth factor (VEGF) and others secreted by MultiStem in response to the injury microenvironment are likely pathway mediators (Van't Hof et al., 2007; Zeng et al., 2007). Significant neo-angiogenesis and improved heart bioenergetics are associated with sustained improvement in ventricular function.

A Phase 1 clinical study with MultiStem, for patients with first-time ST elevation myocardial infarction (STEMI) demonstrated that MultiStem was well tolerated (Penn et al., 2012). After 4 months, a trend towards an increase in cardiac function was observed as measured by left ventricular ejection fraction (LVEF). Based on these results, it would appear likely that cell therapy will play a role in the prevention and treatment of cardiac dysfunction in the ensuing years.

3.1.2 Graft versus Host Disease

The leukemias are cancers of the white blood cells (WBCs) involving bone marrow, circulating WBCs, and organs such as the spleen and lymph nodes. Malignant transformation usually occurs at the hematopoietic stem cell level, although it sometimes involves a committed progenitor cell with more limited capacity for differentiation. Abnormal proliferation, clonal expansion, and diminished apoptosis (programmed cell death) lead to replacement of normal blood elements with malignant cells. Manifestations of leukemia are due to suppression of normal blood cell formation and organ infiltration by leukemic cells. Inhibitory factors produced by leukemic cells and replacement of marrow space may suppress normal hematopoiesis, with ensuing anemia, thrombocytopenia, and granulocytopenia. Organ infiltration results in enlargement of the liver, spleen and lymph nodes, with occasional kidney and gonadal involvement.

Leukemias were originally termed acute or chronic based on life expectancy but now are classified according to cellular maturity. Acute leukemias consist of predominantly immature, poorly differentiated cells (usually blast forms); chronic leukemias have more mature cells. Acute and chronic leukemias are divided into lymphocytic (ALL and CLL) and myelocytic (AML and CML). The most common leukemias are ALL in children (peak incidence ages 2-10), AML in any age group, CLL in mid to old age and CML in young adults. Myelodysplastic syndromes (MDS) involve progressive bone marrow failure but with an insufficient proportion of blast cells (< 30%) for making a definite diagnosis of AML; 40% to 60% of cases evolve into AML (Merck, 2005). Survival in untreated acute leukemia generally is 3 to 6 months.

The goal of treatment is complete remission, including resolution of abnormal clinical features, restoration of normal blood counts and normal hematopoiesis with < 5% blast cells, and elimination of the leukemic clone. Although basic principles in treating ALL and AML are similar, the drug regimens differ. The 4 general phases of treatment for ALL include remission induction, central nervous system (CNS) prophylaxis, post-remission consolidation or intensification, and maintenance. Several regimens emphasize early introduction of an intensive multi-drug regimen. Therapy duration is usually 2.5 to 3 years but may be shorter with regimens that are more intensive in earlier phases and for B cell cases. For a patient in continuous complete remission for 2.5 years, the risk of relapse after therapy cessation is about 20%, usually within 1 year. Thus, when therapy can be stopped,

most patients are cured. Leukemic cells may reappear in the bone marrow, the CNS, or the testes. Bone marrow relapse is of particular concern. Although a new round of chemotherapy may induce a second remission in 80 to 90% of children (30 to 40% of adults), subsequent remissions tend to be brief. Only a few patients with late bone marrow relapses achieve long disease-free second remissions or cure. If a human leukocyte antigen (HLA)-matched sibling is available, stem cell transplantation offers the greatest hope of long-term remission or cure. For AML, treatment includes induction chemotherapy to achieve remission and post-remission chemotherapy (with or without stem cell transplantation) to avoid relapse.

For MDS, prognosis depends greatly on classification and on any associated disease. Patients with refractory anemia or refractory anemia with sideroblasts are less likely to progress to the more aggressive forms and may die of unrelated causes. Azacitidine and decitabine improve symptoms, decrease the rate of transformation to leukemia and the need for transfusions, and probably improve survival. Other therapy is supportive, including red blood cell (RBC) transfusions as indicated, platelet transfusions for bleeding, and antibiotic therapy for infection. In some patients, erythropoietin to support RBC needs, granulocyte colony-stimulating factor to manage severe symptomatic granulocytopenia, and, when available, thrombopoietin for severe thrombocytopenia can serve as important hematopoietic support but have not proved to increase survival. Allogeneic stem cell transplantation is useful, and non-ablative allogeneic bone marrow transplantations are now being studied for patients > 50 years of age, for whom myeloablation is too risky.

Stem cell transplantation is an option for some patients with recurrence of ALL, AML, CML, and MDS. A limitation is the availability of a matched donor and the patient's age and disease status. Myeloablation of the existing bone marrow can lead to many complications, including serious infections. Matched related donor transplantation is more effective than non-related. For the patient in leukemic relapse, HSCT is often a last option. There are complications of such transplantation, some related to the myeloablative drugs and others to the conditioning regimen used to try to prevent rejection.

Acute GvHD is one of the major limitations of allogeneic HSCT. This complication is thought to be initiated by activation of adoptively transferred, mature donor T-cells through recognition of target antigens presented on major histocompatibility complex (MHC) molecules expressed on antigen-presenting cells that reside within host tissues. Moderate to severe GvHD Grades II-IV occurs in 30-50% of matched related HSCTs (Gale et al., 1989; Martin et al., 1991; Weisdorf et al., 2007) and 50-70% of unrelated donor recipients (Hansen et al., 1997; Nash et al., 2000), and is a major cause of morbidity and mortality. Although the incidence of GvHD is influenced by many recipient as well as donor factors, alterations of prophylactic regimens have had only limited evolution over the last fifteen years. It is recognized that innovations in this area are needed which would further reduce the incidence of GvHD without increasing relapse or risk of infection in HSCT patients.

3.1.2.1 Stem Cell Treatment in Graft versus Host Disease

Based on its immunological properties, MultiStem could provide therapeutic benefit following myeloablation and HSCT in hematological malignancy patients as described below.

MultiStem cells are minimally immunogenic and have the potential to diminish GvHD in allogeneic bone marrow recipients. MultiStem cells do not activate allo-T cells *in vitro* in mixed lymphocyte reaction assays (MLR) nor do they generate an allo-immune response when administered *in vivo*. MultiStem cells have been shown to suppress immune responses between allo-reactive T-cells from two unrelated individuals as measured in MLR assays. Based upon these properties, MultiStem has the potential to reduce the incidence and/or severity of GvHD in leukemia patients.

Finally, MultiStem may enhance engraftment of allogeneic bone marrow cells by providing an improved micro-environment through the secretion of cytokines and homing factors. A number of publications document enhanced hematopoietic recovery with co-administration of mesenchymal stem cells (MSC) and allogeneic or xenogeneic bone marrow grafts. Nonclinical studies performed using human MSC and human CD34+ HSC populations show increased chimerism and support the accelerated hematopoietic recovery shown clinically (Auletta et al, 2010). This is likely achieved via several pathways, including production of "homing factors" such as stromal cell-derived factor-1 (SDF-1) that recruit HSC to bone marrow, and production of cytokines supportive of hematopoiesis such as interleukin (IL)-6 (Klyushnenkova et al., 2005).

3.1.3 Stroke

Globally, stroke is one of the leading causes of death and disability. Every year, approximately 15 million people worldwide experience a stroke. Nearly 6 million die and 5 million are permanently disabled (WHF, 2014). Approximately 80-90% are ischemic strokes involving a blockage of a blood vessel(s) in the brain, resulting in a lack of oxygen and nutrients to the underlying parenchymal tissue and subsequent cell and tissue death (Roger et al., 2011; WHF, 2014).

While there can be variability among ischemic stroke victims, the pathology is generally described by a number of defined stages. During the ischemic stage, which may last hours, a clot blocks a vessel, causing loss or reduction of blood flow to cells and tissue downstream of the infarct. This initiates a chain of cellular and inflammatory responses. Within hours of the ischemic event, there is an acute cellular response whereby a complex series of cellular metabolic events can lead to loss of neuronal function and cell death. These cellular responses affect the core ischemic zone, and can have impact on the tissues surrounding the infarct. Following ischemia, inflammatory cytokines are up-regulated initiating a multi-stage inflammatory response to the stroke. Neutrophils infiltrate the ischemic area secreting

additional inflammatory mediators resulting in the destruction of necrotic and neighboring viable tissue. Macrophages and astrocytes are activated stimulating further inflammatory factors, while activated glial cells isolate the area of damage. Reperfusion of the occluded vessel, whether through intervention, spontaneous resolution, or compensation of collateral circulation, can result in a reinforcing cycle / cascade of inflammatory response and damage. The acute inflammatory process remains active for days and may reach its completion within 1-2 weeks after the initial ischemic event.

Current therapy for stroke is limited. Other than one recombinant protein therapy, recombinant tPA, which is directed at the dissolution of the clot in affected blood vessels in adults following stroke, there are no drugs or biologic therapies available for effectively treating ischemic stroke or the damage associated with the ischemic event. Small molecule therapies such as anti-platelet drugs, anti-coagulants, and statins act as prophylactics and have no immediate benefit following an acute attack. Only 5-10% of Americans suffering ischemic stroke receive tPA due to delayed recognition of the symptoms coupled with the limited window for receiving treatment following the stroke. Patients often arrive at hospitals too late for such intervention. The numbers of affected individuals, the costs necessary to facilitate their care and rehabilitation, coupled with the lack of current therapies reiterate that stroke represents a significant unmet medical need.

Drug development efforts to date have focused on stroke prevention (e.g., anti-hypertensives, cholesterol-lowering drugs, and anti-coagulants), thrombolytics, and neuroprotectants. Most recently, results from pre-clinical and clinical studies suggest the potential for stem cell therapy as a treatment for ischemic stroke.

3.1.3.1 Stem Cell Treatment in Ischemic Stroke

Stem cells have demonstrated efficacy when transplanted into animal models of stroke, although the mechanisms through which cells provide benefit in these studies has not been definitively established.

Direct transplantation experiments in the brain have utilized cells derived from bone marrow. Fresh bone marrow transplanted directly into the ischemic boundary zone of rat brain improved functional recovery from middle cerebral artery occlusion (MCAO) (Chen et al., 2000). Similarly, MSC implanted into the striatum of mice after stroke improved functional recovery (Li et al., 2000). Cerebral grafts of mouse bone marrow also facilitated restoration of cerebral blood flow and blood-brain barrier after stroke in rats (Borlongan et al., 2004).

Systemic administration of stem cells via IV or intra-arterial injection has also been shown to have positive effects in animal models of stroke. Intra-carotid artery administration of MSC following MCAO in rats improved functional outcome (Li et al., 2001). Similarly, IV administration of umbilical cord blood stem cells ameliorated motor and neurological

deficits after stroke in rats (Chen et al., 2001). It has also been reported that IV administration of cord blood was more effective than intra-striatal administration in producing functional benefit following stroke in rats (Willing et al., 2003). Intravenous administration of MSC has been found to induce angiogenesis in the ischemic boundary zone following stroke in rats (Chen et al., 2003). Collectively, these studies illustrate the diversity of cell types, routes of delivery, and potential mechanisms of benefit which may contribute to recovery in animal models of stroke.

MultiStem has been shown to improve motor function when transplanted directly into the brain of adult rats (Zhao et al., 2002) or into the hippocampus of neonatal rats following induction of hypoxic-ischemic injury (Yasuhara et al., 2008; Yasuhara et al., 2006a; Yasuhara et al., 2006b). In addition, allogeneic and human MultiStem have shown sustained, statistically significant, dose-dependent benefit when administered IV (without immunosuppressive agents) in rat models of ischemic stroke (Mays et al., 2010).

These data suggest that MultiStem can provide therapeutic benefit through multiple potential biological mechanisms, including: preventing apoptosis, limiting inflammatory damage in the brain, and producing homing factors that may mobilize endogenous stem cells or progenitors thereby further improving CNS function and recovery. Based on these supportive data, the effects of MultiStem are being investigated in patients who have sustained an ischemic stroke.

3.1.4 Inflammatory Bowel Disease

UC is a chronic, relapsing inflammatory bowel disease involving all or a portion of the colon. This disease is the most common form of inflammatory bowel disease worldwide with an estimated incidence of 1.2 to 20.3 cases per 100,000 person-years and a prevalence of 3.6 to 214.0 cases per 100,000 per year (Danese and Fiocchi, 2011). Patients with UC most commonly present with diarrhea, urgency, rectal bleeding, and abdominal pain. Patients may also experience fatigue, fevers, weight loss and dehydration. The symptoms can be incapacitating. The sub-mucosa of the colon becomes progressively dominated by lymphocytic infiltration causing further damage. Many patients will typically suffer a "flare" of disease activity at least once per year followed by a period of remission (Loftus, 2004).

The primary goal of therapy of UC is to induce as well as to maintain remission. Treatment has traditionally involved a step-wise approach of medical therapy. 5-Aminosalicylic acid (5-ASA) agents are the cornerstone of therapy for mild to moderately active UC and are usually used as the first-line therapy (Kornbluth et al., 1997). Although they are generally safe and well tolerated, they only induce remission in approximately 50% of patients (Sutherland et al., 1993). When UC is refractory to 5-ASA agents, corticosteroids are usually the second-line therapy (Kornbluth et al., 1997). However, these medications are

associated with numerous side effects, and many patients develop steroid dependency. Therefore, corticosteroids are used only for inducing remission and are not recommended as maintenance therapy. Alternative therapies to corticosteroids for refractory disease include immunosuppressants such as azathioprine (AZA) or 6-mercaptopurine (6-MP), or in severe cases, cyclosporine (Kornbluth et al., 1997). AZA and 6-MP are most effective in steroid sparing and maintaining remission in UC. They may also have a role in the induction of remission, although the time required before a therapeutic response can be prolonged to 2-6 months (Sandborn, 1998). Side effects of these therapies include pancreatitis, infection, myelosuppression, hepatotoxicity and lymphoma. The most recent advance in medical therapy for UC is the introduction of biological therapy. Infliximab, an anti-tumor necrosis factor (TNF) therapy, induces response following a three-dose induction regimen in approximately two-thirds of patients with moderately to severely active UC, and approximately one third of patients will be in clinical remission at one year (Rutgeerts et al., 2005). As with other immunosuppressants, anti-TNF therapies are associated with a significant risk of side effects, including acute and delayed infusion reactions, development of autoantibodies, infections, lymphoma, neurological disease and hepatotoxicity.

3.1.4.1 Stem Cell Treatment in Ulcerative Colitis

MultiStem represents a mechanistically novel approach for the treatment of UC that has the potential to provide efficacy with a reduced side-effect profile compared to existing treatments. MultiStem appears capable of delivering a therapeutic benefit through more than one mechanism of action. Factors expressed by MultiStem are believed to reduce inflammation and regulate immune system function, protect damaged or injured cells and tissue, promote formation of new blood vessels, and augment tissue repair and healing in other ways (Auletta et al., 2010). MultiStem has been used in adoptive T-cell models of GvHD in rats and mice that involve intestinal injury with disease pathology akin to that occurring in UC. MultiStem reverses this disease pathology by tempering the inflammatory response and intestinal organ damage via mechanisms that include down regulation of TNF-α cytokine networks and effector T-cells. MultiStem provided survival benefit in lethal acute GvHD models in part by reducing intestinal pathology while preserving intestinal function which allows the animals to regain weight. Thus, the nonclinical studies conducted in GvHD rodent models support the clinical investigation of MultiStem in UC patients.

3.1.5 Solid Organ Transplant

End-stage organ failure is a public health concern with few treatment alternatives, with transplantation often being the best option. The field of SOT has advanced over the 4 last decades with significant developments in the fields of surgery, immunology, drug development, and general standards of care. Over 1 million patients worldwide have undergone successful organ transplantation. In the United States and Europe, the most commonly transplanted organs are kidneys, livers, hearts, lungs and pancreases (Bloom et

al., 2005; EMA, 2008). Organ graft rejection continues to be an issue with SOT, and, as the demand for organs continues to exceed the supply, the acceptance of extended criteria donors continues with increased risk of unfavorable transplantation outcomes (EMA, 2008).

The goal of immunosuppression in SOT is to control an undesirable immune response while avoiding the complications of immunodeficiency, especially increased risk of infection and malignancy. The use of immunosuppressants has drastically improved short-term patient and graft survival; however, long-term graft survival has not much improved (van Sandwijk et al., 2013). Current immunosuppressive therapies include glucocorticosteroids; calcineurin inhibitors (CNIs), mammalian target of rapamycin (mTOR) inhibitors, antimetabolites, and antibodies. These immunosuppressants are associated with often severe side effects requiring routine therapeutic drug monitoring due to their narrow therapeutic indices (Johnston, 2013; van Sandwijk et al., 2013). More recent developments aim to promote tolerance through induction or infusion of regulatory T-cells, specialized leukocyte populations that are either selected to have regulatory function during their development or acquire immunosuppressive properties in the local microenvironment of the allograft (van Sandwijk et al., 2013).

The first solid organ transplant indication for MultiStem was liver transplantation. End-stage liver disease can be caused by a variety of hepatotoxic agents (hepatotrophic viruses, alcohol, hepatotoxins), autoimmune processes (autoimmune hepatitis, primary biliary cirrhosis, viral hepatitis), or metabolic defects (α-antitrypsin deficiency and many others). In patients in whom conservative medical management fails or is prone to failure, liver transplantation is the current gold standard of treatment for end-stage liver disease (Adam et al., 2000; Fulginiti et al., 1968).

The clinical success of liver transplantation is evident, with patient and graft survival rates exceeding 75% after 5 years (Lee et al., 2007; Northup et al., 2006). However, next to a continuous shortage of donor organs, the problem of life-long immunosuppression continues to be a major obstacle in transplant medicine, especially considering that further improvements in overall survival may be expected in the years to come. All transplant patients on pharmacological immunosuppression suffer from an increased risk of opportunistic infections, mainly in the early post-transplant period, and from a gradual increase in the incidence of malignant diseases and non-immunological side effects in the later phases after transplantation (Vajdic and van Leeuwen, 2009; Watt et al., 2009).

3.1.5.1 Stem Cell Treatment in Solid Organ Transplant

One strategy to reduce the need for immunosuppressive pharmacotherapy in SOT is cell-based immunoregulation using stem cells. MSCs have emerged as promising candidates for cell-based immunomodulatory therapy promoting operational tolerance of solid organ transplantation in a variety of animal models (Ge et al., 2009; Casiraghi et al., 2008; Popp

et al., 2008; Inoue et al., 2006; Bartholomew et al., 2002). In addition, MSCs possess regenerative potential and may thus participate in the regeneration of marginal organs after transplantation (Hematti, 2008). Thus, MSCs have been shown to be capable of inducing allograft acceptance in rodent models. So far, this phenomenon has only been shown for donor-derived (Ge et al., 2009; Popp et al., 2008; Bartholomew et al., 2002) or haploidentical (Casiraghi et al., 2008) MSCs. However, injecting donor-antigen–bearing cells in a clinical setting carries the risk of recipient sensitization (Dhanireddy et al., 2009; Flye et al., 1995). Also, outside the limited field of living related organ transplantation, making donor-type MSCs available for a clinical study is almost impossible. Recipient-derived cells, on the other hand, carry an increased risk of malignant transformation (Kidd et al., 2009; Worthley et al., 2009; Tolar et al., 2007), and their immunological benefit is unproven. Also, MSCs are characterized by limited in-culture expansion potential and are thus of limited clinical use.

The unique characteristics of MultiStem offering important advantages relative to other cell therapies under development, including MSCs, are as follows:

1) broad differentiation capacity; 2) immunomodulatory activity (immunosuppressive potency and ability to suppress proliferation of T-cells after exposure to allogeneic cell stimuli); 3) well tolerated, and demonstrated to be non-tumorigenic / no observed potential for ectopic tissue formation *in vivo*; and 4) can be manufactured at large scale while maintaining genetic stability and multipotency.

Several clinical studies trying to establish a clinical role of MSCs for various indications, such as GvHD (Le Blanc et al., 2008), kidney failure, inflammatory bowel disease, myocardial infarction, or stroke are underway. These studies use either autologous or donor-type cells, or cells that are genetically related to the donor or recipient in some other way. The cell products used in these studies are prepared on an individualized basis whereas MultiStem is available as a prepared drug product. Recent nonclinical studies have shown that MultiStem effectively prolongs allograft survival *in vivo*. Based on these results, it is believed that MultiStem can be a valuable addition to current immunosuppressive protocols and contribute to reducing the long-term pharmacological side effects and inducing operational transplant tolerance (Orlando et al., 2009). The multipotent and immunologic properties of MultiStem could make it a useful adjunct in SOT by inducing immunologic tolerance or at least enabling the reduction of immunosuppressive drug therapies. Given the clinical need for improved anti-rejection and pro-regeneration treatment after liver transplantation, the risk-benefit equation is appropriate for evaluating MultiStem after allogeneic liver transplantation.

3.1.6 Acute Respiratory Distress Syndrome

ARDS is a common clinical entity and a major cause of morbidity and mortality in the critical care setting. Historically, ARDS has been associated with mortality ranging from 25% to 40%, with worse outcomes in the elderly population (Walkey et al., 2012). According to the NHLBI in 2013 and the ARDS foundation, the annual incidence of ARDS is 190,000 in the US. The NHLBI estimated that in 2009 the annual cost of providing healthcare related to all respiratory conditions, excluding lung cancer, was $113 billion.

ARDS is defined as the sudden failure of the respiratory system and can occur in anyone who is critically ill. This condition can be life-threatening because normal gas exchange does not take place due to severe fluid buildup in both lungs. ARDS is caused mainly by extensive lung inflammation and small blood vessel injury due to sepsis, trauma and/or severe pulmonary infection such as pneumonia. Onset of disease typically occurs within 24-72 hours of the original illness or injury.

3.1.7 Stem Cell Treatment in Acute Respiratory Distress Syndrome

MultiStem, is a cell-based, biological therapy under development for the treatment of ARDS. There is currently no effective drug treatment for ARDS and this project is intended to demonstrate that an allogeneic (off-the-shelf) cell therapy has high potential for treating the critical and highly expensive ARDS – which has not been achieved to date. MultiStem therapy has been shown to be safe and effective in other indications, and its multifactorial immune modulation capacity suggests real potential in ARDS, making this innovation achievable.

MultiStem, which is manufactured from human stem cells obtained from adult bone marrow, has the ability to express a range of therapeutically relevant proteins and other factors. MultiStem can be produced on an industrial scale, in a well validated and reproducible manner. *Ex vivo* and nonclinical studies have shown that MultiStem may be administered without tissue matching or the need for immune suppressive drugs, making it analogous in that regard to type O blood. To date, MultiStem has exhibited a consistently favorable safety profile in nonclinical studies and in clinical trials of MultiStem in the treatment of AMI and GVHD, albeit with more limited clinical experience.

MultiStem is believed to reduce inflammation and regulate immune system function, protect damaged or injured cells and tissue, promote formation of new blood vessels, and augment tissue repair and healing in other ways (Auletta 2010). MultiStem demonstrated a dose dependent inhibition of allogeneic cell or antibody mediated T-cell proliferation *in vitro*. A consistent 50% dose-dependent inhibition of T-cell activation by human MultiStem was observed using different T-cell donors at MultiStem cell to peripheral blood mononuclear cell (PBMC) ratios between 1:5 and 1:20. Human MultiStem was also shown to inhibit murine T-cell proliferation in a dose dependent manner.

MultiStem reduced severity of disease, improved clearance of alveolar edema and returned lung endothelial permeability to normal in our *ex vivo* perfused swine lung model of ARDS. In our sheep model of lipopolysaccharide induced ARDS, vascular pressure was moderated, PO2 levels quickly returned to normal and pulmonary edema cleared (Rojas, 2014).

4 PHYSICAL, CHEMICAL, PHARMACEUTICAL PROPERTIES & FORMULATION

MultiStem is a cell therapy medicinal product originating from adherent adult stem cells taken from the bone marrow of a non-related donor and expanded *ex vivo*.

Laboratory Code	MultiStem
Cell Type	Allogeneic
Tissue Source	Adherent stem cells taken from an adult human bone marrow aspirate

MAPC have been isolated from adult tissues including bone marrow and other non-embryonic sources, have the potential to express proteins indicative of cells representing each of the three germ layers and can be expanded *ex vivo* while retaining their multi-potent differentiation potential. MultiStem is a proprietary subset of the allogeneic bone marrow-derived stem cells. MultiStem refers to the registered (or, trademarked), clinical grade MAPC product. MultiStem cells are derived from a bone marrow aspirate acquired and tested in accordance with 21 CFR Part 1271 Human Cells, Tissues, and Cellular and Tissue Based Products (HCT/Ps) and in compliance with EU regulations on Cells and Tissues; 2004/23/EC, 2006/17/EC and 2006/86/EC.

The MultiStem product is a light, amber-colored homogenous sterile cell suspension composed of MultiStem cells and formulated in cryopreservation medium including Plasma-Lyte A or equivalent, dimethyl sulfoxide (DMSO) and human serum albumin (HSA). The MultiStem product is stored frozen in a container such as a bag or vial in the vapor phase of liquid nitrogen prior to preparation for administration to a subject and the number of containers will vary depending upon the dose, mode of administration, and clinical indication.

4.1 Acute Myocardial Infarction: Dosage Form and Delivery

Once the MultiStem product is thawed, contrast agent is added to each vial and the material filtered through a 41 μm filter prior to administration directly into the adventitia of the target coronary vessel via micro-infusion catheter. The final diluted product consists of Plasma-Lyte A, DMSO (4%), HSA (4%) and 20% contrast agent. Storage and transport of the thawed product is at room temperature using ambient gel packs until catheter loading.

The biocompatibility of MultiStem cells with the transarterial catheter and filter has been demonstrated *in vitro* (Study No. RM-071005-01FR). Infusion of human MultiStem through a transarterial catheter and filter does not affect cell recovery, viability or potency.

4.2 Graft versus Host Disease: Dosage Form and Delivery

The MultiStem diluted product for infusion was prepared from the MultiStem drug product cryobags by diluting the cryobag contents with Plasma-Lyte A (1:1) and passed through a 200 µm blood filter prior to infusion. The final diluted product consisted of Plasma-Lyte A, DMSO (5%) and HSA (2.5%), to produce a final IV bag for patient administration with the targeted cell dose. Storage of the final diluted product was 2-8°C prior to the infusion.

4.3 Stroke: Dosage Form and Delivery

The MultiStem diluted product for infusion in the MASTERS-1 trial was prepared from the MultiStem drug product cryobags by diluting the cryobag contents with Plasma-Lyte A or equivalent (1:1) and passed through a 200 µm blood filter prior to infusion. The final diluted product consisted of Plasma-Lyte A or equivalent, DMSO (5%) and HSA (2.5%), to produce a final IV bag for patient administration with the targeted cell dose. Storage of the final diluted product was 2-8°C prior to the infusion.

The MultiStem diluted product for infusion in the TREASURE and MASTERS-2 trials is being prepared from the MultiStem drug product vials by diluting the vial contents with about 250 mL of Plasma-Lyte A or equivalent. The final diluted product is passed through a 200 µm blood filter prior to infusion. The final diluted product consists of Plasma-Lyte A or equivalent, DMSO (1%) and HSA (1%), to produce a final IV bag for patient administration with the targeted cell dose. Storage of the final diluted product is 2-8°C prior to the infusion.

4.4 Ulcerative Colitis: Dosage Form and Delivery

The MultiStem diluted drug product for use in Ulcerative Colitis was prepared from the MultiStem drug product cryobags where the cells were concentrated and a buffer exchange was performed to formulate the cells into Hypothermosol (HTS) that was the final buffer for the infused product. The infused product contained DMSO (~0.6%), HSA (~0.3) and HTS and was passed through a 200 µm blood filter prior to infusion. The final diluted MultiStem product was stored at 2-8°C until infused.

4.5 Solid Organ Transplant: Dosage Form and Delivery

The MultiStem diluted product for infusion was prepared from the MultiStem drug product cryobags by diluting the cryobag contents with Plasma-Lyte A (1:1). The final diluted product consisted of Plasma-Lyte A, DMSO (5%) and HSA (2.5%). The first dose was run through a 200 µm blood filter and administered through the portal vein after the new liver was transplanted and the patient was stable. The product was prepared just prior to administration. The second dose was initiated 24 hours after the first dose from frozen cryobags where the thawed bag was diluted 1:1 with Plasma-Lyte A, run through a 200 µm

blood filter before it was delivered intravenously. The dilution and administration was performed at the bedside so storage of the product is at room temperature.

4.6 Acute Respiratory Distress Syndrome: Dosage Form and Delivery

The MultiStem diluted product for infusion during the US/United Kingdom B04-02 ARDS trial was prepared from the MultiStem drug product cryobags by diluting the cryobag contents with Plasma-Lyte A or equivalent (1:1) and passed through a 200 μm blood filter prior to infusion. The final diluted product consisted of Plasma-Lyte A or equivalent, DMSO (5%) and HSA (2.5%), to produce a final IV bag for patient administration with the targeted cell dose. Storage of the final diluted product is 2-8°C prior to the infusion.

The MultiStem diluted product for infusion during the ARDS B04-02 ONE-BRIDGE trial in Japan is prepared from the MultiStem drug product vials by diluting the vial contents with about 250 mL of Plasma-Lyte A or equivalent. The final diluted product is passed through a 200 μm blood filter prior to infusion. The final diluted product consists of Plasma-Lyte A or equivalent, DMSO (1%) and HSA (1%), to produce a final IV bag for patient administration with the targeted cell dose. Storage of the final diluted product will be 2-8°C prior to the infusion.

5 NONCLINICAL STUDIES

MultiStem cells were evaluated in multiple *in vitro* and *in vivo* nonclinical studies to assess its immunoregulatory properties, pharmacological potential in various animal models of disease, biodistribution, and safety.

5.1 Nonclinical Pharmacology

5.1.1 *In Vitro* Pharmacology

A series of *in vitro* studies were performed to evaluate the immunoregulatory properties of rat MultiStem and human MultiStem cells. A dose-dependent inhibition of T-cell activation was observed with rat cells and was replicated using human MultiStem cells isolated from different donors. The *in vitro* pharmacology studies conducted with MultiStem are summarized in Table 5-1.

Table 5-1. Overview of *In Vitro* Pharmacology Studies

Study Title	Study Number / Reference	Method	Results
Immune-regulatory properties of MultiStem	RM-051012-02FR	*In vitro* T-cell activation assays testing rat MultiStem immunosuppression. Evaluation of responder T-cells cultured with ConA, or with allogeneic or syngeneic irradiated splenocytes. Proliferation of alloreactive T-cells measured after 4 days of culture by ^{3}H-thymidine incorporation.	Rat MultiStem suppressed ConA-mediated T-cell activation. Syngeneic and third-party MultiStem suppressed T-cell activation by splenocytes from non-matched rats in a dose-dependent manner. ~50% inhibition observed at low MultiStem to T-cell ratios (1:50-1:100).
Summary of human MLR studies	RM-050310-01FR	*In vitro* T-cell activation assays testing human MultiStem immunogenicity and immunosuppression. Proliferation of alloreactive T-cells measured after 6 days of culture by ^{3}H-thymidine incorporation.	Human MultiStem failed to induce proliferative T-cell sensitization *in vitro*. When added to MLRs, MultiStem suppressed T-cell proliferation. Inhibition was similar to, or greater than, inhibition mediated by human MSC.
Immune-regulatory properties of human MultiStem	RM-051108-01FR	*In vitro* assays testing immunosuppressive activity of human MultiStem in MLR or in anti-CD3/anti-CD28 antibody-mediated T-cell activation. Proliferation of alloreactive T-cells measured after 5 days of culture by ^{3}H-thymidine incorporation.	Dose-dependent inhibition of allogeneic cell or antibody-mediated *in vitro* T-cell proliferation by human MultiStem.
Immunosuppressive activity of Cambrex manufactured MultiStem lots in T-cell activation assays	RM-070109-02FR	*In vitro* T-cell activation assays using anti-CD3/anti-CD28 antibody to measure and standardize the immunosuppressive potency of clinical grade human MultiStem.	Consistent dose-dependent inhibition of T-cell activation by clinical grade MultiStem from different donors with a 50% decrease in T-cell activation observed at MultiStem to PBMC ratios between 1:5-1:20.
Immunological properties of third-party MAPCs: effect on T-cell proliferation	Eggenhofer et al., 2013	*In vitro* T-cell activation assays testing human MultiStem immunosuppression. Evaluation of responder T-cells cultured with ConA, or with allogeneic splenocytes. Proliferation measured after 3 (ConA) or 5 days (allostimulus) of culture by flow cytometry.	Third-party human MultiStem suppressed ConA-mediated T-cell proliferation. When added to MLRs, MultiStem suppressed T-cell proliferation.

Table 5-1. Overview of *In Vitro* Pharmacology Studies

Study Title	Study Number / Reference	Method	Results
Supportive-Collaborative Studies			
Clinical scale expanded adult pluripotent stem cells prevent graft-versus-host disease	Kovacsovics-Bankowski et al., 2009	*In vitro* T-cell activation assays testing rat MultiStem immunogenicity and immunosuppression. Proliferation of alloreactive T-cells measured after 4 days of culture by ^{3}H-thymidine incorporation.	Rat MultiStem failed to induce proliferative T-cell sensitization *in vitro*. When added to MLRs, MultiStem suppressed T-cell proliferation in a dose-dependent manner. Inhibition was reversible upon removal of MultiStem from culture, was mediated by soluble factors, and was non-MHC restricted. The overall results are supportive of clinical utility of third-party MultiStem.
Clinical grade MAPCs durably control pathogenic T-cell responses in human models of transplantation and autoimmunity	Reading et al., 2013	*In vitro* T-cell activation assays testing human MultiStem immunogenicity and immunosuppression. Proliferation of alloreactive T-cells measured after 4 days of culture by flow cytometry after stimulation by antigen-driven and antigen-independent stimulii. Inhibition of effector cytokine secretion measured by intracellular flow cytometry.	MultiStem suppressed T-cell proliferation in a dose-dependent manner, including homeostatic T-cell expansion driven by IL-2, IL-7 and IL-15. MultiStem induced Treg in the presence of effector T-cell suppression. T-cell suppression was mediated by induction of indoleamine dehdroxygenase, as inhibitors of this pathway can reverse T-cell suppression.
Direct delivery of syngeneic and allogeneic large-scale expanded MAPCs improves cardiac function after myocardial infarct	Van't Hof et al., 2007	The cytokine, chemokine, and growth factor profiles of conditioned media from three expanded rat MultiStem lines were analyzed by multiplex analysis.	Rat MultiStem lines derived from Sprague-Dawley and Lewis rats produced very similar patterns of secreted factors, highlighted by production of high amounts of GRO/KC, VEGF and MCP-1, and lower levels of RANTES and IL-6.

Table 5-1. Overview of *In Vitro* Pharmacology Studies

Study Title	Study Number / Reference	Method	Results
Supportive-Collaborative Studies			
Migratory pattern of MAPCs	Eggenhofer et al., 2013	*In vitro* investigation of the migratory pattern of MAPCs using Matrigel Invasion Chambers with 24-hour incubation and chemo-attractant (rat splenocytes stimulated with ConA).	MAPCs were substantially less effective at migrating towards an IFN-γ gradient than MSCs. When MSCs were prestimulated with IFN-γ, their migratory capacity decreased more significantly than that of MAPCs. In contrast, IL-10 had a similar effect on both cell populations.
ConA = concavalin A; GRO/KC = GRO1 oncogene and keratinocyte-derived cytokine; IFN = interferon; IL = interleukin; MAPC = multipotent adult progenitor cells; MCP = monocyte chemotactic protein; MHC = major histocompatibility complex; MLR = mixed lymphocyte reaction assay; MSC = mesenchymal stem cell; PBMC = peripheral blood mononuclear cell; RANTES = regulated upon activation normal T-cell expressed and presumably secreted; VEGF = vascular endothelial growth factor			

Immunoregulatory Properties of MultiStem Cells

The immunosuppressive properties of human and rat MultiStem have been evaluated in a series of *in vitro* T-cell activation assays. The allogeneic potential of these cells was tested using MLR. Both rat and human MultiStem had a low immunogenicity profile with immunosuppressive potency *in vitro* and were capable of suppressing T-cell proliferation after exposure to allogeneic cell stimuli. The immunomodulatory properties of rat MultiStem cells were further evaluated, with a focus on possible mechanisms underlying the immunosuppression (Kovacsovics-Bankowski et al., 2009). The authors concluded that *in vitro* inhibition of T-cell proliferation by rat MultiStem cells is non-MHC restricted, reversible, and mediated by soluble factors.

Human MultiStem product lots generated in large-scale expansions consistent with the manufacturing protocol for clinical grade material, did not elicit immune responses in *vitro* and suppressed active immune responses in a dose-dependent manner. These results confirmed that the human MultiStem product produced by a large-scale expansion protocol retained their immunosuppressive properties.

Migratory Pattern of MAPCs

The migratory pattern of MultiStem has been found to differ from that of MSCs, with MultiStem substantially less effective at migrating towards an interferon(IFN)-γ gradient than MSCs. The highly immunosuppressive and low-migration properties of MultiStem cells make them attractive for use in transplant medicine.

5.1.2 *In Vivo* Pharmacology

Numerous *in vivo* pharmacology studies have been conducted with MultiStem in support of various indications, including AMI, GvHD, stroke, UC, SOT and ARDS. These studies are listed in Table 5-2 and discussed below.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Acute Myocardial Infarction				
Direct Delivery of Syngeneic and Allogeneic Large Scale Expanded Multipotent Adult Progenitor Cells Improves Cardiac Function after Myocardial Infarct	Van't Hof et al., 2007	10 million syngeneic or allogeneic rat MultiStem cells/rat heart	AMI was induced in Lewis rats by LAD ligation. Post-infarct, rats were injected with either syngeneic Lewis MAPC expressing βgal, allogeneic Sprague-Dawley MAPC expressing GFP, or PBS (control), administered to the peri-infarct zone. Echocardiography was performed at baseline and at 2 and 6 weeks post-infarct. Rats were sacrificed at various timepoints after MAPC injection, and IHC staining was performed on heart tissue to detect βgal, GFP, and endothelial markers (von Willebrand Factor and anti-α-Smooth Muscle Actin).	Myocardial injection with syngeneic or allogeneic MAPC resulted in an 88% and 55% increase in shortening fraction compared to controls, respectively, with no significant differences between the syngeneic and allogeneic groups. Immunostaining demonstrated significant engraftment of expanded MAPC at 1 day after AMI, with < 10% of either syngeneic or allogeneic cells remaining at 6 weeks. Myocardial MAPC injection after AMI resulted in increased vascular density within the infarct zone.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Acute Myocardial Infarction				
Bioenergetic and Functional Consequences of Bone Marrow Derived Multipotent Progenitor Cell Transplantation in Hearts with Postinfarction LV Remodeling	Zeng et al., 2007	50 million allogeneic pig MultiStem cells/pig heart	AMI was induced in female Yorkshire pigs by LAD ligation. The animals were then randomized into 3 groups: control animals that received vehicle (n = 7), test animals that received allogeneic pig MultiStem while being treated with cyclosporine (n = 7), and test animals that received allogeneic pig MultiStem without cyclosporine treatment (n = 6). The MultiStem and vehicle were administered as direct injections into the peri-scar area. Animals were monitored by MRI and MRS. Four weeks post-surgery, the animals were sacrificed and IHC was conducted on heart tissue (scar, peri-scar, injected area).	Compared to the vehicle treatment, MultiStem resulted in greater LV function ($p<0.05$), improved phosphocreatine/ATP ratio in the border zone of the infarct ($p=0.03$) and increased phosphocreatine/ATP across the left ventricle ($p<0.05$). Approximately 0.3-0.5% cell engraftment was observed, with cells frequently found as vessel wall components. No difference in cell retention or cardiac performance was observed in the presence or absence of cyclosporine. MultiStem was associated with increased vascular density in the infarct and infarct border zones ($p<0.05$), but not in the remote regions of the heart.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Acute Myocardial Infarction				
Evaluation of Safety and Cell Persistence Following Direct and Catheter –based Delivery of MultiStem in Porcine Model of Acute Myocardial Infarction	RM-051011-02FR	50 or 200 million allogeneic pig MultiStem cells/pig heart	The study was divided into two phases. In Phase I, AMI was induced in female Yorkshire pigs by LAD ligation and animals were administered allogeneic pig MultiStem cells expressing βgal or PBS (control) via direct myocardial injection. In Phase II, AMI was induced in female Yorkshire pigs by balloon occlusion and animals were administered allogeneic pig MultiStem cells expressing βgal or PBS (control) via transarterial or intracoronary catheter. In both phases, cell persistence was evaluated at 2 and 8 weeks.	Phase I: Direct injection of MultiStem resulted in cell persistence of < 1%. The range of cell persistence, based on the number of βgal positive cells in one ring from each animal, was 0%-0.4%. However, the total cell persistence might be higher than what was observed in one ring. Phase II: There was a trend of higher cell persistence by transarterial injection compared to intracoronary injection (not statistically significant). Also, irrespective of the dose, there was a trend of lower cell persistence in the 8-week animals compared to the 2-week animals.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
MultiStem Delivered by Transarterial Catheter in a Pig Model of Acute Myocardial Infarction (AMI)	RM-070208-01FR	50 million allogeneic pig MultiStem cells/pig heart	AMI was induced in Yorkshire pigs by intracoronary balloon occlusion of the LAD for 1 hour followed by reperfusion. Subsequently, 6 animals received 50 million pig MultiStem and 4 animals received the placebo control (saline) into the LAD near the occlusion site using a transarterial catheter. At 1 and 4 weeks, heart function was assessed by measuring the LV ejection fraction using MRI. Animals were necropsied at 4 weeks and heart tissue collected.	After 4 weeks post-infarction the control animals had significantly lower ejection fractions than the MultiStem-treated animals (35% vs. 46%, p=0.03). The MultiStem-treated animals showed significant improvement in the ejection fraction from 1 to 4 weeks (33% to 46%, p=0.004) after cell infusion.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Acute Myocardial Infarction				
A GLP Study to Evaluate Safety and Efficacy of Allogenic Pig MultiStem Cells in an Acute Myocardial Infarction Model in Pigs	RM-060523-01FR	20 or 200 million allogeneic pig MultiStem cells/pig heart	AMI was induced in pigs on Day 0 and the animals were randomly assigned to three treatment groups that received low dose (20 million) or high dose (200 million) βgal-labeled allogeneic pig MultiStem cells or vehicle (Plasma-Lyte A) via transarterial catheter two days after the AMI (Day 2). ECGs were conducted on all animals prior to surgery and on all surviving animals immediately post-surgery, on Day 2 prior to injection, and at 1 and 3 months post injection.	Compared to the control or high-dose group, the low-dose group had higher ejection fraction ($p=0.0003$ and $p=0.001$, respectively), smaller increases in end systolic volume ($p=0.014$ and $p=0.012$, respectively), and smaller wall motion scoring index in LAD-related territories ($p=0.002$ and $p<0.001$, respectively). The smaller wall motion scoring index in the low-dose group becomes more significant when compared to control and high-dose scores when the analysis is limited to segments directly affected by the LAD occlusion. Administration of MultiStem was not associated with any adverse clinical observations, bodyweight changes, hematology or coagulation parameters, cardiac markers, alloantibody responses or macroscopic or microscopic findings.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Graft versus Host Disease				
Evaluation of the Role of MultiStem in Syngeneic Bone Marrow Engraftment Post-lethal Irradiation	RM-041006-01FR	5 million rat MultiStem cells (25 million/kg)	Lewis rats received a lethal irradiation dose of 1,200 cGy, given in two separate doses of 600 cGy to decrease systemic toxicity, followed by IV infusion with $2x10^5$ syngeneic bone marrow cells, a dose insufficient for long-term survival. After 24 hours, 6 animals were infused with $5x10^6$ syngeneic MultiStem cells (25 million cells/kg equivalent) and 6 animals received PBS injections. Groups were evaluated daily for survival over 19 days.	Administration of syngeneic MultiStem cells following lethal irradiation and infusion of syngeneic bone marrow cells increased survival rate of Lewis rats by 3 days. However, no long-term benefit was observed, as all rats died by Day 19.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Evaluation of MAPC Benefit in a Rat (P to F1) Acute GvHD Model	RM-060401-01FR	2.5-5 million rat MultiStem cells (12.5-25 million/kg)	F1 recipient rats, acquired from Lewis x Buffalo cross-breeds, were sub-lethally irradiated with 600 cGy in a single dose. The same day, the animals were injected IV with $2x10^7$ Buffalo Bone Marrow cells and $10x10^7$ Buffalo T-cells (splenocytes). Group 1 received PBS vehicle injections without MultiStem, group 2 received 12.5 million Lewis MultiStem cells per kg on Day 1 and group 3 received 12.5 million Lewis MultiStem cells per kg on both Day 1 and Day 8. This timing of administration, prior to the onset of GVHD, is consistent with the evaluation of MultiStem as a prophylactic.	Untreated animals displayed significant weight-loss and other clinical GVHD symptoms, including reduced activity, redness of skin, hair loss and crouching, and generally died between Days 16 and 26. FACS analysis confirmed that clinical GVHD symptoms coincided with engraftment of Buffalo donor T-cells. In MultiStem treated groups, a survival advantage was seen and Log-rank survival analysis showed that the survival profile in the group that received two MultiStem doses was significantly different from the PBS control group ($p=0.016$).

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Graft versus Host Disease				
Evaluation of Human MultiStem in Murine GvHD Model Studies	RM-120808-01FR	0.125 – 1 million human MultiStem cells	B6D2F1(H-2bxd) mice received 13 Gy irraditaion in a split dose separated by 3 hours to reduce the effects of gastrointestinal toxicity prior to receiving bone marrow and T-cells from either naïve allogeneic B6 (H-2b) or syngeneic B6D2F1 donors. Transfer of naïve allogeneic B6 T-cells resulted in an aggressive GVHD response with significant mortality by Day 42 post transplant. Human MultiStem was infused in an escalating dose tier from 125,000 to 1 million cells, and in some cases with repeated administration on a weekly basis. At time of sacrifice animals were assayed for clinical score, histopathology and immune markers of inflammation.	In comparison to disease-induced controls, animals receiving human MultiStem showed a significant survival benefit. A dose-dependent survival response was noted. In addition, significant improvements in gut pathology were observed, consistent with the analysis of inflammatory mediators as described above.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Stroke				
Phase III Neonatal Rat Hypoxic-Ischemic Injury Study: MultiStem IV Dose Escalation	RM-060914-02FR	0.01 – 1 million cells/dose x 1 dose	Hypoxic-ischemic injury was surgically induced in neonatal rats and the animals (8/group) were subsequently administered a single IV dose of 0.01, 0.05, 0.1, 0.2, 0.5 or 1 million rat MultiStem cells (0.05 to 5 million cells/kg) or irradiated non-viable cells (control) 7 days post injury. Animals were evaluated for locomotor deficits and graft cell persistence, endogenous neuroprotection, and toxicological endpoints including clinical observations throughout the 1 year follow-up period.	Statistically significant sustained functional recovery in locomotor function was observed at ≥ 0.1 million cells/animal (0.5 million cells/kg) that persisted for ≥ 1 year post-dose. Improvements in viable endogenous neurons in the hippocampus were also detected in the brains of injured animals treated with rat MultiStem. There were no MultiStem-related clinical observations or changes in body weight or clinical pathology parameters. There was a small increase in spleen weights of MultiStem treated animals as compared to control animals but the mean spleen weights were within the historical control range and there were no correlating macroscopic findings.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Stroke				
Phase III Rat Pre-Clinical Stroke Study: IV Dose Escalation of Human MultiStem	RM-060914-03FR	0.4 – 20 million human MultiStem cells/dose (1.2 to 60 million cells/kg)	A single dose of human MultiStem cells was administered by IV injection to male rats 2 days after induction of MCAL ischemic stroke. Animals received 0.4, 1, 2, 4, 10 or 20 million cells/dose (1.2 to 60 million cells/kg) or 10 million irradiated non-viable human MultiStem cells (negative control). Locomotor and neurological functions were assessed starting at 2 weeks post-MultiStem cell injection and every 2 weeks thereafter until the end of the study (12 weeks).	Statistically significant ($p<0.05$) recovery of locomotor and neurological function was observed from 2 to 12 weeks post-MultiStem cell injection, respectively, at doses ≥ 1 million cells. The dose effects reached a plateau at 4 million cells/animal (~12 million/kg).

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Stroke				
Pre-Clinical Optimization of Immunosuppression, Route of Administration and Window of Therapeutic Benefit in a Rat Model of Ischemic Stroke	RM-050903-01FR	1 million human MultiStem cells IV (3 million cells/kg)	Rats received either 1 million human MultiStem cells (3 million cells/kg) 1 day after stroke (MCAL) surgery, 1 million human MultiStem cells 2 days after stroke surgery, or 1 million human MultiStem cells 7 days after stroke surgery. Some stroke injured animals received 1 million irradiated non-viable human MultiStem cells on Day 7 after stroke surgery as a negative control. At 14 days post-transplantation and then every 14 days for 8 weeks, animals were evaluated for locomotor and neurological deficits. Following testing on Day 56 post-transplantation, animals were sacrificed and evaluations of cell persistence and protection of endogenous neuronal tissue were determined by IHC evaluations and quantitative microscopic analyses.	A single IV dose of 1 million human MultiStem cells (3 million cells/kg) in rats with ischemic stroke injuries administered any time up to 7 days post-injury resulted in statistically significant improvements in both locomotor and neurological test results out to 56 days post MultiStem cell injection. Evaluation of endogenous neuronal survival indicated that earlier administration of MultiStem cells results in statistically significant protection of endogenous neurons.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Stroke				
Pre-Clinical Optimization of Immunosuppression, Route of Administration and Window of Therapeutic Benefit in a Rat Model of Ischemic Stroke	RM-050903-01FR	0.4 million allogeneic rat or xenogeneic human MultiStem cells/animal (1.2 million cells/kg) into brain	Rats received a single dose (0.4 million cells/animal or 1.2 million cells/kg) of allogeneic rat MultiStem cells or xenogeneic human MultiStem cells via direct stereotactic delivery to the brain 7 days after MCAL stroke surgery. All animals which underwent immunosuppression received cyclosporine A daily at a dose of 10 mg/kg, IP, throughout the study. Locomotor and composite neurological testing were performed weekly to evaluate the status of the animals.	Statistically significant improvements in locomotor and neurological testing were observed when compared to non-viable cell treated control animals. The improvement was statistically significant with cyclosporine A or with vehicle. There were no observations of tumor or ectopic tissue formation.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Transplantation of MultiStem in a Rat Occlusion Model for Stroke: Proof-of-Concept Study	RM-040916-01FR	Direct striatal injection of 0.1, 0.2, or 0.4 million human MultiStem cells/animal	Rats received a single dose (0.1, 0.2 or 0.4 million cells) of human MultiStem cells via direct stereotactic delivery to the brain 7 days after after MCAO stroke surgery. At 7 and 14 days post MultiStem-transplantation animals were evaluated for locomotor and neurological deficits using EBST and composite Bederson test, respectively. Following testing on Day 14 post-transplantation, animals were sacrificed and evaluations of cell persistence and cell migration were performed using immunohistochemistry and microscopy techniques.	Significant improvements in locomotor and neurological tests were observed when compared to vehicle-treated animals. There was a dose-dependent improvement in animals receiving 0.2 and 0.4 million cells, showing statistically significant improvement as early as 7 days post-MultiStem injection. No ectopic tissue was observed in the brain at the gross level.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Stroke				
Transplantation of MultiStem in MCA Ligation Rodent Stroke Model: Proof-of-Concept Study	RM-050822-03FR	Direct striatal injection of 0.1, 0.2, or 0.4 human MultiStem cells/animal	Rats received a single dose (0.1, 0.2 or 0.4 million cells) of human MultiStem cells via direct stereotactic delivery to the brain 7 days after after MCAL stroke surgery. At 7 and 14 days post MultiStem-transplantation animals were evaluated for locomotor and neurological deficits using EBST and composite Bederson test, respectively. Following testing on Day 14 post-transplantation, animals were sacrificed and evaluations of cell persistence and cell migration were performed using immunohistochemistry and microscopy techniques.	Statistically significant improvements in locomotor and neurological tests were observed when compared to vehicle-treated animals at 14 days post-MultiStem dose. There was a trend towards increasing improvement at increasing cell dose. Histological evaluation suggested graft cells transplanted into the striatum migrated to the site of injury in the cortex. No ectopic tissue was observed in any animal.
Ulcerative Colitis				
No studies specific to ulcerative colitis have been performed. Models of GvHD in rats and mice involve intestinal injury with disease pathology akin to that occurring in UC; therefore, the nonclinical studies conducted in GvHD rodent models support the clinical investigation of MultiStem in UC patients.				

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Solid Organ Transplant				
Effect of MultiStem on Graft Survival in a Rat Cardiac Transplantation Model	Eggenhofer et al., 2013	Donor-type or third-party MultiStem: 2 to 10 million cells/dose x 1 dose IV or 5 million cells/dose x 2 doses IV or intrasplenically	Lewis rats were used as heart transplant donors, and Black Agouti rats served as recipients. Heterotopic heart transplantation was performed by end-to-side anastomosis of the large cardiac vessels to the recipient intraabdominal aorta and vena cava. Graft rejection time was defined as time from transplantation to the day at which no cardiac contractions were palpable, with verification of rejection by inspection of the allograft via laparotomy in some animals. Cell therapy consisted of donor-type MultiStem cells given as a single IV injection (2, 4 or 10 million cells on Day -4) or two intrasplenic injections (5 million cells on Day -4 and Day 0). Third-party MultiStem cells were given as two IV injections (5 million cells on Day -4 and Day 0) or a single intrasplenic injection (5 million cells on Day 0). MMF diluted in glucose 5% solution was injected into the peritoneal cavity at a dosage of 20 mg/kg/day from Day 0 to Day 7 in designated experiments.	<u>Results with donor-type MultiStem</u> IV MultiStem on Day –4 had a positive effect on allograft survival in 20%–30% of experiments. Dose escalation from 2 to 10 million cells did not increase the beneficial effect. IV administration of 5 million MultiStem cells on Days –4 and 0 had a positive effect on allograft survival in 40% of experiments. Intrasplenic administration of 2x5 million MultiStem cells on Days –4 and 0 had a positive effect on allograft survival in 80% of experiments. <u>Results with third-party MultiStem</u> Intrasplenic administration of 5 million MultiStem cells on Day 0 had a positive effect on allograft survival in 40% or 20% of experiments. IV administration of 2x5 million MultiStem cells on Days –4 and 0 had a positive effect on allograft survival in 60% of experiments. Intrasplenic administration of 2x5 million MultiStem cells on Days –4 and 0 had a positive effect on allograft survival in 60% of experiments.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Human adult bone marrow-derived stem cells decrease severity of lipopolysaccharide-induced acute respiratory distress syndrome in sheep	Rojas et al., 2014	4, 10 or 40 million human MultiStem intrabronchially	Fourteen adult Dorsett Cross sheep received intravenously via the Swan-Ganz catheter 1 and 3.5 µg/kg E. coli endotoxin LPS from E. coli 055:B5 in normal saline over 30 minutes at 0.7 mL/minute to induce moderate-to-severe ARDS, as defined by the ARDS Definition Task Force (Ranieri, 2012). The experimental group received 4, 10 or 40 million MultiStem cells intrabronchially into the lower left lung 30 minutes after the end of LPS infusion. The control group received the same volume intrabronchially of saline.	After administration of endotoxin, there was a rapid decline in oxygenation to hypoxemic values, indicative of severe-to-moderate ARDS. None of the animals treated with saline solution recovered to normal baseline values during the 6 hours that the animals were followed. In contrast, sheep treated with a dose of 40 million MultiStem returned their levels of oxygen in their blood to baseline two hours after the cells were infused. Similarly, improvements in carbon dioxide (CO2) clearance, pulmonary vascular pressures and inflammation were observed and confirmed by histology and by the decrease in lung edema. MultiStem can diminish the impact of endotoxin and accelerate recovery of oxygenation, CO2 removal and inflammation in the ovine model.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Multipotent adult progenitor cells decrease cold ischemic injury in *ex vivo* perfused human lungs: an initial pilot and feasibility study	La Francesca et al., 2014	10 million human MultiStem in 19 mL sterile saline	Four donor lungs not utilized for transplant underwent 8 h of cold storage (4°C). Following rewarming for approximately 30 min, non-HLA-matched allogeneic MAPCs (1×10^7 MAPCs/lung) were bronchoscopically instilled into the left lower lobe (LLL) and vehicle comparably instilled into the right lower lobe (RLL). The lungs were then perfused and mechanically ventilated for 4 h and subsequently assessed for histologic injury and for inflammatory markers in bronchoalveolar lavage fluid (BALF) and lung tissue.	All LLLs consistently demonstrated a significant decrease in histologic and BALF inflammation compared to vehicle-treated RLLs. These initial pilot studies suggest that use of non-HLA-matched allogeneic MAPC during donor lung processing can decrease markers of cold ischemia-induced lung injury.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Safety Pharmacology				
Measurement of Respiratory Rates for Evaluation of Infusional Toxicity after IntravenousInjections of Allogeneic Rat MultiStem	RM-051012-03FR	1.2 – 40 million rat MultiStem cells/dose (6 to 200 million cells/kg) x 1 dose IV 2.5 million rat MultiStem cells/dose (12.5 million cells/kg) x 5 doses IV	Anesthetized Lewis or Buffalo rats were given allogeneic rat MultiStem cells (Sprague-Dawley or Lewis rats, respectively) by IV injection, or Lewis rat derived splenocytes as a control, at doses ranging from 1.2 to 40 million cells/dose. RR was measured by visually counting the number of chest movements per unit of time at baseline (0 min), before cell infusion, and at 1, 5 and 10 minutes after injection of cells. In addition, lethally irradiated Buffalo rats were IV infused with syngeneic bone marrow mononuclear cells, followed by repeated IV injections of allogeneic Lewis rat MultiStem cells at 0 (PBS control) or 2.5 million cells/dose on Days 1, 8, 15, 22 and 29 and RR was measured for 12 minutes following each MultiStem cell administration.	IV infusion of allogeneic MultiStem was well tolerated, without evidence of pulmonary distress following single doses up to 200 million cells per kg or cumulative effects after five repeated infusions of 12.5 million cells per kg.

Table 5-2. Overview of *In Vivo* Pharmacology Studies

Study Title	Study Number / Reference	Dose	Study Protocol	Results and Conclusions
Safety Pharmacology				
Safety of Repeated Administration of Allogeneic MultiStem in Rats	RM-060401-03FR	2.5 million rat MultiStem cells/dose x 1 or 5 doses IV	A repeat-dose study was conducted with rat MultiStem cells in a rat model of HSCT. Buffalo rats were lethally irradiated and infused with 600,000 syngeneic bone marrow mononuclear cells. Rats then received 0 (PBS control) or 2.5 million rat allogeneic MultiStem cells/dose once weekly for 5 doses or as a single dose on Day 2. Respiration rates were measured immediately upon infusion, as a measure of respiratory distress.	No major differences were observed in the RR of animals receiving rat allogeneic MultiStem cells after single or multiple doses as compared to control animals.

AMI = acute myocardioal infarction; βgal = β-galactosidase; EBST = elevated body swing test; ECG = electrocardiogram; FACS fluorescence-activated cell sorting; GFP = green fluorescent protein; GLP = Good Laboratory Practice; GvHD = graft versus host disease; HSCT = human stem cell transplantation; IHC = immunohistochemistry; IV = intravenous; IP = intraperitoneal; LAD = left anterior descending artery; LV = left ventricular; MAPC = multipotent adult progenitor cells; MCAL= middle cerebral artery ligation; MCAO = middle cerebral artery occlusion; MMF = mycophenolate mofetil; MRI = Magnetic resonance imaging; MRS = magnetic resonance spectroscopy; PBS = phosphate-buffered saline; RR = respiratory rate

5.1.2.1 Acute Myocardial Infarction

In vivo studies using rat and pig AMI models showed that direct myocardial injection of allogeneic MultiStem post-AMI results in significant increases in functional performance compared to vehicle controls. In the rat, myocardial MultiStem injection of 10 million cells after AMI resulted in significant increase in vascular density within the infarct zone, with < 10% of MultiStem remaining at 6 weeks. In pig studies, approximately 0.3-0.4% cell engraftment was observed following direct injection into the peri-scar area (5 injections of 10 million cells each), with cells frequently found as vessel wall components. Pig studies also showed increased vascular density around MultiStem injection areas. Administration of cyclosporine A did not affect cell retention or improvement of cardiac performance, supporting therapeutic benefit of allogeneic MultiStem, without need for tissue matching or co-administration of immunosuppressive agents.

Studies in the pig AMI model showed higher cardiac persistence of MultiStem upon transarterial injection (Mercator MedSystems catheter) as compared to intracoronary injection (Edwards catheter). Allogeneic pig MultiStem (50 million cells) delivered by transarterial catheter also provided statistical benefit to left ventricular heart function at 4 weeks post dose. It was also shown that a dose of 20 million cells produced significantly less ventricular remodeling compared to a dose of 200 million cells. The results from these studies support the therapeutic hypothesis of improvement of heart function following MultiStem treatment of acute ischemic injury by enhancement of re-vascularization.

5.1.2.2 Graft versus Host Disease

Studies were conducted to evaluate the efficacy of MultiStem cells in rodent models of HSCT and acute GvHD.

The effects of rat MultiStem cells were evaluated in a rat model of HSCT in which rats were sublethally or lethally irradiated followed by IV injection of bone marrow mononuclear cells and then IV infusion of MultiStem cells 24 hours later. These studies showed that rat MultiStem cells, at a dose of 2.5 million cells, do not interfere with the autologous or syngeneic hematopoietic reconstitution of either sublethally or lethally irradiated rats. In addition, there was a short-term survival benefit after IV administration of a dose of 5 million MultiStem cells in that the 50% mortality rate was extended from 11 days for controls to 14 days for MultiStem-treated rats; however, no long term survival benefit was observed as all rats died by Day 19.

Studies were conducted to determine the efficacy of rat or human MultiStem cells in a rat or mouse acute GvHD model, respectively. In an acute GvHD rat model, animals receiving IV doses of 2.5 million allogeneic rat MultiStem cells (12.5 million cells/kg) on Days 1 and 8 showed a statistically significant dose-dependent survival advantage over control groups. Additionally, human MultiStem cell treatment resulted in significant efficacy in a

murine model of GvHD. Intravenous administration of 1 million human MultiStem cells on Days 1 or 4 after transplantation reduced splenic cell proliferation and activation of T-cells at Day 14, reduced the levels of circulating TNF-α at Day 14, reduced lung, liver and GI tract pathology typically associated with acute GvHD, and protected against early weight loss at Day 21.

5.1.2.3 Ischemic Stroke

A series of *in vivo* experiments with rat or human MultiStem cells were conducted to determine their efficacy in two different rodent models of ischemic stroke.

In a neonatal rat model of hypoxic-ischemic injury, a single dose of 0.2 million rat MultiStem cells/animal (1 million cells/kg) via direct intracranial or IV injection resulted in statistically significant improvement in locomotor tests and viable neurons in the hippocampus when compared to controls at 2 weeks post-MultiStem cell injection. In a subsequent dose escalation phase it was shown that statistically significant sustained functional recovery in locomotor function was observed at ≥ 0.1 million cells/animal (0.5 million cells/kg) that persisted for 1 year post-dose. Improvements in viable endogenous neurons in the hippocampus were also detected in the brains of injured animals treated with rat MultiStem cells.

Ischemic stroke was induced in adult rats by temporarily occluding the MCAO or permanently surgically ligating the distal portion of the MCAL. Human cells were administered directly into the brain of injured rats in both stroke models. In both studies, cells were found to be safe, and result in statistically significant improvement in gross motor function, 2 weeks after cell administration. Subsequently, human MultiStem cells were administered intracranially versus intravenously at different dose levels, at different times after the induction of the injury (1 day, 2 days, or 7 days after initiation of stroke), or with and without immunosuppressive drugs to address translational questions relevant to potential clinical testing in humans. Locomotor and composite neurological testing was performed weekly to evaluate the status of the animals. Results of this study demonstrated statistically significant ($p<0.05$) recovery of locomotor and neurological function was observed from 2 to 12 weeks post-MultiStem cell IV injection, respectively, at doses of ≥ 1 million cells. The dose effects of cells given intravenously reached a plateau at 4 million cells/animal (~12 million/kg). A dose of 1 million cells when delivered 1, 2 or 7 days post-MCAL surgery provided sustained and statistically significant motor and neurological benefit up to 56 days post-surgery. When delivered 1 or 2 days post-surgery, statistically significant neuroprotection was observed in terms of the number of viable neurons.

Direct stereotactic delivery of a single dose of 0.4 million cells/animal (1.2 million cells/kg) of either allogeneic rat MultiStem or xenogeneic human MultiStem, into the brains of rats 7 days after MCAL stroke surgery, resulted in statistically significant improvements in

locomotor and neurological testing when compared to non-viable cell treated control animals. The improvement was statistically significant with and without cyclosporine A, indicating immunosuppression is not required for the cells to exert their benefit in this model.

These cumulative data demonstrated the potential of MultiStem to provide therapeutic benefit for treatment following ischemic stroke.

5.1.2.4 Ulcerative Colitis

The nonclinical strategy for the evaluation of MultiStem in treatment of UC is to use the data from the nonclinical pharmacology studies that support the clinical investigational use of MultiStem cells in the treatment of GvHD. The rationale for using data from the nonclinical GvHD studies to support the use of MultiStem cells in the UC setting in humans is based on the significant commonality of pathology between these two disorders, both of which are immune system related.

Administration of MultiStem cells in nonclinical models of GvHD indicated that these cells distributed to the intestinal tract after IV dosing and provided protection from inflammatory damage. MultiStem cells provided survival benefit in lethal acute GvHD models in part by reducing intestinal pathology while preserving intestinal function allowing the animals to regain weight. MultiStem cell treatment resulted in significant efficacy in a murine model of GvHD with almost complete reversal of GI tract pathology including observed changes in intestinal crypts and villi. That MultiStem cells afford protection from intestinal disease in nonclinical GvHD studies provides the rationale for evaluation of MultiStem benefit in UC patients suffering from long-term inflammatory disease.

5.1.2.5 Solid Organ Transplant

In support of the solid organ transplant indications, a heterotopic cardiac transplant model study was conducted with MultiStem cells in rats. A mycophenolate-based immunosuppression regimen was used in this study based on literature showing that mycophenolate-based immunosuppression prolongs donor-MSC–induced allograft survival (Popp et al., 2008), whereas calcineurin inhibitor (CNI)-based immunosuppression reduces the positive immunomodulatory effect of MSCs (Inoue et al., 2006).

A modest increase in graft survival was seen after IV injection of a single dose of donor-type MultiStem cells on Day -4. Dose escalation from 2 to up to 10 million cells did not increase the beneficial effect of MultiStem. Intravenous injection of 2 doses of donor-type MultiStem (5 million cells/dose) on Days -4 and 0 markedly improved graft survival, and a further improvement in graft survival was seen when donor-type MultiStem (5 million cells/dose) was applied intrasplenically. Intravenous injection of 2 doses of third-party

MultiStem (5 million cells/dose) significantly prolonged survival of fully mismatched allogeneic grafts. As already observed with donor-derived MultiStem, intrasplenic injection of third-party MultiStem (5 million cells/dose) further improved graft survival, implying that the liver is a prime site of immunological engagement in allograft rejection.

This study demonstrated that MultiStem cells mediate long-term acceptance of fully mismatched vascularized heart grafts when administered concurrently with low-dose CNI–free immunosuppression in rats.

In further cardiac transplant studies, MAPC have been shown to confer long term protection from allogeneic immune rejection by inducing regulatory T-cells in the tissue environment subject to immune rejection (Eggenhofer et al., 2013). Protection can be long lasting (>100 days), and allogeneic tissues can be re-transplanted to allogeneic recipients at 100% frequency without immunosuppressive drug or cell treatment. MAPC induce a protective immune homeostasis preventing recurrent allogenic recognition and rejection responses.

5.1.2.6 Acute Respiratory Distress Syndrome

Because the most common cause of ARDS in humans is sepsis, infusion of endotoxin has been used as an animal model of sepsis related lung injury. Emerging evidence suggest protective effect of MAPC on ARDS. Intravenous (IV) or intrabronchial infused exogenous MAPCs can orchestrate the multiple processes that mitigate ARDS and induce repair in the lung. The *ex vivo* perfused swine lung model demonstrated that MAPCs reduced the severity of endotoxin induced lung injury, decreased hypoxemia and improved the clearance of alveolar edema. Concurrently, an *in vivo* sheep model of mild to moderate LPS-induced ARDS demonstrated that after administration of endotoxin there was a decline in the levels of PO2, followed by a slow recovery. Animals dosed with 4 million MAPCs saw a moderate effect. However, animals dosed with 40 million MAPCs sustained a fast recovery, reaching baseline an hour after cell therapy. Endotoxin induced increase in vascular pressure was moderated in animals treated with MAPCs. MAPCs treatment induced lower plasma levels of pro-inflammatory cytokine IL-8 and reduced numbers of neutrophils.

These data in a sheep model of ARDS using MAPCs provide an important link between the experimental data obtained in rodents using related adherent stem cells and the potential to translate cell based therapy to subjects with ARDS, by showing that human MAPCs have efficacy in the setting of an endotoxin induced lung injury in the of a large animal lung.

5.1.3 Safety Pharmacology

Rat MultiStem cells were assessed in two rat respiratory safety pharmacology studies due to the observed accumulation of cells in the lung following IV administration. These studies in healthy rats or HSCT model demonstrated that IV administration of allogeneic rat MultiStem cells as a single dose up to 200 million cells/kg, or repeated doses (5 weekly injections of 12.5 million cells/kg per dose), did not cause pulmonary distress as measured by respiratory rate (RR).

5.1.4 Pharmacodynamic Drug Interactions

5.1.4.1 Acute Myocardial Infarction

In vitro studies were conducted to evaluate the growth, viability, and functionality (as measured by VEGF secretion) of MultiStem cells in the presence of drugs potentially used during treatment of patients following an AMI including clopidogrel, atorvastatin, metoprolol, acetyl salicylic scid, abciximab, eptifibatide, N-acetylcysteine, captopril, heparin and stents coated with rapamycin or paclitaxel (Study No. RM-070822-01FR). Only rapamycin reduced cell growth and VEGF production in MultiStem cells. These results confirm that rapamycin was functioning as a cell proliferation inhibitor at the concentrations tested.

5.1.4.2 Graft versus Host Disease

In vitro studies were performed to evaluate whether combinations of immunosuppressive drugs routinely used for prophylactic treatment of GvHD after allogeneic HSCT have any adverse effects on the viability, growth, and immunosuppressive activity of MultiStem cells (Study Nos. RM-060615-01FR; RM-070301-01FR). Combinations of methotrexate with tacrolimus or cyclosporine did not have a detrimental effect on short-term MultiStem viability or plating efficiency; however, MultiStem expansion in culture was entirely inhibited in the long-term (14 days) presence of these drugs at physiological levels, confirming that the drugs act as cell-proliferation inhibitors. These drugs did not impact the immunosuppressive activity of MultiStem cells as measured in T-cell activation assays.

Since patients that will be receiving MultiStem cells would also be receiving a HSCT prior to the infusion of MultiStem, the patients would be undergoing a conditioning regimen for this indication. The combinations of immunosuppressive and chemotherapeutic drugs used in the pre-conditioning include fludarabine, cyclophosphamide, anti-thymocyte globulin, and cyclosporine prior to bone marrow transplant and MultiStem infusion. After bone marrow transplant and MultiStem administration, patients will continue on a regimen of drugs including cyclosporine, hydrocortisone, methylprednisolone, and Granulocyte-Colony Stimulating Factor. Fludarabine and cyclophosphamide are stopped 2 days before

bone marrow transplant; therefore, these drugs were not tested. The other drugs did not interfere with MultiStem cell viability *in vitro* (Study No. RM-060301-09FR).

5.1.4.3 Stroke

The multi-drug regimens used in the acute-onset treatment of ischemic stroke patients include drugs described in Section 5.1.4.1. Medications to stabilize the patient and treat the ischemic attack may include statins, beta-blockers, antiplatelets, and anticoagulants. Examples of these drugs were tested with MultiStem and shown to have no impact on cell viability, growth or activity. In addition, Activase (recombinant tissue plasminogen activator) may be given to patients who have had a stroke within 4.5 hours of presenting in the urgent care facility. With a half-life of 3-5 minutes, Activase and its breakdown products will have cleared systemically at the time of MultiStem administration. Therefore, Activase was not tested in the *in vitro* studies described in Section 5.1.4.1.

5.1.4.4 Ulcerative Colitis

In vitro studies were conducted to evaluate the potential interaction of MultiStem cells with a range of drugs that may be used concomitantly in UC, including 5-aminosalicylic acid (5-ASA), budesonide, azathioprine, 6-mercaptopurine (6-MP), and a TNF inhibitor, adalimumab (Pfizer internal study). The studies showed that the concomitant administration of these drugs with MultiStem drug product are unlikely to have a significantly adverse effect on MultiStem cell viability or immunosuppressive activity.

5.1.4.5 Solid Organ Transplant

The possible interaction between MultiStem and a CNI-free immunosuppressant (i.e., mycophenolate) has been investigated (see Section 5.1.2.5). MultiStem mediated long-term acceptance of fully mismatched vascularized heart grafts when administered concurrently with mycophenolate immunosuppression in rats.

5.1.4.6 ARDS

In vitro studies were conducted to investigate possible interactions with MultiStem and drugs indicated for ARDS. No effect on growth or viability was shown for budesonide, prednisone, and a TNF inhibitor, adalimumab (Pfizer internal study, Study No. RM-100329-01FR). Viability was not affected at 24 hours when treated with Zosyn a combination of piperacillin and tazobactam (a penicillin antibiotic), cefepime (a cephalosporin antibiotic), vancomycin (a glycopeptide antibiotic), fentanyl, Versed, propofol, cisatracurium, omeprazole, norepinephrine or vasopressin (Study No. RM-141203-01FR). A macrolide antibiotic, rapamycin (Sirolimus) showed decreased cell growth and reduced VEGF production (Study No. RM-070822-01FR1). Long term exposure of the immunosuppressant, cyclosporine A, showed MultiStem growth inhibition

but had no effect on T-cell activation (Pfizer internal study). A commonly used drug in shock, hydrocortisone, had no effect on MultiStem (Study No. RM-060301-09FR).

A central line catheter is the primary venous entry point in patients with ARDS. These catheters are often coated with silver and antibiotics like chlorohexidine. As MultiStem is delivered primarily intravenously in the clinical indications, biocompatibility of the cells with the infusion tubing was tested and showed no negative effects on viability and recovery of MultiStem (Study No. RM-FR-023-15).

5.2 Pharmacokinetics and Product Metabolism in Animals

5.2.1 Absorption

The product is administered via IV infusion or locally (*e.g.*, to the heart for AMI) and therefore there are no relevant absorption processes.

5.2.2 Distribution

The nonclinical assessment of the pharmacokinetics of MultiStem cells focused on biodistribution and residual presence in tissue.

The biodistribution of rat MultiStem cells, following IV infusion of cells in a mouse model of GvHD, has been evaluated using *in vivo* imaging of cells labeled with a luciferase reporter. Imaging of animals in the model showed an initial accumulation of rat MultiStem cells in the lungs in the hours immediately following infusion, followed by re-distribution to the gastrointestinal (GI) tract over the following 24-48 hours. In the majority of samples, the bioluminescence signal was below the limit of detection by 10 days post dose suggesting the majority of the administered cells were cleared in this time frame (Study No. RM-080714-01FR).

Distribution to the GI tract was only observed in the circumstance of injury. Similar studies in an ischemic heart injury model showed distribution to the heart with no detectable accumulation in the GI tract (Study No. RM-080603-01FR). The persistence of MultiStem cells at 4, 8 and 12 weeks post infusion in sub-lethally irradiated NOD/SCID mice was assessed in blood/bone marrow and selected tissues using flow cytometry and immunofluorescence analysis, respectively (Study No. RM-051017-01FR; RM-060522-02FR). The reproductive organs and gut tissue sections were analyzed for the presence of MultiStem using a human-specific antibody to β2-microglobulin . In this study, no evidence of engraftment was observed in these tissues. The blood and bone marrow of these animals were evaluated for MultiStem presence using flow cytometry. One male animal was found to have low levels (1.09% human CD45+, mouse CD45-) in blood and no conclusive staining in bone marrow, but findings in all other animals were negative.

The biodistribution after transarterial catheter-based administration of MultiStem was examined in a pig model of AMI. Analysis of heart tissue at 2 and 8 weeks after administration of MultiStem demonstrated < 1% cell persistence in tissue sections of the heart (Study No. RM-051011-02FR).

In addition, 2- and 4-week biodistribution studies in the brains of stroke injured rats were conducted, and no detectable signal was found by qPCR for human cells in the animal brains at either time point (Study No. RM-061212-01FR).

5.2.3 Metabolism

Nonclinical metabolism studies have not been conducted since these are not relevant to a cell-based therapy.

5.2.4 Excretion

Nonclinical excretion studies have not been conducted since these are not relevant to a cell-based therapy.

5.2.5 Pharmacokinetic Drug Interactions

Nonclinical pharmacokinetic drug interaction studies have not been conducted as conventional studies of *in vitro* enzyme inhibition or P450 induction are not relevant to a cell-based therapy.

5.3 Toxicology

5.3.1 Brief Summary

Rat, pig and human MultiStem cells were assessed in a series of nonclinical studies outlined in Table 5-3.

The toxicology program focused on areas of particular importance for cell-based therapies, including single- and repeat-dose toxicity, immunogenicity, and tumorigenicity.

- Single-dose toxicity studies in mice and rats have been conducted using the IV and SC routes of administration with both human and rat MultiStem cells. The cells were well tolerated at up to 10 million cells/dose (500 million cells/kg administered SC) and up to 40 million cells/dose (200 million cells/kg administered IV) in mice and rats, respectively.

- Allogeneic rat MultiStem cells have been administered to rats in an IV study for up to 5 weeks in duration (once weekly dosing). The NOAEL in the 5 week study was 2.5 million cells/dose (12.5 million cells/kg). Two IV doses of 10 million cells/dose (50 million cells/kg) have been given one week apart to rats with no adverse effects observed.

- In studies using pig cells in AMI pig models, up to 200 million cells were delivered by a transarterial catheter into the pig coronary artery and observed to be well tolerated.

- Immunogenicity, as measured by alloantibody or T-cell responses, has not been observed with allogeneic rat MultiStem cells in rats.

- IV and SC studies to assess the ability of MultiStem cells to transform into ectopic tissue or tumors have been conducted in nude mice. MultiStem cells have not been shown to induce ectopic tissues or tumors.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Single-Dose Toxicity				
Evaluation of Clinical Pathology Following MultiStem Intravenous Injection	RM-060403-01FR	1 million cells/dose x 1 dose (mice) 10 million cells/dose x 1 dose (rats)	Two different human MultiStem lots (BMC108.1 and BMC112.1) and one rat MultiStem lot (lrBMC9.1) were tested. Sixteen NOD/SCID mice received 1 million human MultiStem cells per animal, infused in 100 µL/animal. In the control group 8 animals received PBS vehicle at 100 µL/animal. Two rats were injected with 10 million rat MultiStem cells, infused in 200 µL/animal. The control and test articles were administered by IV injection in a single bolus via tail vein.	IV administration of a single dose of human MultiStem cells in NOD/SCID mice, or syngeneic MultiStem in Lewis rats was well tolerated. There was no apparent hematology or clinical chemistry effects.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Single-Dose Toxicity				
Evaluation of Safety and Cell Persistence Following Direct and Catheter-based Delivery of MultiStem in Porcine Model of Acute Myocardial Infarction	RM-051011-02FR	50 million cells/site x 5 sites in peri-infarct zone x 1 dose 50 or 200 million cells/dose x 1 dose (intracoronary or transarterial catheter)	The study was divided into two phases. In Phase I, AMI was induced in female Yorkshire pigs by LAD ligation and animals were administered allogeneic pig MultiStem cells expressing βgal or PBS (control) via direct myocardial injection. In Phase II, AMI was induced in female Yorkshire pigs by balloon occlusion and animals were administered allogeneic pig MultiStem cells expressing βgal or PBS (control) via transarterial or intracoronary catheter. The animals were allowed to recover for 2 or 8 weeks. Clinical observations and body weights were evaluated weekly. Physical examinations were conducted pretest. Blood and urine samples for clinical pathology evaluations were collected from all animals prior to randomization and prior to necropsy.	During both Phases I and II, there were no test article-related effects on body weights, hematology or clinical chemistry parameters. During Phase I (direct injection), 3 of 13 animals died of refractory ventricular fibrillation after the initial ligation of the LAD and prior to treatment with the test article. One animal was initially placed on study as an 8-week animal. This animal was found dead on Day 16 and was reported as a 2-week animal. Upon necropsy, death was considered to be related to infarct, not test article. All other Phase I animals survived to the scheduled necropsy. During Phase II (catheter-based injection), 8 of 22 animals died during the 60-minute occlusion of the LAD or at reperfusion of the LAD and prior to treatment with the test article. All other Phase II animals survived to the scheduled necropsy.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Single-Dose Toxicity				
Transarterial Infusion of Pig MultiStem in the Coronary Artery with Severe Vascular Trauma and Pre-implanted Stent(s)	RM-061011-02FR	100 million cells/dose x 1 dose via transarterial catheter	Juvenile female domestic farm pigs were implanted with overlapping stents in segments of the LAD and LCX (50-60% overlapping). Vascular trauma was induced in the vessels distal to the pre-implanted overlapping stents by over-inflating angioplasty balloons to cause vascular injury. MultiStem cells expressing βgal were delivered via transarterial catheter to the injured vessel segments with overlapped stents. Intravascular ultrasound was used to evaluate the integrity of the stents before and after introduction of the transarterial catheter. Coronary angiography was used to assess vessel patency. Animals were sacrificed and the blood vessel segments, including stented and injured ones, were visually assessed for any damage. The heart was pressure fixed with 10% formalin for 30 minutes, and the stented and injured blood vessel segments were excised for further histological analysis to determine if catheter injection causes further injury to traumatized vessels.	Angiography results revealed no findings of significance, no disturbance to the overlapped stents or maintenance of vessel patency. Visual inspection of the catheters after cell injection revealed no significant damage to the catheters after passage through pre-implanted overlapping stents. Analysis of the injury scores determined by histological analysis of the vessels indicated that there was no significant difference ($p=0.92$) in the injury to the control vessels compared to the vessels that had cell injections. X-gal staining of the surrounding tissue at the injection site revealed the presence of positive staining indicating the successful delivery of βgal labeled MultiStem cells. Intravascular ultrasound data revealed no significant changes in the stent architecture before or after the transarterial catheter

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Single-Dose Toxicity				
A GLP Study to Evaluate the Safety and Efficacy of Allogeneic Pig MultiStem Cells in an Acute Myocardial Infarction Model in Pigs	RM-060523-01FR	20 or 200 million cells/dose x 1 dose via transarterial catheter	AMI was induced in pigs on Day 0 and the animals were randomly assigned to three treatment groups that received low dose (20 million) or high dose (200 million) βgal-labeled allogeneic pig MultiStem cells or vehicle (Plasma-Lyte A), via transarterial catheter two days after the AMI (Day 2). Four animals from each of these groups were assessed for acute safety on Day 9. All other animals were assessed for long-term safety and efficacy and remained on study for a 91-(+8) day recovery period. Primary endpoints were safety (lack of arrhythmia, acute morbidity/mortality, gross necropsy) and efficacy of the cells as demonstrated by better cardiac function in the cell-treated groups compared to the control group.	MultiStem cells were not associated with any adverse clinical observations, bodyweight changes, hematology or coagulation parameters, alloantibody responses or macroscopic or microscopic findings. Mild changes in clinical chemistry values were evident and were considered to be related to the infarct procedure and tended to resolve between 30 and 90 days post injections. MultiStem cells did not affect cardiac biomarkers. Increases in Troponin I and CK-MB were noted in all groups and were considered to be a result of the infarct procedure. Increases in total CK noted on Day 2 at 6 hours post-injection were considered to be a result of the surgical procedure.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Single-Dose Toxicity				
Acute Observations and 28-Day Histopathology of Porcine Coronary Arteries after Saline/Contrast or MultiStem Infusion with MDL09-2040-145-34	RM-110308-01FR	50 million cells/dose x 1 dose via transarterial catheter	Female Domestic Yorkshire crossbred swine received infusions of human MultiStem cells or vehicle (in 4% DMSO and 20% contrast solution) administered into the LAD by transversing a pre-implanted stent using 2 different models of micro-infusion catheter. The animals were allowed a 27-day recovery period following implantation/dosing. Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Clinical observations and body weights were evaluated weekly. Physical examinations were conducted pretest. Blood samples for clinical pathology evaluations were collected from all animals pretest, on Day 0 (following intervention), and prior to necropsy. At study termination, complete necropsy examinations were performed and select tissues were microscopically examined. Hearts were removed and histopathology and histomorphometry evaluations were performed.	Infusion of the test article using the injection catheters resulted in a severe chronic inflammatory infiltrate, characterized by numerous lymphoid nodules admixed with lymphocytes, plasma cells, macrophages and fewer numbers of eosinophils and pigmented macrophages. Occasionally, mineral deposits surrounded by multinucleated giant cells were observed. Generally, no appreciable difference in the microscopic findings at coronary artery sites 1 cm proximal to the infusion sites was observed between control and treated sites. No difference in microscopic findings was observed between the two delivery devices. There were no delivery device, test or vehicle related effects on body weight, clinical chemistry, coagulation times or hematology parameters in either treatment group at the post intervention and recovery intervals compared to pretest.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Single-Dose Toxicity				
28-Day Histopathology of Porcine Coronary Arteries after Vehicle/Contrast, Porcine MultiStem or human MultiStem Infusion with MDL09-2040-145-34	RM-110822-01FR	50 million cells/dose x 1 dose via transarterial catheter	Female Domestic Yorkshire crossbred swine received infusions of human or pig MultiStem cells or vehicles (in 4% DMSO and 20% contrast solution or Plasma-Lyte A and 20% contrast solution) administered into the LAD by transversing a pre-implanted stent using a micro-infusion catheter. The animals were allowed a 27-day recovery period following implantation/dosing. Observations for morbidity, mortality, injury, and the availability of food and water were conducted twice daily for all animals. Clinical observations and body weights were evaluated weekly. Physical examinations were conducted pretest. Blood samples for clinical pathology evaluations were collected from all animals pretest, on Day 0 (following intervention), and prior to necropsy. At study termination, complete necropsy examinations were performed and select tissues were microscopically examined. Hearts were removed and histopathology and histomorphometry evaluations were performed.	No adverse test or control article related events were observed when evaluating clinical observations, body weight values, clinical pathology parameters or macroscopic observations. At the microscopic level, infusion of the first vehicle control alone (4% DMSO, 4% HSA) resulted in mild chronic inflammatory infiltrate at a lower incidence when compared to the infusion of pig or human Multistem cells delivered in either the first (4% DMSO, 4% HSA) or the second (Plasma-Lyte A) vehicle control. A similar inflammatory pattern was not present at infusion sites receiving the second vehicle control alone (Plasma-Lyte A). The mild to moderate chronic inflammatory infiltrate observed at the infusion sites treated with the pig or human Multistem cells with either the first (4% DMSA, 4% HSA) or the second (Plasma-Lyte A) vehicle control was characterized by formation of many lymphoid nodules admixed with lymphocytes, plasma cells, eosinophils, and macrophages.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Single-Dose Toxicity				
Evaluation of Human MultiStem Engraftment and Differentiation in a NOD-SCID Mouse Model	RM-050822-01FR	1 million cells/dose x 1 dose	One million human MultiStem cells (lot BMC98) were infused into sub-lethally irradiated NOD/SCID mice, for an approximate final dose of 50 million cells/kg. Engrafment was assessed at 4, 8, and 12 weeks. At 4, 8 and 12 weeks 7/8, 9/7, 11/13 (male/female, respectively) were infused with MultiStem cells; while PBS vehicle control group consisted of 4/3/3 males and 4/4/4 females at 4, 8, 12 weeks, respectively. Engraftment was assessed at 12 weeks. At sacrifice, tissue was collected from the injection site, spleen, liver, kidney, heart, brain, lung, pancreas, gut, and testes/ovaries for histological analysis. Histological sections of gut and gonads were also stained for human β2-microglobulin to identify human MultiStem cells in the mouse tissue background. Flow cytometric analysis of blood samples was used to determine the presence of human MultiStem cells in the hematopoietic system.	MultiStem cells did not cause lesions or form tumors/ectopic tissue. Evidence for hematopoietic engraftment of human cells (1-2%) was observed in a single MultiStem injected animal.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Phase III Neonatal Rat Hypoxic-Ischemic Injury Study: MultiStem IV Dose Escalation	RM-060914-02FR	10 thousand – 1 million cells/dose x 1 dose	Hypoxic-ischemic injury was surgically induced in neonatal rats and the animals were subsequently administered a single dose of 10,000 to 1 million rat MultiStem cells (50,000 - 5 million cells/kg) or 500,000 irradiated non-viable cells (control) by IV injection 7 days post injury. A subset of animals was evaluated for toxicological endpoints for a 1-year period including clinical observations, body and organ weights, and clinical and gross pathology under GLP conditions.	There were no MultiStem-related clinical observations or changes in body weight, clinical pathology or gross pathology parameters. There was a small increase in spleen weights of MultiStem-treated animals compared to control animals but the mean spleen weights were within the historical control range and there were no correlating macroscopic findings.
Single-Dose Toxicity				
Phase III Rat Pre-Clinical Stroke Study: IV Dose Escalation of Human MultiStem	RM-060914-03FR	0.4 – 20 million cells/dose x 1 dose	A single dose of human MultiStem cells was administered by IV injection to male and female rats 2 days after induction of ischemic stroke via MCAL. Animals received 400,000 to 20 million cells/dose (2 to 100 million cells/kg) or 10 million irradiated non-viable human MultiStem cells (negative control). A subset of animals was evaluated for toxicity (clinical and gross pathology, body and organ weights, and histopathology) for 1 year post-MultiStem cell injection. Clinical pathology and gross assessment of tissues was performed on animals from every group under GLP conditions.	There were no test article-related clinical observations or alterations in hematology, coagulation or serum chemistry parameters, final body weight, organ weights or gross pathology. Microscopic evaluation of multiple tissues from animals in the non-viable cell control, 1 million and 20 million MultiStem treatment groups revealed no abnormal tissue or findings.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Pivotal Study of Human MultiStem in a MCAO Stroke Model in Rats	RM-061212-01FR	1 or 20 million cells/dose x 1 dose	Short-term safety evaluations after IV infusion of human MultiStem in rats following MCAO occlusion. Animals were euthanized at either Day 14 or Day 28 after investigational product infusion.	There were no test article-related clinical observations, effects on body weight, food consumption or hematology and serum chemistry parameters. There were no test article related macroscopic findings or effects on organ weights.
Evaluation of the Safety of Intraportal Injection of MultiStem in a Rat Model	Eggenhofer et al., 2013	1.5 million cells/dose x 1 dose	ACI (MHC haplotype: RT1a) rats received 1.5 million rat MultiStem cells from Lewis rats in 500 µL PBS into the portal vein after appropriate anesthesia. Histomorphology was evaluated for sections of the hepatic parenchyma of untreated control rats and of rats at 5 minutes, 30 minutes, 2 hours, and 24 hours after injection.	There were no histomorphological differences in hepatic parenchyma between the control animals and animals that received an intraportal injection of MultiStem. None of the hepatic parenchyma specimens showed any pathologic changes, particularly in terms of necroses, infarctions, or corpuscular emboli.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Repeat-Dose Toxicity				
Safety of Repeated Administration of Allogeneic MultiStem in Rats	RM-060401-03FR	2.5 million cells/dose x 1 or 5 doses	A repeat-dose study was conducted with rat MultiStem cells in a rat model of HSCT. The repeat-dose study was conducted in Buffalo rats lethally irradiated and infused with 600,000 syngeneic bone marrow mononuclear cells. Rats then received 0 (PBS control) or 2.5 million rat allogeneic MultiStem cells/dose (12.5 million cells/kg) once weekly for 5 doses or as a single dose on Day 2. Respiration rates were measured immediately upon infusion, as a measure of respiratory distress. Rats also were monitored and graded twice a week for clinical parameters, including body weights, activity, posture, and paleness. Blood samples were drawn at various time points for analysis of circulating blood cells and comprehensive chemistry assessment. Sera were analyzed to evaluate allosensitization of T-cells in injected animals. Upon sacrifice, gross necropsy and histopathological analysis was performed on key tissues.	There was no MultiStem-related morbidity or mortality. There were no biologically or toxicologically important MultiStem-treatment related changes in clinical observations, clinical chemistry parameters or histopathology as compared to PBS-treated control animals. There was no evidence of respiratory distress upon infusion. There were no consistent treatment-related changes in immunogenicity as measured by alloantibody formation or allo-T-cell sensitization. The NOAEL was 2.5 million cells/dose (12.5 million cells/kg).

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Repeat-Dose Toxicity				
Evaluation of Hematopoietic Reconstitution in Irradiated Rats in the Presence or Absence of MultiStem	RM-060401-02FR	2.5 million cells/dose x 1 or 2 doses	The impact of rat MultiStem cells on the reconstitution of the hematopoietic system in non-immunocompromised rats was evaluated after sub-lethal irradiation (600 cGy), or after lethal irradiation (1,200 cGy), followed by HSCT. MultiStem was administered at a dose of 2.5 million cells on either one occasion (on Day 1), or two occasions (on Day 1 and Day 8). Hematopoietic reconstitution was evaluated by measurement of the number of neutrophils, white blood cells and/or platelets.	MultiStem did not interfere with reconstitution of the hematopoietic system, neither after sub-lethal irradiation, nor after lethal irradiation followed by HSCT. The rates and reconstitution levels of neutrophils, white blood cells and/or platelets in MultiStem recipients were the same as in control animals.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Repeat-Dose Toxicity				
Safety of Administration of Human MultiStem Processed in HypoThermosol in a Mouse Model of Graft versus Host Disease	RM-100730-01FR	1 million cells/dose x 5 doses	A repeat-dose study was conducted in a murine model of GvHD. Mice were induced for GvHD on Day 0 and 1 million human MultiStem cells (50 million cells/kg) in HTS were administered by IV injection once every three days (Days 1, 4, 7, 10 and 13) for 14 days. Untreated control groups included mice not induced for GvHD and mice induced for GvHD; treated control groups included mice induced for GvHD and administered either HTS or Plasma-Lyte A by IV injection at the same volume as the mice receiving MultiStem cells in HTS. Clinical observations for infusional toxicity and clinical evaluations for GvHD symptoms were completed. Histopathology analysis was performed on key tissues.	Acute infusion-related toxicity was not observed in any group. There were no biologically or toxicologically relevant differences in weight-loss or clinical scores between any of the groups. Gross pathology observations were as expected for GvHD animals and there were no other gross pathology findings. At the end of the in-life portion of the study there were no deaths in the non-GvHD group, 1/8 animals died in the GvHD (no vehicle) and Plasma-Lyte A control groups, and 4/8 and 3/8 animals died in the HTS and MultiStem in HTS groups, respectively. The early deaths in all groups were likely related to GvHD model-based radiation injury. Slight increases in the severity of lung pathology (GvHD related lesions of increasing thickness characterized by perivascular lymphocyte infiltration) were observed in HTS and MultiStem in HTS groups as compared to the treated and non-treated control groups.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Other Toxicity Studies - Tumorigenicity				
Evaluation of Human MultiStem Tumorigenicity in Nude Mice under GLP Conditions	RM-060522-02FR	1 million cells/dose x 1 dose	A tumorigenicity safety study was conducted with human Multistem cells in immunodeficient nude mice. Human Multistem cells, B16-F10 melanoma (positive control), or Plasma-Lyte A were injected IV into the tail vein of 20 animals (10 male and 10 female) per group. Detailed clinical examinations were conducted weekly to evaluate external organs, respiratory and circulatory effects, salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and bizarre behavior. Tissue pathology of the injection area (tail) and other relevant tissues was performed.	Administration of MultiStem was well tolerated by the mice. There were no adverse clinical observations or effects on body weights associated with MultiStem. In the B6-F10 group, 10 of 10 male and 9 of 10 female mice exhibited metastatic lung tumors. There was no test article-related macroscopic or microscopic pathology in the cellular test article or vehicle control groups.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Other Toxicity Studies - Tumorigenicity				
Evaluation of Human MultiStem Tumorigenicity in Nude Mice	RM-051017-01FR	7.4 – 10 million cells/dose x 1 dose	A tumorigenicity safety study was conducted with human MultiStem in immunodeficient nude mice. Mice (n = 2-5/sex/group) were injected subcutaneously with HT1080 (positive control), or one of four human MultiStem cell lines (BMC104.2, BMC105, BMC94 and BMC87.2). Animals were evaluated semi-weekly for tumor formation at the site of injection. Animals were sacrificed *in extremis* if tumors grew to a volume of >2000 mm^3 or after 12 weeks on study, whichever occurred first. Tissue pathology of the injection site as well as lymph nodes, liver lung, spleen, and kidneys was performed for all animals. Organ weights were assessed and the tissues preserved for microscopic examination.	Administration of MultiStem test article was well tolerated. There were no adverse clinical observations or effects on body weights associated with MultiStem administration and none of the mice formed tumors. In the HT1080 group, 4 of 5 male and 2 of 5 female mice formed tumors. There were no test article-related macroscopic observations or organ weight changes in the cellular test article groups.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Other Toxicity Studies - Tumorigenicity				
Evaluation of Tumor Formation in Nude Mice Following Subcutaneous Injection of Human MultiStem	RM-070117-01FR	10 million cells/dose x 1 dose	A study was conducted to evaluate tumorigenic potential of clinical-grade MultiStem following subcutaneous injection in athymic nude (nu/nu) mice. These mice, failing to mount a cell-mediated response against foreign materials, support the growth of tumor cell lines, permitting the assessment of allogeneic and heterogeneic cells to form neoplasms *in vivo*. Animals were randomized into 3 groups (n = 10 female mice/group): Group 1 was injected with MultiStem (10 million cells), Group 2 received 18C1-10T-cells (10 million cells, positive control) and Group 3 received PBS (vehicle control). All animals were observed daily and the injection site palpated twice a week for lesion development for a period of up to 84 days. Tissue pathology of the injection site, lungs, scapular lymph nodes, and gross lesions from each mouse was performed.	All positive control mice were necropsied 13 days post-injection (PI) as all mice (10/10) showed large masses (> 10mm diameter) at the injection site. All mice in the negative control group injected with PBS were necropsied 84 days PI. None of the animals (0/10) showed evidence of solid tumor formation. All mice in the test group injected with MultiStem were necropsied 84 days post-dose. None of these animals (0/10) showed evidence of solid tumor formation.

Table 5-3. Overview of Toxicology Program

Study Title	Study Number	Dose	Study Protocol	Results and Conclusions
Other Toxicity Studies – Antigenicity (Immunogenicity)				
Evaluation of T-Cell Sensitization and Alloantibody Generation after Intravenous Injection of Allogeneic MultiStem in Rats	RM-051026-01FR	1 – 10 million cells/dose x 1 or 2 doses 2.5 million cells/dose x 5 doses	Lewis or Buffalo rats were infused IV with allogeneic MultiStem cells from either Sprague-Dawley or Lewis rats, with control arms receiving splenocytes or PBS. Specific cohorts received booster injections with the same dose and type of cell at 35 days after the first cell injections. Specific experiments involved lethal irradiation of Buffalo rats followed by administration of autologous bone marrow cells at doses sufficient for survival, followed by 5x weekly infusions with allogeneic MultiStem cells over a period of 4 weeks.	Infusion with high doses (62.5 million cells/kg) of allogeneic MultiStem did not induce alloantibody formation or allosensitized T-cells. After infusion with allogeneic splenocytes significant allo T-cell sensitization and alloantibody generation was observed. Repeated IV administration (5x) of a high dose (12.5 million cells/kg) of allogeneic MultiStem did not result in the generation of detectable levels of alloantibodies, or in significant T-cell sensitization against allogeneic MultiStem.
Other Toxicity Studies – Genomic Stability				
Genomic Stability and Microarray Analysis of MultiStem	RM-051012-01FR	N/A	Two independent human MultiStem products (hBMC103.2 and hBMC104.2) were evaluated by transcription profiling, chromosome SNP analysis, gene methylation array analysis, and G-banding/karyotyping for normal and equivalent cytogenetics at early and late population doublings.	The MultiStem products displayed normal karyotype and G-banding, absence of significant chromosomal rearrangement by SNP analysis, and equivalency in transcriptional profiling and gene methylation profile between population doubling ~20 and 40.

AMI = acute myocardial infarction; βgal= β-galactosidase; DMSO = dimethyl sulfoxide; GLP = good laboratory practices; GvHD = graft versus house disease, HSCT = hematopoietic stem cell transplantation; h = hours; IV = intravenous; LAD = left anterios descending artery; MCAL = middle cerebral artery ligation; LCX = left circumflex artery; N/A = not applicable; PBS = phosphate buffer saline; SNP = Single Nucleotide Polymorphism; X-gal = 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

5.3.2 Single-Dose Toxicity

Two different human MultiStem drug product lots or one rat MultiStem cell lot were administered as single IV doses to non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice or Lewis rats, respectively. All MultiStem cells were well tolerated at 1 million cells/dose (50 million cells/kg) in mice and at 10 million cells/dose (50 million cells/kg) in rats. There were no treatment-related effects on clinical signs, body weights or other parameters in either mice or rats during the conduct of the study. There were some changes noted in the clinical pathology parameters; however, none of the findings were determined to be definitely related to MultiStem administration, nor were they deemed to be clinically meaningful.

In support of the AMI clinical program, the safety of local administration of pig MultiStem cells was evaluated in three single-dose studies in the porcine model of AMI and two studies in uninjured porcine arteries. Pig MultiStem cells were injected directly into the peri-infarct zone (50 million cells/site x 5 sites) or via intracoronary or transarterial catheters (20-200 million cells). These studies demonstrated the safety of transarterial catheter-based delivery of MultiStem. Additionally, this mode of delivery did not increase the injury to traumatized vessels or pre-implanted stents. Administration of pig or human MultiStem cells into uninjured porcine arteries did not produce any adverse effects up to 28 days post dose. However, human MultiStem infusions in a 4% DMSO and 20% contrast solution administered by transversing a pre-implanted stent resulted in a severe chronic inflammatory infiltrate as a possible immune response to the introduction of a xenogeneic test article into the myocardium of non-immunocompromised animals.

The potential of human MultiStem cells to engraft and potentially differentiate was assessed in the NOD/SCID mouse model. A single IV dose of 1 million human MultiStem cells (50 million cells/kg) was well tolerated with no evidence of adverse effects during 12 weeks of observation. Histopathology analysis revealed no pathologic lesions, tumors, teratomas, or ectopic tissue formation. Low levels (1% to 2%) of persistence of human MultiStem cells in peripheral blood were observed in one animal. No evidence was observed for the presence of MultiStem cells in either blood or bone marrow samples from the remaining MultiStem-treated animals.

In support of the clinical program for ischemic stroke, three *in vivo* single dose safety studies were performed to measure the effect of up to 20 million cells/dose of rat or human MultiStem transplanted into groups of neonatal or adult rats having undergone surgical induction of hypoxic-ischemic injury or ischemic stroke. In all three studies there were no MultiStem-related changes in body weight, food consumption, organ weights, clinical pathology, gross pathology, or histology when rats were necropsied and evaluated between 14 days (one study) and 1 year post-dose (two studies).

The safety of intraportal injection of MultiStem was evaluated in support of the SOT clinical program. Administration of 1.5 million cells of allogeneic rat MultiStem into the portal vein of rats produced no adverse effects on the hepatic parenchyma.

5.3.3 Repeat-Dose Toxicity

A repeat-dose study was conducted with rat MultiStem cells in a rat model of HSCT. Two females in the rat MultiStem cell group died on Days 17 and 18 due to insufficient bone marrow transplant and one male in the rat MultiStem cell group died on Day 14 due to irradiation-related complications. There was no other morbidity or mortality. There were no biologically or toxicologically important MultiStem-treatment related changes in clinical observations, clinical chemistry parameters, or histopathology as compared to control animals. There were no consistent treatment-related changes in immunogenicity as measured by alloantibody formation or allo-T-cell sensitization. The NOAEL for rat MultiStem cells following once weekly IV administration for 5 weeks was 2.5 million cells/dose (12.5 million cells/kg).

In another study in the rat model of HSCT, it was shown that rat MultiStem administered at a dose of 0.2 to 2.5 million cells as a single dose or 2.5 million cells/dose (12.5 million cells/kg) for one or two doses (on Day 1 and Day 8) did not interfere with reconstitution of the hematopoietic system after sub-lethal or lethal irradiation followed by autologous bone marrow infusion.

A repeat-dose study was also conducted in a murine model of GvHD to evaluate the safety and efficacy of human MultiStem in HTS when administered by IV injection once every three days (Days 1, 4, 7, 10 and 13) for 14 days. Control groups included untreated, HTS and Plasma-Lyte A. Day 14 was selected for necropsy, as this was deemed optimal for evaluation of animals with progressing GvHD without significant impact of GvHD-related mortality. Early deaths in all groups were likely related to GvHD model-based radiation injury. Slight increases in the severity of lung pathology (GvHD-related lesions) were observed in HTS and MultiStem in HTS groups as compared to the Plasma-Lyte A and non-treated control groups. The relationship between the slight increases in the severity of lung pathology to HTS or MultiStem in HTS administration could not be determined. In summary, HTS or MultiStem in HTS administered IV in mice with early onset GvHD did not elicit any biologically or toxicologically significant effects greater than those observed in control animals induced for GvHD.

5.3.4 Reproductive and Developmental Toxicity

Formal reproductive and developmental toxicity studies have not been conducted with human MultiStem cells.

5.3.5 Local Tolerance

Local tolerance studies with human MultiStem cells have not been conducted for IV infusion. However, injection sites were evaluated macroscopically and microscopically in several pharmacology and toxicology studies. Any findings were attributed to local trauma from injection. There were no MultiStem-related injection site macroscopic or microscopic findings observed in any studies using Plasma-Lyte A or HTS as diluents.

Other routes of administration of MultiStem, including intracranial, pericardial, and intraportal vein, have also been shown to be well tolerated in animal models of ischemic stroke in rats, AMI in pigs and healthy rats, respectively.

5.3.6 Other Toxicity Studies

5.3.6.1 Antigenicity (Immunogenicity)

In vivo immunogenicity with rat MultiStem cells has been assessed by evaluation of alloantibody formation or allo-T-cell sensitization in a stand-alone repeat-dose study in healthy rats and HSCT rat model and as part of a repeat-dose toxicity study in the rat model of HCST. Single or repeated IV dosing (5 weekly doses) did not result in the generation of detectable levels of alloantibodies, or in significant T-cell sensitization against allogeneic MultiStem cells.

In a safety pharmacology study, cytokine and histamine levels were assessed after two doses of rat MultiStem cells or one dose of human MultiStem cells; there were no changes in cytokine or histamine levels that were considered biologically significant at a dose of 10 million cells/dose (50 million cells/kg) during the course of the study. Acute hypersensitivity reactions directly related to MultiStem cell administration were not observed in any nonclinical single or repeated-dose IV studies using human or rat MultiStem cells.

In the Phase 1 AMI clinical trial, both humoral and cellular responses were examined in both the registry control and treatment patients to determine if an immune response occurred after exposure to MultiStem. Surprisingly, 50% of the registry control patients had detectable antibodies against HLA antigens at baseline. In the treatment group, 89% of the patients had no allogeneic antibodies and 2 patients had detectable antibodies against HLA antigens present on MultiStem. However, both patients had high allogeneic antibody reactivity before MultiStem delivery, making it difficult to ascertain whether a specific response to MultiStem was generated. No cellular immune response to MultiStem was observed as determined by using an MLR assay.

5.3.6.2 Tumorigenicity Studies

Three studies have been conducted in nude mice to evaluate the potential for tumor formation after IV or SC administration of human MultiStem cells.

There was no indication of malignancy or teratomas in the mice that received either MultiStem cells or vehicle (see Table 5-3).

In assessing biodistribution and persistence of rat or human MultiStem cells in rodent models it appears that most of the injected MultiStem cells are undetectable within a few days, but very low numbers of MultiStem cells may persist for up to 52 weeks in the hippocampus of rats administered cells by IV injection. Gross pathology and histopathology evaluations of multiple tissues have been conducted in pharmacology, toxicology, and tumorigenicity studies up to 1 year in duration after single or multiple IV, SC or intra-cranial infusions of rat or human MultiStem cells. There

were no MultiStem cell-related alterations in gross pathology or histopathological evaluation of multiple tissues that would be indicative of tumorigenicity identified in any of the studies.

5.3.7 Safety of Diluents

Most of the nonclinical studies supporting the safety of MultiStem cells were conducted with Plasma-Lyte A as the diluent for the cells and/or as a vehicle control.

The safety of HTS as a diluent was also assessed *in vitro* in a mast cell assay and *in vivo* in single dose mouse and rat studies and in repeat-dose mouse and cynomolgus monkey studies. HTS caused species-specific acute hypersensitivity reactions in rats due to one of its components, Dextran-40. However, HTS was well tolerated with no hypersensitivity observed in mice and cynomolgus monkeys.

5.3.7.1 Genomic Stability and Microarray Analysis of MultiStem Cells

The long term culturing of MultiStem cells will most likely be necessary to provide sufficient numbers of cells for therapeutic treatment. Therefore, the genomic stability of two lots of the cells in long-term culture was examined to confirm that genetically normal and stable cells are provided for clinical use (Boozer et al, 2009). Genomic analysis of early (~20 population doublings [PD]) and late human MultiStem cell cultures (~40 PD) demonstrated that little if any changes occurred in the genome with long-term culture (~40 PD). An examination of gene expression data revealed no major differences between the two lots. Karyotype analysis of early and late MultiStem cell cultures demonstrated that both cultures had normal phenotype as assessed by G-banding. A more detailed analysis of genomic stability was assessed by Single Nucleotide Polymorphism (SNP) analysis and showed similar results. Finally, the methylation profile analysis demonstrated few differences between early and late human MultiStem cell cultures. These results show that human MultiStem cells maintain their genomic stability during the culture periods required for manufacture of clinical grade MultiStem.

5.3.8 Target Organ Toxicity

Single and multiple IV dose nonclinical studies have been conducted with rat or human MultiStem cells in mice and rats. Syngeneic and allogeneic MultiStem cells were well tolerated after both single and multiple dose IV administration with no identified adverse effects or organ toxicity. Other routes of administration, including pericardial and intraportal vein, have also been shown to be well tolerated in animal models.

With all human stem cell products there are theoretical risks associated with the potential for cell-mediated immunogenicity and tumorigenicity. Allogeneic human MultiStem cells were not immunogenic in rats based on results from MLR and other T-cell activation assays. The cells have also been shown to be immunosuppressive (likely one of their primary mechanisms of pharmacological action) during *in vitro* assays, lending additional support for the lack of immunogenicity of human MultiStem. In assessing biodistribution and persistence of rat or human MultiStem cells in rodent models it appears that most of the injected cells are cleared in a few days

from the animals. However, there was no evidence of MultiStem cell-related alterations in gross pathology or histopathological evaluation of multiple tissues that would be indicative of tumorigenicity identified in any of the nonclinical studies. Thus, the risk for tumorigenicity or teratoma formation after administration of human MultiStem cells appears to be low.

5.3.9 Integrated Overview and Conclusions

Numerous nonclinical studies in various animal models of different diseases and injuries have shown repeatedly that MultiStem is safe. The MultiStem cell therapy product is non-immunogenic, as demonstrated by suppression of T-cell proliferation *in vitro,* lack of alloreactive antibody formation *in vivo,* and equivalent efficacy in the absence of immunosuppressant drugs.

The MultiStem product is also non-tumorigenic, as illustrated by multiple GLP histopathology studies lasting as long as 1 year. After IV infusion, MultiStem initially accumulates in the lung, but causes no pulmonary effects, followed by efficient cell homing to sites of injury. MultiStem cells are generally undetectable within a few days after infusion.

Single doses of 40 million MultiStem cells/dose (200 million cells/kg administered IV) or 10 million MultiStem cells/dose (500 million cells/kg administered SC) have been shown to be well tolerated in rats or mice, respectively. MultiStem cell safety was evaluated in a repeat-dose IV study in a rat HSCT model and there were no adverse effects and no target organs of toxicity identified. The NOAEL for rat MultiStem cells in the rat HSCT model following once weekly IV administration for 5 weeks was 2.5 million cells/dose (12.5 million cells/kg).

The nonclinical safety profile of human MultiStem cells has been adequately characterized and support its safe progression in clinical development for various indications.

6 EFFECTS IN HUMANS

6.1 Pharmacokinetics and Product Metabolism in Humans

The evaluation of absorption, metabolism, and excretion is not relevant to a cell-based therapy. MultiStem is neither metabolized nor excreted in a conventional sense. Clinical pharmacology data do not currently exist for MultiStem due to the lack of methods to track stem cells compared to traditional pharmacokinetic methods used in the study of chemical and biological products.

6.2 Safety and Efficacy

The clinical experience with the MultiStem product to date comprises five completed, one terminated, and four ongoing clinical trials. These trials are evaluating the safety, tolerability, and preliminary efficacy of MultiStem for different indications, i.e., treatment following AMI (separate AMI-07-001 and B02-02 trials), prophylaxis of GVHD during and after HSCT (GvHD-2007-001), treatment for acute ischemic stroke (B01-02 and B01-03) and UC (B3041001), support during and after liver transplant (MiSOT-I), and treatment of ARDS (B04-01 and B04-02). These trials use different MultiStem formulations and concentrations, but identical cellular constituents. The 5 completed trials are in AMI (AMI-07-001), UC (B3041001), ischemic stroke (B01-02), ARDS (B04-01), and GvHD (GvHD-2007-001). The 1 terminated trial is in liver transplant. Ischemic stroke (B01-03 and B01-04) and ARDS (B04-02) and AMI (B02-02) trials are ongoing with 94, 28, 8 and 34 subjects enrolled and dosed, respectively, as of this document's cutoff date.

MultiStem was delivered locally to the heart in completed and ongoing AMI trials and was administered IV in the completed GvHD, ischemic stroke, ARDS and UC trials and the ongoing ARDS and ischemic stroke trials. The terminated MiSOT-I liver transplant study delivered MultiStem via portal and systemic IV circulation.

As outlined in Table 6-1, the product has been shown to be well tolerated in a total of approximately 324 participants in MultiStem clinical trials to date (25-November-2019 cut-off) using doses ranging from 20 million cells administered through local cardiac injection to ≥ 1.2 billion cells administered through systemic IV injection. In addition MultiStem has been shown to be well tolerated using doses of 50 and 100 million cells administered via trans-arterial catheter to patients with AMI receiving percutaneous coronary intervention.

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics / Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI / LPO
Completed/Terminated Studies									
Acute Myocardial Infarction-STEMI (AMI) (A Phase I, Multicenter, Dose-Escalation Trial Evaluating the Safety of Allogeneic AMI MultiStem® in Patients with Acute Myocardial Infarction)	13554/NA United States 9 sites	Open-label, multi-center, dose-escalation trial to evaluate the safety of specified doses of AMI MultiStem administered via trans-arterial catheter in patients with acute myocardial infarction receiving percutaneous coronary intervention	1. Assessment of acute adverse events during the first 24 hours 2. Assessment of post-acute adverse events up to 30 days	Cohorts at single 20, 50 or 100 million cell doses and registry cohort	2 year follow-up	Males or females, 18-85 years of age who had a diagnosis of first time ST elevation myocardial infarction (STEMI) with reduced ejection fraction (LVEF between 30 and 45%) 28-37 subjects	Completed 25 enrolled 19 completed 20 Million cohort 6 enrolled 5 completed 1 withdrawn 50 Million cohort 7 enrolled 6 completed 1 withdrawn 100 Million cohort 6 enrolled 6 completed Registry cohort 6 enrolled 6 completed	23 completed 2 premature termination	09 / 2008 02 / 2010
Graft versus Host Disease (GvHD) (A Phase I, Multicenter, Dose-Escalation Trial Evaluating Maximum-Tolerated Dose of Single and Repeated Administration of Allogeneic MultiStem® in	13507 / 2010-018760-16 United States, Belgium 7 sites	Open-label, multicenter, two-armed, dose escalation trial to assess the safety of MultiStem in subjects with hematological malignancy after hematopoietic	Maximum-tolerated dose, as determined by the CRM, evaluating dose limiting toxicities through 30 days after administration of the last MultiStem dose	First arm: single doses at 1, 5, or 10 million cells/kg Second arm: 1 or 5 million cells/kg administered weekly for three weeks or 5	100 day follow-up	Males or females, 18-65 years of age inclusive, scheduled for a HSCT due to a diagnosis of acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous	Completed 36 enrolled 30 completed 1 million x1 6 enrolled 5 completed 1 withdrawn 5 million x1 3 enrolled 2 completed 1 withdrawn 10 million x1 9 enrolled	30 completed 6 premature termination	10 / 2008

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics / Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI / LPO
Patients with Acute Leukemia, Chronic Myeloid Leukemia, or Myelodysplasia)		stem cell transplantation (HSCT) in patients receiving MultiStem via systemic IV infusion		million cells/kg administered weekly for 5 weeks		leukemia, or myelodysplastic syndrome 36 subjects	7 completed 2 withdrawn 1 million x3 3 enrolled 3 completed 5 million x3 3 enrolled 2 completed 1 withdrawn 5 million x5 12 enrolled 11 completed 1 withdrawn		11 / 2011
Ulcerative Colitis (UC) (A Phase 2 Randomized, Placebo-Controlled, Parallel Group, Multi-center Study to investigate the Safety and Efficacy of MultiStem (PF-05285401) in subjects with moderate to severe ulcerative colitis)	14512 / 2010-022766-27 United States, Belgium, Canada, Germany, Hungary, Italy, Slovakia, Sweden 52 sites	Multi-center, double-blind, dose-escalation trial to evaluate the safety, tolerability and efficacy of intravenous doses of MultiStem in moderate-to severe ulcerative colitis in patients receiving MultiStem via systemic IV infusion	1 Incidence and severity of adverse events (at Weeks 4, 8, 12 and 16) 2 Change from baseline of endoscopic score at Week 8 as measured by modified Baron score. 3 Change from baseline of Mayo rectal bleeding sub-score at Week 4. 4 Change from baseline of Mayo rectal bleeding sub-score at Week 8	Cohorts 1 and 2: dose escalation from 300 to 750 million total cells/ placebo with crossover at 8 weeks; Cohort 3 at 750 million total cells or placebo with second dose at 8 weeks Group 1 D1 750 mm W8 placebo Group 2 D1 750 mm	1 year follow-up	males or females, ≥18 years of age with diagnosis of active moderate-to-severe ulcerative colitis 128 subjects	Completed Cohort 1 (300 million) 9 enrolled- 8 Day 1 dosed 6 Week 8 dosed 3 complete 5 withdrawn Cohort 2 (750 million with crossover) 9 enrolled- 9 Day 1 dosed 9 Week 8 dosed 4 complete 5 withdrawn Cohort 3 88 enrolled 88 Day 1 dosed 84 Week 8 dosed 58 completed	105 enrolled 65 completed 40 premature termination	02 / 2011 11 / 2014

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics / Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI / LPO
				W8 750 mm Group 3 D1 placebo W8 750 mm Group 4 D1 placebo W8 placebo			30 withdrawn 750 million x2 Group 1 & 3 (750 x1) 42 enrolled Group 2 (750 million x2) 21 enrolled- Group 4 (Placebo) 21 enrolled		
Stroke (Double-Blind, Randomized, Placebo-Controlled, Phase 2 Safety and Efficacy Trial of MultiStem in Adults With Ischemic Stroke)	13852 / 2012-005749-18 United States, United Kingdom 36 sites	Randomized, double-blind, placebo-controlled, multicenter, dose-escalation trial to assess safety and efficacy of MultiStem in adults with ischemic stroke in patients receiving MultiStem via systemic IV infusion	1 The primary efficacy variable is the proportion of subjects with a modified Rankin Scale of ≤2 at Day 90 in the MultiStem treatment group compared to subjects in the placebo treatment group 2 The primary safety variable is the DLTs between MultiStem and placebo in infusion related allergic reactions, related AEs and related	Cohorts 1 and 2: dose escalation from 400 million to 1.2 billion total cells or placebo (3:1) given once at baseline; Cohort 3 at 1.2 billion total cells or placebo (1:1) given once at baseline	1 year follow-up	Males or females, 18-83 years of age inclusive who have suffered a cortical ischemic stroke within the past 1-2 days 136 subjects	Completed 134 enrolled 114 completed cohort 1-400 million/placebo (3:1) 8 enrolled 8 completed 6 treatment 2 placebo cohort 2-1.2 billion/placebo (3:1) 8 enrolled 8 completed 6 treatment 2 placebo cohort 3-1.2 billion/placebo (1:1) - blinded 118 enrolled 98 completed 20 withdrawn	134 enrolled 114 completed 20 premature termination	12 / 2011 12 / 2015

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies
***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics / Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI / LPO
			neurologic worsening						

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics / Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI / LPO
Liver Transplant (Phase 1: Safety and feasibility of MultiStem for immunomodulation therapy after liver transplantation: A phase I study of the MiSOT study consortium)	NA / 2009-017795-25 Germany 1 site	Investigator initiated, single center, open-label, dose-escalation trial to evaluate the safety and feasibility of MultiStem after allogeneic liver transplantation in patients receiving MultiStem via trans-portal and systemic IV infusion	The primary endpoint is the occurrence of dose limiting toxicity, defined as at least one grade 3 toxicity until day 30	Two infusions with first at time of transplant via portal circulation and second systemically two days later with up to 150 to 600 million cells per infusion	Up to 6 year follow-up	Males or females, 18-65 years of age inclusive, undergoing liver transplant 12-24 subjects	Cohort 1 (150 million) 3 enrolled 3 completed 0 withdrawn	3 enrolled 3 completed 0 premature termination	08 / 2014 04 / 2016 terminated due to poor enrollment

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics / Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI / LPO
Acute Respiratory Distress Syndrome (MUST-ARDS: A Phase 1/2 Study to Assess the Safety and Efficacy of MultiStem Cell Therapy in Subjects with Acute Respiratory Distress Syndrome)	16460 / 2015-001586-96 US & UK sites 12 sites	Multicenter, safety and efficacy trial conducted in 3 sequential cohorts including dose-escalation with open label and randomized, double-blind, placebo-controlled cohorts	The primary endpoints are safety and tolerability within 4 hours of dosing and SUSARs within the first 24 hours	Single infusions up to 96 hours post-ARDS diagnosis via systemic circulation with 300 or 900 million cells per infusion	Up to 1-year follow-up	Males or females, 18-90 years of age inclusive, with confirmed ARDS 40 subjects	36 enrolled 21 completed 15 withdrawn	36 enrolled 21 completed 15 premature termination	3/2016 7/2019
Ongoing Studies									
Acute Myocardial Infarction-NSTEMI (A Phase 2 prospective, randomized, double-blind, sham-controlled, parallel-group, multi-center trial of AMI MultiStem in subjects with non-ST elevation acute myocardial infarction)	13554/NA United States 10-12 sites	Multi-center, randomized, double-blind, sham-controlled, parallel-group trial to evaluate the safety and efficacy of AMI-MultiStem administered via a micro-infusion catheter in subjects with non-ST	The primary endpoint is safety through Day 30 and efficacy (myocardial perfusion by MRI) at Day 120	One infusion to the heart via a micro-infusion catheter following successful PCI, 50 million cells or Sham	Up to 1 year follow-up	Males or females, 18-85 years of age inclusive, undergoing PCI following NSTEMI 90 subjects	34 enrolled 29 completed 4 withdrawn	34 enrolled 29 completed 4 premature termination	1/2016 target 2/2020

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies
***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics / Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI / LPO
		elevation acute myocardial infarction (NSTEMI) receiving percutaneous coronary intervention (PCI)							

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI LPO
Stroke ((Placebo-Controlled, Double-Blind, Phase 2/3 Efficacy and Safety Trial of HLCM051 (MultiStem®) in Patients With Ischemic Stroke))	13852 /NA Japan 44 sites	Randomized, placebo-controlled, double-blind, multicenter, phase 2/3 trial to evaluate the efficacy and safety of intravenous administration of HLCM051 compared with placebo in subjects with acute ischemic stroke (within 36 hours of onset)	1) Proportion of subjects with an excellent outcome defined by the functional assessments [Time Frame: Day 90] Excellent outcome>is defined as modified Rankin Scael score of ≤1 (scale, 0 to 6), National Institutes of Health Stroke Scale score of ≤1 (scale, 0 to 42), and Barthel Index score of ≥95 (scale, 0 to 100). 2) Comparison between the HLCM051 and the placebo groups in key adverse events [Time Frame: within Day90]	Infusion of 1.2 billion total cells or placebo (1:1) given once at baseline via systemic circulation	1-year follow-up and additional safety follow-up to 2 years	Males or females, 20 years and older of age inclusive who have suffered a cortical ischemic stroke within 18 to 36 hours _____ 220 subjects	94 enrolled 24 completed (1 year) 5 withdrawn	94 enrolled 24 completed (1 year) 5 premature termination	11/2017 _____ target 6/2021 (1 year)

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI LPO
Stroke ((MultiStem® Administration for Stroke Treatment and Enhanced Recovered Study (MASTERS-2))	13852 /2019-001680-69 USA, EU, Asia Pacific 50 sites	Randomized, placebo-controlled, double-blind, multicenter international, Phase 3 trial to evaluate the efficacy and safety of intravenous administration of MultiStem compared with placebo in subjects with acute ischemic stroke (within 36 hours of onset)	1) Differences between the MultiStem and placebo treatment groups in the distribution of Day 90 mRS scores will be evaluated by shift analysis. 2) Differences between the MultiStem and placebo treatment groups for: 2a) proportion of subjects achieving an excellent functional outcome at Day 365; 2b) proportion of subjects achieving an excellent functional outcome at Day 90; 2c) proportion of subjects with a mRS score of less than or equal to 2 at Day 90.	Infusion of 1.2 billion total cells or placebo (1:1) given once at baseline via systemic circulation	1-year follow-up	Males or females, 18 years and older of age inclusive who have suffered a cortical ischemic stroke within 18 to 36 hours of dosing _____ 300 subjects	28 enrolled 6 completed 3 withdrawn	28 enrolled 6 completed 3 premature termination	7/2018 _____ target 9/2021

Table 6-1. Overview of Designs and Status of Completed/Terminated and Ongoing MultiStem Studies

***enrollment as of 25-November-2019**

Indication (Study Title)	IND / EudraCT Country Number of Sites	Study Purpose and Design	Primary End Point	MultiStem Dose and Schedule	Duration	Patient Population Demographics Planned Enrollment	Status	Number of Patients Completed / Premature Termination	FPI LPO
Acute Respiratory Distress Syndrome (An open-label, standard treatment as a control, multicenter phase II study to evaluate the efficacy and safety of HLCM051 (MultiStem®) in patients with acute respiratory distress syndrome (ARDS) caused by pneumonia) (ONE-BRIDGE)	NA/NA Japan 22 sites	A Phase 2 randomized, standard treatment as a control, open-label, multicenter study to evaluate the efficacy and safety of intravenous administration of MultiStem compared to standard care only in subjects with ARDS caused by pneumonia	Number of days of survival free from mechanical ventilation (ventilator-free days) during 28 days after administration of the investigational product	Single, 900 million total cells infused up to 72 hours post-ARDS diagnosis given via systemic circulation along with ARDS standard care or standard care only (2:1)	6-month follow-up	Males or females, 20 to 90 years of age diagnosed with ARDS caused by pneumonia 30 subjects	8 enrolled 1 completed 2 withdrawn	8 enrolled 1 completed 2 premature termination	4/2019 target 12/2020

Table 6-2 presents a cumulative summary of all serious adverse reactions that occurred in any clinical trial with MultiStem until 25-November-2019.

Table 6-2. Cumulative Summary of Serious Adverse Reactions

System Organ Class (SOC) / Preferred Term (PT)	MultiStem	Blinded – MultiStem/Placebo
TOTAL SERIOUS ADVERSE REACTIONS	**6**	**3**
SOC Immune System Disorders		
PT Hypersensitivity	1	0
SOC Blood and lymphatic system disorders		
PT Pancytopenia	1	0
SOC Injury, poisoning and procedural complications		
PT Procedural complication	0	1**
PT Procedural intestinal perforation	0	1**
SOC Nervous system disorders		
PT Convulsions	1*	0
Dyskinesia	0	1
SOC Cardiac disorders		
PT Atrial fibrillation	1	0
Coronary artery dissection	0	1***
SOC Hepatobiliary disorders		
PT Hyperbilirubinemia	1	0
SOC Neoplasms benign, malignant and unspecified		
PT Acute myeloid leukemia	1	0
SOC General disorders and administration site conditions		
Chills	1	0

* This case was assessed as possibly related by the investigator in the B01-02 ischemic stroke trial, but downgraded by the sponsor as a manifestation of the underlying disease.
**This case was reported related to a study procedure, which occurred after enrollment and informed consent, but before investigational product dosing.
***This case was reported by the Investigator as unrelated to investigational product and definitely related to the micro-infusion investigational catheter procedure.

6.2.1 Summary of Trial in Patients with Acute Myocardial Infarction (AMI-07-001)

MultiStem was evaluated in a Phase 1 dose-escalation, open-label, safety trial (AMI-07-001) in patients following an AMI (Penn et al., 2012). There were no clinically significant changes to vital signs or evidence of allergic reaction associated with MultiStem administration observed immediately following dosing. Over the 30-day post-acute observation period, no infusional toxicities or clinically significant cardiac adverse events or arrhythmias deemed to be definitively related to MultiStem occurred.

Below is a listing of serious adverse events considered to be at least possibly related to MultiStem by Investigator and/or Sponsor.

Table 6-3. Listing of Serious Adverse Events Related to MultiStem for the AMI-07-001 Trial

System Organ Class (SOC) / Preferred Term (PT)	MultiStem	Blinded – MultiStem/Placebo
SOC Cardiac disorders		
PT Atrial fibrillation	1	0

6.2.2 Summary of Trial for Prophylaxis in Graft versus Host Disease (GVHD-2007-001)

MultiStem was evaluated in the completed Phase 1 dose-escalation, open-label safety trial (GvHD-2007-001) in patients undergoing HSCT. MultiStem was administered after transplant as a potential prophylactic agent to prevent GvHD.There were no allergic reactions related to MultiStem infusion. No HLA antibody responses were detected and no MultiStem drug product chimerism was observed up to Day 100 post transplant. The safety profile was consistent with the events that would be expected from the high risk, HSCT population studied.

Below is a listing of two serious adverse events considered to be at least possibly related to MultiStem by Investigator and/or Sponsor. The acute myeloid leukemia case was a relapse of the existing disease that the patient was being treated for and was considered possibly related to MultiStem by the Investigator and unrelated by the Sponsor. The Investigator considered the case of "Hyperbilirubinemia" to be possibly related to therapy with MultiStem although the Sponsor assessed the event unrelated to MultiStem.

Table 6-4. Listing of Serious Adverse Events Related to MultiStem for the GVHD-2007-001 Trial

System Organ Class (SOC) / Preferred Term (PT)	MultiStem	Blinded – MultiStem/Placebo
SOC Hepatobiliary disorders		
PT Hyperbilirubinemia	1	0
SOC Neoplasms benign, malignant and unspecified		
PT Acute myeloid leukemia	1	0

6.2.3 Summary of Trial in Patients with Ischemic Stroke (B01-02)

MultiStem was evaluated in a Phase 2 double-blind, randomized, placebo-controlled safety and efficacy trial in adults following an acute ischemic stroke. The total trial duration for safety and efficacy follow-up was 12 months. As of 25-November-2016, the study has been

completed and final data have been reported. The trial is also referred to as MASTERS-1 (MultiStem Administration for Stroke Treatment and Enhanced Recovery Study).

In total, 71 subjects received a single dose of MultiStem and 63 subjects received a single dose of placebo 24-48 hours after stroke onset during the study. Six subjects received 400 million cells and 65 subjects received 1.2 billion cells.

Although an efficacy analysis of Excellent Outcomes (modified Rankin Scale score ≤ 1, National Institutes of Health Stroke Scale score ≤ 1, and Barthel Index score ≥ 95) showed a percentage difference which favored MultiStem treatment, the prospective analysis was not statistically significant for the primary and secondary efficacy endpoints for the trial (Hess et al., 2017). However, the post-hoc analyses demonstrated that MultiStem treatment had an effect as measured by multiple endpoints (e.g., Global Stroke Recovery test statistic, distribution of modified Rankin Scale scores, and Excellent Outcome), especially for subjects who received treatment ≤ 36 hours; this effect became more pronounced through 1 year. Based on these results, a Phase 2/3 trial in Japan referred to as TREASURE and a Phase 3 trial in North American, Europe, and the Asia Pacific region referred to as MASTERS-2 will be conducted to confirm these efficacy results.

Treatment with MultiStem was shown to be safe and generally well tolerated throughout all dose cohorts in MASTERS-1. In particular, the highest dose studied (1.2 billion total cells) was shown to be safe and well tolerated during the study. No subjects had infusion-related allergic reactions, neurological worsening, or reported dose limiting adverse that trigger protocol stopping rules. The majority of adverse events were mild or moderate in severity and consistent with the disease state being studied. There were no clinically significant differences between treatment groups in laboratory findings or vital signs.

Additionally, the results showed MultiStem to be associated with a favorable impact on a range of complications and outcomes following ischemic stroke.Treatment with MultiStem was associated with a reduced incidence of deaths, life-threatening adverse events, urinary tract infection, and secondary infection rates compared to placebo. Additionally, the mean duration of hospitalization and the mean duration of stay in an intensive care unit were shorter in the MultiStem group compared to the placebo group.

No serious adverse events were determined to be related to MultiStem use by the Sponsor.

6.2.4 Summary of Trial in Patients with Ulcerative Colitis (B3041001)

MultiStem was evaluated in a completed Phase 2 dose-escalation safety and efficacy trial (B3041001) in patients with moderate to severe UC. This trial was sponsored by Pfizer Inc and has been completed.

Overall, the completed study showed a favorable safety profile for MultiStem with repeat IV infusions at Day 1 and Week 8 of 300 or 750 million total cells. There were no differences between MultiStem and Placebo for clinical laboratory parameters and vital signs. Adverse events reported were what was expected for this population of patients with active moderate-to-severe UC. No deaths were reported in this study and the number of subjects discontinuting due to adverse events was small and not different between groups.

Weekly repeat infusions of 300 million total cells of MultiStem for the initial patients of Cohort 1 (Day 1, Week 1 and Week 2) were associated with delayed onset infusion reactions (pyrexia and chills) and hypersensitivity, which were transient and treated with standard medications. Weekly re-challenge with MultiStem was avoided during the rest of Cohort 1 and in subsequent Cohort 2 (300 million cells) and Cohort 3 (750 million cells). Repeat dosing was limited to a maximum of two infusions of MultiStem at Day 1 and Week 8, which appeared to be generally well tolerated in this patient population with most adverse events being mild to moderate in severity.

Table 6-5 has a listing of serious adverse events considered to be at least possibly related to MultiStem by Investigator and/or Sponsor from this completed study. A serious adverse event of hospitalization for "hypersensitivity" was reported in Cohort 1. This occurred after the second infusion of investigational product (i.e., 1 week between doses). The causality of this event was possibly related to MultiStem or the solution used to dilute the stems cells prior to infusion. The event was reported by the Investigator as an allergic reaction characterized by fever and chills, which subsided after treatment with IV fluids, oxygen, famotidine, paracetamol, diphenhydramine hydrochloride, and IV steroids. No rash or respiratory issues were noted. The patient recovered, received no further infusions, and was followed in the trial to completion. The protocol was modified to allow multiple dosing, but with 8 weeks between MultiStem readministration for Cohort 2 and 3.

A serious adverse event of "pancytopenia" was reported by the Investigator in Cohort 3 of the trial. Three days after the second infusion of the product at Week 8, the patient was admitted to the hospital with the initial diagnosis of "aplastic anemia", which was later revised to "pancytopenia". The patient recovered from the event. The Investigator classified the event as related to the investigational product. Pfizer's assessment concluded that although there were other risk factors which could contribute to the reported event, based on the information provided and an unclear temporal association, a causal relationship between the event "pancytopenia" and the blinded study drug could not be excluded. However, Pfizer considered it very likely that a causal relationship between the reported event and the concomitant UC medication, 6-MP, exists that would explain the patient's recovery, and the improvement in platelet counts in particular.

Table 6-5. Listing of Serious Adverse Events Related to MultiStem for the B3041001 Trial for Ulcerative Colitis

System Organ Class (SOC) / Preferred Term (PT)	MultiStem	Blinded – MultiStem/Placebo
SOC Immune System Disorders		
PT Hypersensitivity	1	0
SOC Blood and lymphatic system disorders		
PT Pancytopenia	1	0
SOC Injury, poisoning and procedural complications		
PT Procedural complication	0	1*
PT Procedural intestinal perforation	0	1*

*This case was reported related to a study procedure, which occurred after enrollment and informed consent, but before investigational product dosing.

6.2.5 Summary of Trial in Patients undergoing Liver Transplantation (MiSOT-I)

The MiSOT-I protocol was a Phase 1, open label, dose escalation, single arm, single center, safety and feasibility study of MultiStem in patients undergoing allogeneic liver transplantation. Standard of care pharmacological immunosuppression can achieve reasonable survival of liver grafts and patients. The side effects of this treatment; however, are clinically significant and diminish the overall success of organ transplantation as a curative therapy. It was therefore the objective of this study to implement cellular immunomodulation therapy as an adjunct to standard pharmacological immunosuppression with the ultimate goal of significantly reducing drug-based immunosuppression. The primary objective of this trial was to evaluate short and longer-term safety of MultiStem. As of 25-November-2016, the trial has been terminated due to poor enrollment. Three subjects were enrolled with final study visits completed for the trial. MultiStem was well tolerated with no infusion-related reactions being reported and adverse events were consistent with the disease state being studied. No serious adverse events were reported to be related to MultiStem use.

6.2.6 Summary of Trial in Non ST-Elevation Acute Myocardial Infartion (B02-02)

The B02-02 protocol is a Phase 2 multi-center, randomized, double-blind, sham-controlled, parallel-group trial to evaluate the safety and efficacy of AMI-MultiStem administered via a micro-infusion catheter in subjects with non-ST elevation acute myocardial infarction (NSTEMI) receiving percutaneous coronary intervention. Approximately 90 subjects will be randomized 1:1 to 50 million cells of AMI-MultiStem or Sham. The primary objectives of this trial are to evaluate safety 30-days post-infusion and assess efficacy (myocardial perfusion as measured by cardiac MRI) at day 120. As of 25-November-2019, 34 subjects have been enrolled in the trial with no infusion-related reactions being reported and adverse events have been consistent with the disease state being studied. The study is ongoing

with subject follow-up although no further subjects will be enrolled due to poor enrollment. See listing in Table 6-6 of serious adverse events considered to be at least possibly related to MultiStem by Investigator and/or Sponsor. No serious adverse events have been reported to be related to MultiStem use. One reported serious adverse event of coronoary artery dissection was reported related to investigational procedure, but not investigational product.

Table 6-6. Listing of Serious Adverse Events Related to MultiStem for the B02-02 Trial for AMI

System Organ Class (SOC) / Preferred Term (PT)	MultiStem	Blinded – MultiStem/Placebo
SOC Cardiac disorders		
Coronary artery dissection	0	1*

*This case was reported by the Investigator as unrelated to investigational product and definitely related to the micro-infusion investigational catheter procedure.

6.2.7 Summary of Trial in Acute Respiratory Distress Syndrome (B04-01)

The ARDS B04-01 protocol is a completed Phase 1/2 double-blind, randomized, placebo-controlled safety and efficacy trial that was conducted in the United States and the United Kingdom. The total trial duration for safety and efficacy follow-up was 12 months. As of 25-November-2019, the study has been completed and final data have been reported.

The study was conducted in 3 sequential cohorts, starting with 2 open-label cohorts (at different dose levels) followed by a randomized, double-blind, placebo-controlled cohort:

- Cohort 1: 3 subjects received 300 million cells via intravenous infusion.
- Cohort 2: 3 subjects received 900 million cells via intravenous infusion.
- Cohort 3: 30 subjects were randomized 2:1 to receive MultiStem therapy or placebo via intraveous infusion. In Cohort 3, MultiStem therapy was administered at the highest tolerated dose from Cohorts 1 and 2, which was 900 million cells.

Inclusion criteria included male or female subjects that were 18 to 90 years of age (inclusive) with new acute onset of moderate to severe ARDS (as per the Berlin definition). Once all diagnostic criteria for ARDS and other eligibility criteria had been met, subjects then had to achieve a 2-hour stable baseline period (pre infusion stability) prior to receiving treatment. The study infusion had to be administered within 96 hours of fulfilling the diagnostic criteria of moderate to severe ARDS.

A total of 36 subjects were randomized and received treatment. That was 3 subjects in Cohort 1, 3 subjects in Cohort 2, and 30 subjects in Cohort 3 (20 receiving MultiStem and

10 receiving placebo). The median age across all cohorts was approximately 60 years. The majority of subjects were White and body mass index was similar in all cohorts.

No infusion related adverse events of special interest were seen during the first 4 hours post-infusion or within the first 3 days post-infusion and no serious adverse events possibly related to MultiStem were reported. The majority of adverse events were moderate or severe in severity and consistent with the disease state being studied. No changes in laboratory values, vital signs or physical examination findings were seen that indicated an adverse effect of MultiStem therapy.

Other data collected during this exploratory study showed lower mortality of 25% in the MultiStem treatment group compared to 40% in the placebo group from Day 0 to Day 28. In addition, the MultiStem group had a higher median number of ventilator-free days from Day 0 to Day 28 (18.5 days) compared to the placebo group (6.5 days). The number of Intensive Care Unit-free days from Day 0 to Day 28 was higher in the MultiStem group (median of 12.5 days) compared to the placebo group (4.5 days).

6.2.8 Summary of Japanese Trial in Ischemic Stroke (B01-03)

The TREASURE trial (protocol B01-03) is an ongoing Phase 2/3 double-blind, randomized, placebo-controlled efficacy and safety trial in Japanese patients who have suffered an ischemic stroke. This trial is being conducted in Japan and the Sponsor is HEALIOS K.K.

Approximately 220 subjects will be randomized 1:1 to 1.2 billion cells of HLCM051 (MultiStem) or placebo. A single systemic infusion will be given within 36 hours from onset of an ischemic stroke and subjects will be followed for 1 year as the primary follow-up period with additional safety information collected at 2 years.

The primary objectives of this trial are to evaluate efficacy and safety. The primary efficacy objective will evaluate the proportion of subjects with an excellent outcome at Day 90 (modified Rankin Scale score of ≤ 1, National Institutes of Health Stroke Scale score of ≤ 1, and Barthel Index score of ≥ 95). The primary safety objective will examine comparisons between the HLCM051 and the placebo groups in key adverse events through Day 90.

As of 25-November-2019, the study has enrolled 94 subjects. Table 6-7 has a listing of serious adverse events considered to be at least possibly related to MultiStem by Investigator and/or Sponsor.

A serious adverse event of dyskinesia was reported. The Investigator reported the verbatim term of involuntary movement with onset of hemiballism/hemichorea observed on the left upper extremity 7 days after the stroke and 6 days following administration of blinded investigational product. Symptoms were reported as moderate in severity and gradually

improved over the following 10 days. The dyskinesia event was reported as a serious adverse event because discharge from the acute care hospital and transfer to rehabilitation was delayed due to the participant's dyskinesia symptoms, which resolved 26 days after being initially reported. Given the temporal sequence of the event 6 days following blinded investigational product administration, a potential causal relationship between administration of blinded investigational product and dyskinesia could not be ruled out by the Investigator.

Table 6-7. Listing of Serious Adverse Events Related to MultiStem for the B01-03 Trial for Ischemic Stroke

System Organ Class (SOC) / Preferred Term (PT)	MultiStem	Blinded – MultiStem/Placebo
SOC Nervous system disorders		
Dyskinesia	0	1

6.2.9 Summary of International Trial in Ischemic Stroke (B01-04)

The MASTERS-2 trial (protocol B01-04) is an ongoing Phase 2/3 double-blind, randomized, placebo-controlled efficacy and safety trial in adult subjects who have suffered a moderate to moderately-severe, acute cortical ischemic stroke. This trial will be conducted in the US and Europe and potentially other regions such as Asia Pacific. The Sponsor is Athersys, Inc.

Approximately 300 subjects will be randomized 1:1 to 1.2 billion cells of MultiStem or placebo. A single systemic infusion will be given within 18-36 hours from onset of an ischemic stroke and subjects will be followed for 1 year.

The primary objectives of this trial is to evaluate efficacy. The primary efficacy objective will evaluate differences between the MultiStem and placebo treatment groups in the distribution of Day 90 mRS scores by shift analysis. The key secondary efficacy variables will examine differences between the MultiStem and placebo treatment groups for the:

- Proportion of subjects achieving an excellent outcome at Day 365 defined by all of the following criteria: mRS score of ≤ 1, NIHSS total score of ≤ 1, and Barthel Index score of ≥ 95;
- Proportion of subjects achieving an excellent outcome at Day 90 defined by all of the following criteria: mRS score of ≤ 1, NIHSS total score of ≤ 1, and Barthel Index score of ≥ 95; and
- Proportion of subjects with a mRS score of ≤ 2 at Day 90.

As of 25-November-2019, the study has enrolled 28 subjects. No serious adverse events have been reported to be related to MultiStem use.

6.2.10 Summary of Japanese Trial in Acute Respiratory Distress Syndrome (B04-02)

The ONE-BRIDGE study (protocol B04-02) is an open-label, standard treatment as a control, multicenter Phase 2 trial to evaluate the efficacy and safety of MultiStem in Japanese patients with acute respiratory distress syndrome (ARDS) caused by pneumonia. This trial is being conducted in Japan and the Sponsor is HEALIOS K.K.

30 subjects will be randomized 2:1 to open-label, 900 million cells of HLCM051 (MultiStem) or standard ARDS care only. A single systemic infusion will be given within 72 hours of ARDS diagnosis.

The primary objective of this trial is to evaluate efficacy. The primary efficacy objective will evaluate the number of days of survival free from mechanical ventilation (ventilator-free days) during 28 days after administration of the investigational product. The primary safety objectives will examine comparisons between the HLCM051 and the standard care only groups in adverse events, vital signs, and laboratory test values through Day 180.

As of 25-November-2019, the study has enrolled 8 subjects. Table 6-8 has a listing of serious adverse events considered to be at least possibly related to MultiStem by Investigator and/or Sponsor.

A serious adverse event of chills was reported. Per protocol, the study participant received, open-label, investigational product (MultiStem - 900 million cells), by single intravenous infusion following diagnosis of ARDS. The Investigator reported onset of chills ("shivering") about 20 minutes following the end of MultiStem administration. Moderate generalized tremor was accompanied by coughing, increased ventilated tidal volume, and decreased pulse oximetry measurements. The chills event was reported as severe in severity and resolved about 20 minutes after onset. Treatment consisted of up-titration of sedative medications and ventilator and airway management. Resolution of the event was followed by observation of increased body temperature lasting several hours. This event met seriousness criteria based on being judged an Important Medical Event by the Investigator. Given the temporal sequence of the event following MultiStem administration, a potential causal relationship between administration of MultiStem and chills could not be ruled out by the Investigator.

Table 6-8. Listing of Serious Adverse Events Related to MultiStem for the B04-02 Trial for ARDS

System Organ Class (SOC) / Preferred Term (PT)	MultiStem	Blinded – MultiStem/Placebo
SOC General disorders and administration site conditions		
Chills	1	0

6.3 Reference Safety Information

A summary of the treatment-related serious adverse events experienced during each clinical trial with MultiStem through 25-November-2019 are presented in Table 6-3, Table 6-4, Table 6-5, Table 6-6, Table 6-7, and Table 6-8 along with a summary in Table 6-2. These results show that there are limited serious adverse events seen with no events occurring in more than one subject. Therefore, all serious adverse events at least possibly related to the study drug will be considered as unexpected and will be reported to the Competent Authorities as per current legislation.

7 SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

7.1 Mode of Action and Intended Indications

MultiStem is a cell therapy medicinal product originating from adherent adult stem cells taken from the bone marrow of a non-related donor and expanded *ex vivo*. MultiStem appears capable of delivering a therapeutic benefit through more than one mechanism of action. Factors expressed by MultiStem are believed to reduce inflammation and regulate immune system function, protect damaged or injured cells and tissue, promote formation of new blood vessels, and augment tissue repair and healing (Auletta et al., 2010).

Based on these multipotent and immunoregulatory properties, MultiStem is being investigated in numerous indications including treatment of AMI, prevention of GvHD, treatment of ischemic stroke and UC, as an adjunct to immunotherapy in SOT, and in treatment of ARDS. The rationale for MultiStem in these indications is discussed in Section 3.1.

7.2 Posology and Method of Administration

MultiStem is a sterile aqueous suspension of viable human cells. Administration of MultiStem may differ by indication and clinical trial (see Section 4). Details of the dosing regimen are included in the clinical trial protocols.

7.2.1 Acute Myocardial Infarction

MultiStem was administered directly into the adventita of the target coronary vessel in patients with ST elevation AMI. To date, single doses of 20, 50 or 100 million total cells have been administered in this indication.

MultiStem is being administered directly into the adventita of the target coronary vessel in patients with non-ST elevation AMI. Single doses of 50 million total cells are being administered in this indication.

7.2.2 Graft versus Host Disease

MultiStem is administered via the IV route in patients who have undergone myeloablation and HSCT resulting in the potential for developing GvHD. To date, single doses of 1, 5 or 10 million cells/kg body weight and multiple doses of 1 or 5 million cells/kg weekly for three weeks or 5 million cells/kg weekly for five weeks have been administered in this indication.

7.2.3 Stroke

MultiStem is administered via the IV route in patients with ischemic stroke. To date, single doses of 400 million or 1.2 billion total cells have been administered in this indication.

7.2.4 Ulcerative Colitis

MultiStem is administered via the IV route in patients with UC. To date, single doses of 300 or 750 million total cells or two doses of 750 million total cells per dose have been administered in this indication.

7.2.5 Solid Organ Transplant

For patients undergoing liver transplantation, MultiStem is administered via the portal vein and via the IV route for subsequent doses. To date, multiple doses have been administered of 150 to 600 million total cells per the initial portal vein infusion followed by IV infusion for the second dose.

7.2.6 Acute Respiratory Distress Syndrome

MultiStem is administered via the IV route in patients with ARDS. Single doses of 300 million or 900 million total cells have been administered in this indication.

7.3 Risks, Side Effects, Precautions, and Special Monitoring

As of 25-November-2019, approximately 324 patients have received more than 403 infusions of MultiStem including an estimated 61 ischemic stroke patients, 5 ARDS patients and an estimated 17 AMI patients from ongoing studies. The patients confirmed to have received MultiStem in completed or terminated studies include 19 AMI patients receiving transcoronary injection; 36 GvHD patients receiving IV infusion through a central line, 84 ulcerative colitis (UC); 71 acute ischemic stroke; 26 ARDS; and 2 expanded access use patients receiving IV infusion through a peripheral line; and 3 liver transplant patients via the portal circulation and through a peripheral line. There were no infusional or allergic reactions reported in the completed AMI, GvHD, ARDS, and stroke trials and adverse events reported were consistent with the disease state under study. There have been no adverse events definitely associated with MultiStem use in any of the completed trials or two expanded access use cases. In the completed UC trial, one serious adverse event of hypersensitivity, as further described below, and one serious adverse event of pancytopenia have been reported and considered possibly related to MultiStem or the product used to dilute the stem cells. In the ongoing open-label ARDS trial in Japan (B04-02), a serious adverse event of chills has been reported possibly related to MultiStem.

The cellular component of MultiStem is sourced from bone marrow from a human donor. As such it carries a potential risk of viral and non-viral contamination or infection when infused into patients. The bone marrow donation program has been accredited by the FDA and is compliant with EU regulations on cells and tissues for acquisition of human tissues, providing safeguards against unintentional contamination of the sample following collection and to ensure that quality of the cells is maintained. The manufacturing process

used for expansion and harvest of cells has a number of steps where there is a risk for introduction of adventitious viral and non-viral agents. Thus, the manufacturing process involves a number of quality oversight procedures to minimize and identify such potential risks, including manufacturing under current Good Manufacturing Processes, certification or testing of all raw materials that come into contact with cells, and product sterility testing at multiple stages.

The major potential risks of administration of MultiStem, similar to that of cell-based therapies in general, include the immunogenic risk to patients and the potential for infusional toxicity. Although there is very limited persistence of MultiStem cells in animals and no evidence of toxicity or tumorigenicity in the numerous animal studies conducted, these cannot be excluded as potential risks.

To date, there has only been one serious adverse event of allergic reaction following MultiStem drug product administration in the patients exposed. This occurred in a UC trial patient following their second infusion and was characterized by fever and chills, as measured by changes in temperature. The reaction resolved after treatment at the hospital, including IV steroids. The diluent (HTS) used in the UC trial is different from the diluent (Plasma-Lyte A or equivalent) used in the AMI, GvHD, ARDS or ischemic stroke trials. There has only been one serious adverse event of chills possibly related to MultiStem drug product administration in the patients exposed. This occurred in an open-label ARDS trial patient and was characterized as shivering about 20 minutes following the end of MultiStem administration with the event being reported as severe in severity and resolved about 20 minutes after onset. There have been no allergic reactions or serious adverse events definitely related to MultiStem in any of the completed AMI, GvHD, ARDS, and stroke trials. In the GvHD study, no HLA antibody responses were detected and no MultiStem drug product chimerism was observed up to Day 100 post-transplant. The safety profile in the AMI, GvHD, stroke, ARDS and UC trials was otherwise consistent with the events that would be expected from the high risk populations being studied. Additionally, a series of *in vivo* safety studies were conducted to evaluate the immune responses occurring upon IV administration of multiple high doses of allogeneic MultiStem (5 to 50 million cells/kg) in rats. Infusion with high doses (50 million cells/kg) of allogeneic MultiStem did not induce alloreactive antibody formation or allo-sensitization. With allogeneic cell-based therapies there is a risk of triggering an immunological response (host versus graft reaction). MultiStem has been shown not to activate allogeneic T-cells in MLRs, and has also been shown to suppress an allo-reaction between two mismatched lymphocytes. This allows MultiStem to be administered to patients without the need for tissue matching.

There have been no cases of other infusional toxicities reported in the completed AMI, GvHD, stroke, ARDS, and UC trials, or in the expanded access use cases. Moreover, infusional toxicity was evaluated in multiple animal studies. The results indicated that IV

infusion of allogeneic MultiStem was well tolerated, without evidence of pulmonary distress, mortality or biologically significant change in body weight after infusion of single doses and no cumulative side effects after five repeated infusions of MultiStem. Single doses of 40 million MultiStem cells/dose (200 million cells/kg administered IV) or 10 million MultiStem cells/dose (500 million cells/kg administered SC) have been shown to be well tolerated in rats or mice, respectively. Additionally, 200 million MultiStem cells delivered into the adventitial layer of the coronary artery by a transarterial catheter were well tolerated in a pig AMI model. However, in pigs anesthetized with isoflurane, which causes a significant reduction in blood pressure on its own (~35-40% lower than normal), transient hypotension has been observed shortly (1-2 minutes) following initiation of rapid intravenous infusion of MAPC cells and lasting for several minutes during the infusion, without evidence of impact on pulmonary function. This has not been observed in human subjects in any of the ongoing or completed trials, and has not been observed in safety pharmacology studies in rodents evaluating rapid intravenous infusion of high doses of MultiStem cells or in other animal models with intravenous MAPC administration.

Although the risk of allergic reaction or infusional toxicity cannot be ruled out, they can be mitigated by dose escalation designs and by careful safety evaluations conducted before escalating the doses in subsequent cohorts of patients. Furthermore, vital sign and temperature measurements should be taken during and after each MultiStem administration. Any event of infusional toxicity or allergic response, defined as clinically significant deviations in blood pressure, heart rate, respiratory rate, temperature, and oxygen saturation, should also be recorded. In the event of infusional toxicity or allergic response and if flushing, sudden rash, or difficulty breathing occur, the infusion should immediately be slowed or stopped, or in cases of severe reactions (Grade 3 or higher) the infusion should be immediately terminated. Administration of MultiStem cells should be performed in an inpatient setting with access to equipment and staff qualified to provide appropriate emergency care.

Biodistribution and persistence of MultiStem cells was evaluated in NOD/SCID mice and in rodent disease models. Most of the MultiStem cells were cleared from tissues within a few weeks of administration. There was no evidence of tumorigenicity in SC and IV nude mouse tumorigenicity studies or in any other nonclinical studies where tissues were evaluated. Whereas embryonic stem cells in their undifferentiated state can cause the formation of teratomas when administered to animals, this effect has never been observed with MultiStem in nonclinical tumorigenicity studies or with other adult-derived stem cell products.

7.4 Interaction with Other Medicinal Products and Other Forms of Interaction

Clinical pharmacology studies have not been conducted with the MultiStem cell product. Human MultiStem cell viability and activity were assessed *in vitro* in the presence of drugs used as standard of care for each indication under clinical investigation (see Section 5.1.4). The results of these studies indicate that concomitant drug regimens should not significantly impact the viability or functionality of MultiStem cells.

7.5 Undesirable Effects

The clinical experience with the MultiStem product to date comprises two completed and three ongoing trials, which are being conducted using different MultiStem formulations and concentrations, but identical cellular constituents. While MultiStem was delivered locally to the heart in the AMI trial, it was infused as single and multiple doses IV in patients for prevention of GvHD and in patients with ischemic stroke. MultiStem was also infused as single and multiple IV doses in patients during the completed UC trial. The safety profile has been consistent with the events that would be expected from the high risk populations being studied.

7.6 Overdose

The highest single dose administered to date (25-November-2019) in clinical studies was approximately 1.25 billion cells with no dose limiting toxicities or infusional toxicities observed. No specific antidote exists for the treatment of MultiStem overdose.

7.7 Pregnancy and Lactation

Formal reproductive and developmental toxicity studies have not been conducted with human MultiStem cells.

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