Study Protocol

Study Title	An Observational, Practice-Based, Open Label, Feasibility Study to Observe the Efficacy and Safety of Intramuscular Administration of Stempeucel® in Malaysian Patients with Critical Limb Ischemia (CLI) Due to Buerger's Disease.
Study Number	CBR-BD-22-003
Methodology	Single arm, practice-based, feasibility study
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Protocol Version and Date	Version 3, 20-July-2022 (Supersedes Version 2)

CONFIDENTIALITY STATEMENT

SPONSOR SIGNATURE OF PROTOCOL

Protocol Number:	CBR-BD-22-003
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Medical Monitor:	To be decided
Clinical Trial Manager:	Dr. Abid Nordin, CRA; Cell Biopeutics Resources Sdn. Bhd.
Quality Assurance:	To be decided
Biostatistician:	To be decided
Data Manager:	To be decided
Central Laboratories:	To be decided
Protocol Version and Date:	Version 3, 20-Jul-2022

I have prepared the protocol for study code: CBR-BD-22-003, version 3.0 dated 20 July 2022. To the best of my knowledge, the protocol is accurate and complete.

Protocol Author Signature: Name and Title:	 Dr. Abid Nordin, CRA; Cell Biopeutics Resources Sdn. Bhd.
Signature Date:	20-Jul-2022

I have reviewed the protocol for the study code: CBR-BD-22-003, version 3.0 dated 20 July 2022. To the best of my knowledge, the protocol is accurate and complete. I approve this version as the final copy and consider it acceptable for regulatory submission.

Sponsor Signature: Name and Title:	 Dr. Jezamine Lim, CEO; Cell Biopeutics Resources Sdn. Bhd.
Signature Date:	20-Jul-2022

INVESTIGATOR SIGNATURE OF PROTOCOL

The trial will be conducted in accordance with the ICH E6, the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

By signing this cover page, I attest that I have read and understand the contents of this protocol. I agree to adhere to the design, conduct and reporting requirements of the study as stated in the clinical protocol and to my obligations to The Sponsor as described in the protocol and executed contracts between myself, my Institution and The Sponsor. I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

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Protocol Version and Date:	Version 3, 20-Jul-2022
Principal Investigator Signature: Name and Title:	 Prof. Dato' Dr. Hanafiah Harunrashid
Co-Investigator Signature: Name and Title:	 Dr. Lenny Suryani Safri
Co-Investigator Signature: Name and Title:	 Dr. Mohd Azim Md Idris
Signature Date:	20-Jul-2022

CONFIDENTIALITY STATEMENT

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List of Abbreviations

ABPI	Ankle Brachial Pressure Index
AE	Adverse Event
ALT	Alanine Aminotransferase
AMI	Acute Myocardial Infarction
ASC	Adipose-derived Stromal Cell
AST	Aspartate Aminotransferase
CBR	Cell Biopeutics Resources Sdn. Bhd.
CLI	Critical Limb Ischemia
COA	Certificate of Analysis
CRF	Case Report Form
2D ECHO	Two-Dimensional Echocardiography
DMP	Data Management Plan
DMSO	Dimethyl sulfoxide
DSA	Digital Subtraction Angiogram
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
e-CRF	Electronic CRF
EPCs	Endothelial Progenitor Cells
GCP	Good Clinical Practice
GEE	Generalized Estimating Equations
GMP	Good Manufacturing Practice
HED	Human Equivalent Dose
HLA	Human Leukocyte Antigen
HSA	Human Serum Albumin
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent Ethics Committee
IM	Intramuscular
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IV	Intravenous
LC	Liver Cirrhosis
MNCs	Mononuclear cells
MSCs	Mesenchymal Stem Cells
NCERT	National Stem Cell Ethics and Research Sub-Committee
NPRA	National Pharmaceutical Regulatory Authority
NOAEL	No Observable Adverse Effect Level
NOEL	No Observed Effect Level
OA	Osteoarthritis
PA view	Postero-anterior view
PAD	Peripheral Arterial Disease
PBMNCs	Peripheral Blood Mononuclear Cells
PI	Principle Investigator
RDC	Remote Data Capture
SAE	Serious Adverse Event
SCF	Stem Cell Factor
SD	Standard Deviation
SDFI	Stromal Cell Derived Factor
SE	Standard Error

SOPs	Standard Operating Procedures
SPOC	Standard Protocol of Care
TBI	Toe Brachial Index
TD	Therapeutic Dose
TNF-α	Tumor Necrosis Factor- alpha
UKMMC	Universiti Kebangsaan Malaysia Medical Centre
UKMREC	Universiti Kebangsaan Malaysia Research Ethics Committee
VEGF	Vascular Endothelial Growth Factor

Study Summary

Title	An Observational, Practice-Based, Open Label, Feasibility Study to Observe the Efficacy and Safety of Intramuscular Administration of Stempeucel® in Malaysian Patients with Critical Limb Ischemia (CLI) Due to Buerger's Disease
Study Design	Single arm, practice-based, feasibility study
Study Duration	Estimated duration for the main protocol (e.g. from starts of screening to last subject processed and end of the study) is approximately 18 months
Study Center	Universiti Kebangsaan Malaysia Medical Centre (UKMMMC), Jalan Yaacob Latif, Bandar Tun Razak, 56000 Kuala Lumpur, Wilayah Persekutuan, Malaysia
Objectives	To observe the efficacy and safety of Stempeucel® (adult human bone marrow derived, cultured, pooled, allogeneic mesenchymal stromal cells) in Malaysian patients with critical limb ischemia (CLI) due to Buerger's disease.
Investigational Medicinal Product	**Description** • Ex-vivo cultured allogeneic mesenchymal stem cells (MSCs) supplied in cryo-bags consisting of 150 or 200 million, suspended in 50 ml of Plasmalyte A containing 1.5% human serum albumin (HSA) and 3% dimethyl sulfoxide (DMSO). **Dosage** • Dosing of Stempeucel® is based on body weight. The recommended dose is 2 million cells/kg body weight. **Administration** • 40 – 60 injections administered as 0.6 ml/kg (200 million bag) or 0.8 ml/kg (150 million bag) intramuscularly into different points on the muscle. Additional injections of 2 ml (200 million bag) or 3 ml (150 million bag) administered around the ulcer
Number of Subjects	3 patients
Eligibility Criteria	**Inclusion criteria:** • Patients between 18-65 years old • Patients diagnosed with Buerger's disease by Shionoya criteria • Patients not eligible for or have failed surgical or percutaneous revascularization (No option patients) • Patients with at least one ulcer (between 0.5 to 10 cm^2 size) • Ankle brachial pressure index (ABPI) ≤ 0.6 (toe brachial index (TBI) if ABPI out of range; TBI ≤ 0.5) • Patients who are able and willing to provide consent and agrees to comply with study procedures and follow-up evaluations **Exclusion Criteria:** • Patients diagnosed with atherosclerotic peripheral arterial disease • Patients eligible for surgical or percutaneous revascularization • Patients with a history of participating in another stem cell trial or therapy within 3 months • Patients who are unsuitable to participate the clinical trial as determined by investigators

Evaluation Parameters	**Primary Endpoint:** • Relief of the rest pain • Healing of ulcerations or reduction of ulcer area • Improvement in ankle brachial pressure index (ABPI) • Improvement in total walking distance as measured by treadmill • Major amputation free survival • Angiogenesis **Secondary Endpoints:** • The type of AE(s), number of AE(s) and proportion of patients with AE(s). • Assessment of clinical laboratory parameters • Physical examination findings and assessment of vital signs • Assessment of ECG parameters
Data Analysis	**Data Management:** • Electronic case record form (eCRF) will be used for data entry. • Oracle clinical (or other suitable alternatives with audit trail) will be used for data management. **Statistical Method:** • The SPSS® package (IBM Inc., USA, version 22) will be used for statistical evaluation. • All patients in the study with relevant efficacy and safety data will be considered for the analysis. • Efficacy analysis will be done using GEE (Generalized Estimating Equations) method or paired t test as appropriate. • Adverse events monitored using information voluntarily disclosed by the patients and as observed by the PI will be summarized descriptively by total number of AE(s). • AEs will be categorized as: all AEs, all treatment-emergent AEs, all severe AEs, treatment-related AEs and severe treatment-related AEs. These events will be reported as appropriate and summarized.

1. Research Background and Rationale

Peripheral vascular disease of the lower extremities comprises a clinical spectrum that extends from asymptomatic patients to patients with chronic CLI that might result in amputation and limb loss. Incidence of CLI is estimated to be approximately 500 to 1000 patients per million per year (1).

Chronic CLI includes all patients with chronic ischemic rest pain, ulcers or gangrene attributable to objectively proven arterial occlusive disease. CLI arises as a result of atherosclerosis or vasculitis in leg arteries which severely impairs the patient functional status and QOL, and is associated with an increased cardiovascular mortality and morbidity. The annual overall major cardiovascular event rate (non-fatal MI, stroke and vascular death) in peripheral arterial disease (PAD) patients is 4–7% (2).

Narrowed vessels that cannot supply sufficient blood flow to exercising leg muscles may cause claudication, which is brought on by exercise and relieved by rest. As vessel narrowing increases, CLI can develop when the blood flow does not meet the metabolic demands of tissue at rest. 10% to 40% of the patients are at the risk of primary amputation (3). Patients with CLI have one year and 10 years mortality of approximately 20% and 75% respectively (4).

Prognosis of chronic CLI is poor and no effective treatments have been established in patients who are not amenable for the traditional revascularization therapies such as angioplasty and bypass procedures due to the inappropriate anatomy of the leg arteries or frequent re-occlusion following revascularization. Therefore, it is necessary to establish novel revascularization treatments like neo-angiogenic therapies to improve prognosis of these patients.

Thromboangiitis obliterans (Buerger's disease) is a non-atherosclerotic segmental inflammatory disease that most commonly affects the small and medium-sized arteries, veins, and nerves of the arms and legs (5). Pathologically, there is a highly cellular and inflammatory thrombus with relative sparing of the blood-vessel wall (6).

Although Buerger's disease has a worldwide distribution, it is more prevalent in the Middle East and Far East than in North America and Western Europe (7, 8). The prevalence of the disease among all patients with peripheral arterial disease varies from as low as 0.5 to 5.6 percent in Western Europe to as high as 45 to 63 percent in India (9-11). Buerger's disease primarily affects young male smokers; in the

past, they represented over 90% of affected people. Recently, however, women have made up an increasing percentage of affected people-more than 20% in some studies (12, 13). There is an extremely high prevalence in India among people of low socioeconomic class who smoke bidis (homemade cigarettes with raw tobacco) (14).

Buerger's disease usually begins with ischemia of the distal small arteries and veins. As the disease progresses, it may involve more proximal arteries. Involvement of the large arteries is unusual.

No specific laboratory test to diagnose Buerger's disease is available. However antinuclear antibody level, erythrocyte sedimentation rate, rheumatoid factor, VDRL antigen, hepatitis serologies, and serum complement levels (C-C4) can be useful in excluding other causes of vasculitis. In addition to a complete blood count, antithrombin III, antiphospholipid antibodies, factor V Leiden, protein C, and protein S levels as well as prothrombin time and partial thromboplastin time can help to exclude the possibility of hyper coagulopathies. Most test results of patients with Buerger's disease are within the normal ranges; however, elevated erythrocyte sedimentation rate, fibrinogen level, and platelet count may occur in patients with an active ulcer or necrosis (15).

Complete discontinuation of cigarette smoking or other use of tobacco in any form is the most effective treatment for Buerger's disease. Supportive care is directed towards maximizing blood supply including avoiding vasoconstriction from exposure to cold or drugs. Avoidance of thermal, chemical or mechanical injury, especially from poorly fitting footwear or minor surgery of digits, and fungal infection is recommended. Surgical revascularization is usually not possible for patients with Buerger's disease, because of the diffuse segmental involvement and distal nature of the disease. Sympathectomy may be helpful in healing of superficial ischemic ulcerations (12, 16).

Allogeneic MSCs derived from bone marrow and ex-vivo cultured, being both myogenic and angiogenic are being explored for their therapeutic potential for treating limb ischemia patients who have no option other than amputation.

Human MSCs are present as a rare population of cells in bone marrow, representing 0.001 to 0.01% of the nucleated cells, but they can rapidly grow in in-vitro culture without losing their stemness. MSCs can be expanded in vitro $\geq$ 2 million –fold and retain their ability to differentiate into several mesenchymal lineages (17).

MSCs can differentiate not only into mesodermal lineage such as myocardium, bone, cartilage and skeletal muscle but also into other lineages like neurons and endothelial cells, both in vivo and in vitro (18).

Adult MSC do not express human leukocyte antigen (HLA) class II antigens on the cell surface and do not elicit a proliferative response from allogeneic lymphocytes, thus, suggesting that the cells are not inherently immunogenic. The MSC also do not express co-stimulatory molecule B7-1, B7-2, CD40 R and CD40 ligand and hence do not activate allo-reactive T cells. Allogeneic MSCs do not induce lymphocyte proliferation when used in a mixed lymphocyte reaction (MLR), which is an in vitro model of immune cell activation. (19, 20).

Phenotypically, MSCs express a number of markers – CD105, CD73, CD44, CD90 (Thy-1), CD71 and Stro-1 as well as the adhesion molecules CD106 (VCAM-1), CD166 (ALCAM), ICAM-1 and CD29. Adult human MSCs do not express the hematopoietic markers CD45, CD34, CD14 or CD11. They also do not express CD80, CD86, CD40 (co-stimulatory molecules), or endothelial cell marker CD31 (PECAM-1), CD18 (LCAM) or CD56 (NCAM-1) (adhesion molecules).

MSCs can be isolated from bone marrow, cultured ex vivo, and expanded many fold (21). Cultured-expanded MSCs appear to represent a homogeneous population by flow cytometric measures of cell-surface markers. These cells retain the ability to undergo in-vitro differentiation to osteogenic, adipogenic and chondrogenic lineages, even when clonally expanded (22). MSCs are known to give rise to limb-bud mesoderm (osteoblasts, chondrocytes, adipocytes, stroma cells, and skeletal myoblasts) and can also differentiate into cells of visceral mesoderm (endothelial cells) (23). MSCs can facilitate vasculogenesis by increasing vascular endothelial growth factor (VEGF) levels (24).

MSCs display membrane-bound and soluble secreted molecules involved with cell attachment to neighbouring cells and to the extra cellular matrix. (21) This cell surface configuration may enable MSCs to home from bloodstream to mesenchymal tissue (25).

Bone marrow derived MSCs have several advantages over other type of stem cells like

- Non-embryonic source
- Reduced likelihood of neoplasia

- Ease of isolation
- High expansion potential
- Immune privileged
- Genetic stability
- Reproducible attributes from isolate to isolate
- Reproducible characteristics
- Compatibility with tissue engineering principles
- Potential to enhance repair in many vital tissues.

Potential beneficial mechanisms of MSC therapy in CLI would likely be by inducing regeneration:

1. Neo-angiogenesis – helps in development of new collateral blood vessels and thereby improves circulation or microcirculation in the ischemic region

2. Myo-genesis – regeneration of damage skeletal muscle.

Adult stem cells derived from bone marrow contain not only endothelial progenitor cells (EPCs) but also secrete angiogenic factors and cytokines and implantation of bone marrow-mononuclear cells (BM-MNCs) into ischemic tissues augment collateral vessel formation (26, 27).

Intramuscular implantation of human peripheral blood mononuclear cells (PBMNCs) and platelets into ischemic limbs effectively induce collateral vessel formation mainly by supplying VEGF (28). Mononuclear cells (MNCs) from adult human peripheral or cord blood have been shown to improve capillary density. (29).

MSCs promote new vessel formation and vascular remodelling in the upper and lower limbs. Angiopoietins and VEGF are both involved in angiogenesis and new vessel maturation, and MSCs have been shown to promote angiogenesis through the up-regulation of both in an experimental model of wound healing. (30)

Injury or inflammation is a prerequisite for the participation of stem cells to home and differentiate on to this micro- environment. The increased vascular permeability and expression of adhesions like proteins like integrin assist in stem cell homing. The migratory capacity of stem cells is dependent on natural growth factors such as VEGF, stromal cell derived factor (SDFI) and stem cell factor (SCF).

The expression of VEGF, SDFI and SCF is highly unregulated in the hypoxic damaged tissue and is responsible for the recruitment of the stem cells to assist in the repair mechanism.

1.1 Preclinical Experience

The low immunogenicity of allogeneic MSCs has been demonstrated in – vivo in animal models using infusion of allogeneic MSCs. Studies have shown that the MSCs possess the ability to engraft, persist and function in an unrelated mismatched allogeneic host (31).

Several preclinical studies on stem cells in animal models of hind limb ischemia have given encouraging results.

Bone marrow contains pluripotent CD34+ cells, which are known to give rise to hematopoietic cells. In vitro studies show that they can differentiate into mature endothelial cells. (32,33) A study demonstrated in the dog that CD34+ cells seeded into grafts could enhance vascular graft endothelialisation and vessel formation (34).

In a rat ischemic hind limb model, using nonradioactive coloured microspheres and by determining the femoral arteriovenous oxygen difference post-ligation at 2 weeks, the severity of the ischemic insult was demonstrated. To assess angiogenesis, histologic evaluation and angiography were done. It was concluded that bone marrow cells induced angiogenesis and improved deteriorated exercise capacity in the animal (35). Also, the angiogenic effect was examined in the ischemic hind limb in a diabetic rat model, wherein diabetes mellitus was induced by streptozotocin (36).

A study has shown in animals that BM-MNCs implantation into ischaemic limbs promotes collateral vessel formation, with incorporation of EPCs into new capillaries, and that local concentrations of angiogenic factors (basic fibroblast growth factor, VEGF, and angiopoietin-1) or angiogenic cytokines [interleukin 1β and tumour necrosis factor α (TNF α)] were increased in implanted tissues (26). This protocol was rapidly applied clinically with positive outcome (37).

Preclinical animal toxicology studies on the human MSC have been completed by Stempeutics and have been found to be safe.

Preclinical safety studies were conducted using Stempeucel®. They include

Acute toxicity studies (14 days single dose and repeat dose) by intravenous (IV) and intramuscular (IM) administration of Stempeucel®.

Sub-chronic toxicity studies (90 days single dose) in two animal species (rats and rabbits) by 2 routes (IV and IM) of administration and conducted using human equivalent doses (HED) in respective animals.

Erythrocyte micronucleus test showed no genotoxic potential of Stempeucel®.

Acute toxicity studies in rats showed that the minimum lethal dose, maximum tolerated dose and the median lethal dose of MSCs were – 20 X 10^6 MSCs/kg body weight. Daily dose of 15 X 10^6 MSC/kg body weight was administered intravenously and intramuscularly to rats for 14 days. No observed effect level (NOEL) of ex vivo cultured human adult-MSCs in rats was found to be greater than 15 X 10^6 MSCs/kg body weight.

Further acute toxicity study was conducted by calculating the HED in rats and rabbits by IM and IV routes. The therapeutic dose (TD) was assumed as 2 X 10^6 cells/kg body weight in humans. The HED (of TD) was calculated to be 12.6 X 10^6 and 6.53 X 10^6 cells/kg body weight in rats and rabbits, respectively. The study was conducted using 20 times these doses in rats and rabbits i.e. 252 X 10^6 Cells/kg body weight in rats and 130.6 X 10^6 Cells/kg body weight cells in rabbits. There was no lethality in the rats administered 20 times the TD by IV and IM routes. No lethality was recorded in rabbits administered 20 X TD by IM route. However, there was 40% mortality in rabbits when the MSC was administered at 20 X TD by IV route. Keeping these results in mind, sub-chronic toxicity tests in rats and rabbits were undertaken using TD (1 X TD), 5 X TD and 10 X TD.

In sub-chronic (90 days) toxicity studies by IM and IV routes of administration, no abnormalities in physical, physiological and neurological parameters were observed in rats or in rabbits. No observable adverse effect level (NOAEL) was recorded in rats when a single dose of 126 X 10^6 cells/kg body weight (10 X TD) Stempeucel – CLI™ was administered.

In sub-chronic toxicity study of rabbits, one female animal in high dose group died on the 12th day and one male animal in vehicle control group died on the 44th day after exposure to MSC and vehicle respectively in IM route study (Pre-terminal death of 8.3%). Mortality was 4.2% by IV at dose of 65.3

X 10^6 cells/kg body weight. When single dose of Stempeucel – CLI™ was administered, NOEL was 65.3 X 10^6 cells/kg body weight (10 X TD) by IM and so also was NOAEL by IV route in rabbits up to 90 days of exposure.

1.2 Clinical Experience

Accumulated evidence indicates that adult stem cells derived from bone marrow contain not only EPCs but also angiogenic factors and cytokines and that implantation of BM-MNCs into ischemic tissues augment collateral vessel formation (26, 27). EPC in the CD34+ stem-cell fraction of adult human peripheral blood takes part in postnatal neovascularisation after mobilization from bone marrow (32).

It was shown that IM implantation of human PBMNCs and platelets into ischemic limbs effectively induce collateral vessel formation mainly by supplying VEGF. (28) Also, it has been shown that MNCs from adult human peripheral or cord blood improved capillary density in limb ischemia (29).

The results of a randomized, double-blinded, multicentric, placebo-controlled, single dose, Phase I/II study (Protocol number.: SRPL/CLI/07-08/001) conducted by Stempeutics to assess the safety and efficacy of ex-vivo cultured adult allogeneic mesenchymal stem cells, administered intramuscularly in patients with CLI demonstrated the stem cell therapy to be safe with no major adverse events (AEs) including abnormal laboratory values. The study was conducted in 20 patients equally divided into stem cell arm and placebo arms. The allogeneic MSCs did not appear to elicit an immunological response as demonstrated by lymphocyte and cytokine profile analyses. A few efficacy parameters showed positive trend with improvement in ABPI (33).

A Phase II clinical study was conducted by Stempeutics in patients with critical limb ischemia due to Buerger's disease. This phase II clinical trial was done to evaluate the efficacy and safety of (90) patients who were enrolled in the study This was a non-randomized, open label, single-centre, three arm study to evaluate efficacy and safety of ex-vivo cultured adult bone marrow derived allogeneic MSCs (Stempeucel®) as compared to control group (standard protocol of care) in the treatment of CLI due to Buerger's disease. The study population consisted of patients of either sex, aged between 18-65 years with Buerger's disease as diagnosed by Shionoya criteria. Eleven centers in India participated in the trial. A total of 90 patients were enrolled into the study groups in a sequential manner to the dose group of 1 million cells/kg body weight (36 patients), 2 million cells/kg body weight (36 patients), and the

SPOC group (18 patients). The patients were followed for up to one year in the study for efficacy assessment and 2 years for both efficacy and safety assessment. The primary end points of this study were relief of rest pain and healing of ulcers in patients with Critical Limb Ischemia due to Buerger's disease. The data of patients showed reduction in both relief of rest pain and decrease in ulcer area which was statistically significant in patients who received 2 million cells/kg body weight as compared with the placebo group (34).

A phase I clinical trial, named ACellDREAM, was done to evaluate the feasibility and safety of intramuscular injections of autologous adipose-derived stromal cell (ASC) in non-revascularizable CLI patients. Seven patients were enrolled, on the basis of the following criteria: (i) lower limb rest pain or ulcer; (ii) ankle systolic oxygen pressure <50 or 70 mm Hg for non-diabetic and diabetic patients, respectively, or first-toe systolic oxygen pressure <30 mm Hg or 50 mm Hg for non-diabetic and diabetic patients, respectively; (iii) not suitable for revascularization. Abdominal fat was processed, cultured and harvested for 2 weeks to get ASCs. More than 200 million cells were obtained, with almost total homogeneity and no karyotype abnormality. The expressions of stemness markers Oct4 and Nanog were very low, whereas expression of telomerase was undetectable in human ASCs compared with human embryonic stem cells. ASCs were then intramuscularly injected into the ischemic leg of patients, with no complication. It was noted that trans-cutaneous oxygen pressure increased in most patients. Ulcer and wound healing showed improvement. This demonstrates the feasibility and safety of autologous ASC transplantation in patients with objectively proven CLI suitable for revascularization. The improved wound healing also supports a putative functional efficiency (35).

A recent prospective combined-centre pilot study done in eight patients with stable CLI in Norway using autologous BM-MNCs showed that five patients reported pain relief after four months. According to visual analogue scale and physiological tests, all were either stable or showed improvement. (36)

A clinical trial was conducted in China on 50 Type 2 diabetic patients with lower limb ischemia to assess the efficacy and safety of autologous transplantation of BM-MSCs on diabetics with lower limb ischemia. Main ischemic symptoms, including rest pain and intermittent claudication, were improved significantly in transplanted patients. The ulcer healing rate of the transplanted group was significantly higher than that of the control group. Improvement was seen in ABPI with new vessel formation on MRA along with lower amputation rate (37).

A trial to evaluate the safety and feasibility of regenerative therapy with intra-arterial infusion of MSCs from adipose tissue, in diabetic patients with chronic CLI without revascularization options has been completed. During one year follow-up, clinical, angiologic and angiographic measures was monitored to assess the impact of the cell infusion on the evolution of the ischemic limb (38).

In one of the clinical trials in 51 CLI patients, BM-MNCs were implanted in 25 patients with PAD and 26 patients with Buerger's disease. Forty-six CLI patients who had no BM-MNC implantation served as control subjects. The 4-year amputation-free rates after BM-MNC implantation were 48% in PAD patients and 95% in Buerger's disease, and they were 0% in control PAD patients and 6% in control Buerger's disease. The 4-year overall survival rates after BM-MNC implantation were 76% in PAD patients and 100% in Buerger's disease, and they were 67% in control PAD patients and 100% in control Buerger's disease. In Buerger's disease, ABPI and transcutaneous oxygen pressure were significantly increased after 1 month and remained high during 3-year follow-up. However, in patients with PAD, ABPI and transcutaneous oxygen pressure significantly increased after one month and gradually decreased during 3-year follow-up and returned to baseline levels. These findings suggest that BM-MNC implantation is safe and effective in patients with CLI, especially in patients with Buerger's disease (39).

The safety of Stempeucel® has been established in four therapeutic areas in 6 clinical trials: two trials each on CLI and osteoarthritis (OA), one trial each on acute myocardial infarction (AMI) and liver cirrhosis (LC).

Stempeutics has conducted 6 clinical trials. The completed clinical trials are AMI (SRPL/AMI/07-08/001; Phase I/II), CLI (SRPL/CLI/07-08/001; Phase I/II), OA-India (SRPL/OA/09-10/001; Phase II) and OA-Malaysia (SRM/OA/10-11/001; Phase II). CLI (SRPL/CLI/10-11/001) and LC (SRPL/LC/09-10/001).

1.3 Completed Trials

AMI Phase I/II (SRPL/AMI/07-08/001): In this trial, 20 patients were randomized into 2 arms; 10 patients received Stempeucel® and 10 patients received placebo. The MSCs were injected IV in the patients at a dose of 2 million cells/kg body weight. Stempeucel® was well tolerated and safe in the trial as evidenced by physical examination, vital signs monitoring and laboratory evaluations including renal

and liver function testing. The Stempeucel® did not elicit an immunological response in the recipients as evidenced by immunological parameters, which were within normal limits and comparable to the placebo group.

CLI Phase I/II (SRPL/CLI/07-08/001): A total of 20 patients were enrolled in this trial. 10 patients each were randomized to Stempeucel® arm and placebo arm. Stempeucel® were injected IM at a dose of 2 million cells/kg body weight. Stempeucel® was well tolerated in the trial as evidenced by physical examination, vital signs monitoring and laboratory evaluations including renal and liver function testing. The immunological parameters were also found to be within normal limits and comparable to the placebo group.

OA Phase II (India) (SRPL/OA/09-10/001): Sixty patients were enrolled in this study. Fifteen patients were randomized to the stem cell arm and the placebo arm in a ratio of 2:1 at each dose level. The doses used in this study were: 25 million, 50 million, 75 million and 150 million cells intra-articularly. Stempeucel® was well tolerated and have a favourable safety profile when injected intra-articularly. The incidence of treatment emergent adverse events which were related to the treatment was higher in 75 million group and 150 million group when compared to that of 25 million and 50 million group.

OA Phase II (Malaysia) (SRM/OA/10-11/001): A total of 72 patients were enrolled. Thirty six patients were randomized to the stem cell arm and the placebo arm in a ratio of 2:1 at each dose level. The doses used in the study were 25 million cells and 50 million cells. Similarly, safety results from this trial too demonstrated Stempeucel® has a favourable safety profile when injected intra-articularly for OA of knee. The incidence of treatment emergent adverse events which were related to the treatment was higher in 25 million group when compared to that of 50 million group.

CLI Phase II (SRPL/CLI/10-11/001): A total of 90 patients were enrolled into 3 arms: 1 million cells/kg body weight (36 patients), 2 million cells/kg body weight (36 patients), and the control group (18 patients). In this study, one of the primary endpoint was healing of ulcer and reduction of ulcer area in the target limb. The ulcer area assessment was performed at baseline, at one month (visit 4), 3 months (visit 5) and 6 months (visit 6) follow-up. Over the study period, ulcer area decreased in all the treatment groups. In the 1 million cells/kg body weight, the mean (SD) ulcer area reduced from 3.10 (4.73) cm^2 at baseline to 0.88 (3.58) cm^2 at 6 months (visit 6). The mean (SD) change from baseline was reduction of 3.32 (4.81) cm^2 at 1 month (visit 4) (55.2% reduction from baseline) and 5.02 (6.08) cm^2 at 6 months

(visit 6) (93.2% reduction from baseline). The mean (SD) ulcer area in the 2 million cells/kg dose group was 4.09 (5.81) cm^2 at baseline, which reduced to 0.21 (0.49) cm^2 at 6 months (visit 6). The mean (SD) change from baseline was reduction of 0.95 (2.27) cm^2 at 1 month (visit 4) (23.2% reduction from baseline) and 3.64 (4.93) cm^2 at 6 months (visit 6) (92.36% reduction from baseline). The mean (SD) ulcer area in SPOC group was 1.78 (1.60) cm^2 at baseline, which reduced to 0.12 (0.30) cm^2 at 6 months (visit 6). The mean (SD) change from baseline was reduction of 0.44 (1.53) cm^2 at 1 month (visit 4) (17.87% reduction from baseline) and 1.54 (1.62) cm^2 at 6 months (visit 6) (89.30% reduction from baseline). Longitudinal analysis of ulcer area using GEE in mITT population showed that overall there was a significant decrease in the ulcer size by 18% (SE=0.03) across all groups together per month (p-value = <0.0001). As compared to SPOC group, 1 million cells/kg body weight group had 2% (SE=0.06) decrease in ulcer size per month (p=0.6967; CI = 0.87, 1.10). As compared to the SPOC group, 2 million cells/kg dose group had 11% (SE=0.05) decrease in ulcer size per month (p=0.0253; CI = (0.80, 0.99), which was statistically significant. Hence the second primary endpoint, healing of ulcers was achieved in the D2m dose group.

Based on the above results, the primary endpoint of ulcer healing i.e., reduction in ulcer area have reached statistical significance in the 2 million cells/kg body weight dose group. And also based on the safety results, Stempeucel® have a favourable safety profile when injected IM to CLI patients at either dose of 1 million or 2 million cells/kg body wt.

LC Phase II (SRPL/LC/09-10/001): In this trial, a total of 60 patients were enrolled. There are three dose groups in this study: 2.5, 5 and 7.5 million cells/kg body weight. The third dose group (7.5 million cells/kg body weight was put on clinical hold as recommended by DSMB. Twenty patients were exposed to Stempeucel® in the 2.5 and 5 million cells/kg body weight dose groups each. The planned follow-up duration for this study was two years.

Stempeucel® has been proved to be safe and efficacious (or indicatively efficacious) in different clinical indications as mentioned above. It has proven ulcer healing ability and relief of rest pain as shown in the CLI due to Buerger's disease clinical trial. In the preclinical animal model of diabetic ulcer, it has shown improvement in healing of ulcers.

From the successful small number of randomized clinical trials, it is reasonable to conclude that the use of bone marrow adult MSCs are safe in the long-term.

1.4 Study Rationale

Research involving human pluripotent/multipotent stem cells promises new treatment and possible cure for many chronic and debilitating diseases including Parkinson's disease, Heart disease, Multiple Sclerosis, Chronic CLI, Burns and Spinal Cord Injury. Various preclinical animal studies have shown the potential of bone marrow derived MSCs in re-vascularizing ischaemic limbs and promoting collateral vessel formation. Multipotent MSCs have the potential to facilitate the formation of new blood vessels and skeletal muscle.

Clinical studies indicate that stem-cell transplantation is feasible and may have beneficial effects in CLI due to Buerger's disease (40). Injury or inflammation is a prerequisite for the participation of circulating stem cells to home and differentiate on to this micro- environment. The increased vascular permeability and expression of adhesion proteins like integrin assist in stem cell homing. The migratory capacity of stem cells is dependent on natural growth factors such as VEGF, SDFI and SCF. The expression of VEGF, SDFI and SCF is highly unregulated in the hypoxic muscular tissue and is responsible for the recruitment of the stem cells to assist in the repair mechanism and consequent improvement in limb function.

In addition to the above regenerating potential of MSCs, they have several advantages; they can be easily isolated and can be rapidly cultured ex-vivo to more than million folds without losing their stemness. The low immunogenicity of these cells has been demonstrated in vivo in animal models using infusion of allogeneic MSCs. Several preclinical and clinical studies have shown that administration of allogeneic MSCs in an unrelated mismatched allogeneic host does not stimulate the formation of allo-specific antibodies or lead to a T cell sensitization of the recipient to alloantigen in different animal models. They have also shown that the MSCs possess the ability to engraft, persist and function in an unrelated mismatched allogeneic host. In addition to the property of low immunogenicity, MSCs have been shown to actively modulate the immune response (41).

1.5 Potential Risks

Human MSCs cultured in the laboratory possess the ability to differentiate into other tissues such as bone and cartilage under appropriate conditions. However, none of earlier pilot clinical trials have demonstrated abnormal tissue growth and injection related toxicity with allogeneic MSCs.

The propagation of MSC during culture requires serum from fetal bovine origin (free of Bovine Spongiform Encephalitis and Transmissible Spongiform Encephalopathy), media supplements and chemicals of animal origin and could therefore have immune responses in recipients to animal proteins. Allogeneic MSCs are derived from HLA non-matched donors and could sensitize recipients. Hence AE and SAE reporting in this study will look at these possibilities and document the same.

1.6 Potential Benefits

Various preclinical animal studies have shown that Allogeneic MSCs have the potential to re vascularize ischemic limbs and promoting collateral vessel formation. Potential beneficial mechanisms of MSC therapy in CLI would be

- Neo-angiogenesis –development of new collateral blood vessels with improvement of microcirculation in the ischemic region – relief of leg pain with healing of leg ulcer in the ischemic limb.

- Myo-genesis – regeneration of damage skeletal muscle fibers.

2. Research Objectives and End Points

2.1 Objective

To observe the efficacy and safety of Stempeucel® (adult human bone marrow derived, cultured, pooled, allogeneic mesenchymal stromal cells) in Malaysian patients with critical limb ischemia (CLI) due to Buerger's disease.

2.2 Endpoints

2.2.1 Primary endpoint

- Relief of the rest pain – measured by VAS score
- Healing of ulcerations or reduction of ulcer area, or improvement in the rate of healing of ulcer in the target limb – measured using Wound Zoom or any other suitable app or by photography
- Improvement in ankle brachial pressure index (ABPI) – measured by Doppler
- Improvement in total walking distance – measured by treadmill
- Major amputation free survival

2.2.2 Secondary endpoints

- The type of AE(s), number of AE(s) and proportion of patients with AE(s).
- Assessment of clinical laboratory parameters
- Physical examination findings and assessment of vital signs
- Assessment of ECG parameters
- Angiogenesis – measured by digital subtraction angiogram (DSA)

3. Number of Subjects and Calculation Basis

- Total 3 subjects
- This study is an exploratory study and it is desirable to target the minimum number of subjects who can satisfy the research purpose. Furthermore, based on our clinical experience in HCTM, only 1 or 2 patients were admitted annually with Buerger's disease. Hence, in this study, 3 research participants will be selected.

4. Eligibility Criteria

4.1 Inclusion criteria

1) Males or females (willing to use accepted methods of contraception during the

course of the study) in the age group of 18-65 years.

2) Buerger's disease as diagnosed by Shionoya criteria
3) Patients should have at least one ulcer (target ulcer): area between 0.5 to 10 cm^2 (both inclusive)
4) Ankle Brachial Pressure Index (ABPI) ≤ 0.6. If ABPI is ≥ 1.1 then Toe Brachial Index (TBI) will be performed and TBI should be ≤ 0.5
5) Patients who are able to understand the requirements of the study, and willing to provide voluntary written informed consent, abide by the study requirements, and agree to return for required follow-up visits

4.2 Exclusion criteria

1) Patients diagnosed with atherosclerotic peripheral arterial disease
2) Patients eligible for surgical or percutaneous revascularization
3) Patients with a history of participating in another stem cell trial or therapy within 3 months
4) Patients who are unsuitable to participate the clinical trial as determined by investigators

5. Study Conduct

5.1 Study Design Rationale

The market approval granted for Stempeucel® in India has validated the efficacy and safety of the product. Nevertheless, considering the infancy of stem cell use in Malaysia, particularly with Buerger's disease, the study team are keen to administer this innovative therapy under the scrutiny of strict and safe environment offered by a clinical trial setting.

Hence, the study will be conducted as a clinical trial in accordance to "Malaysian Guidelines for Stem Cell Research and Therapy" by Medical Development Division, Ministry of Health Malaysia, 2017, the EMA guidelines on human cell based medicinal products, 2009, and International Council for Harmonisation (ICH) Good Clinical Practices (GCP); approvals will be taken from relevant ethics committees and approval from NPRA before starting the study.

5.2 Trial Design Flow Chart

A schematic overview of study design is shown in Figure 1.

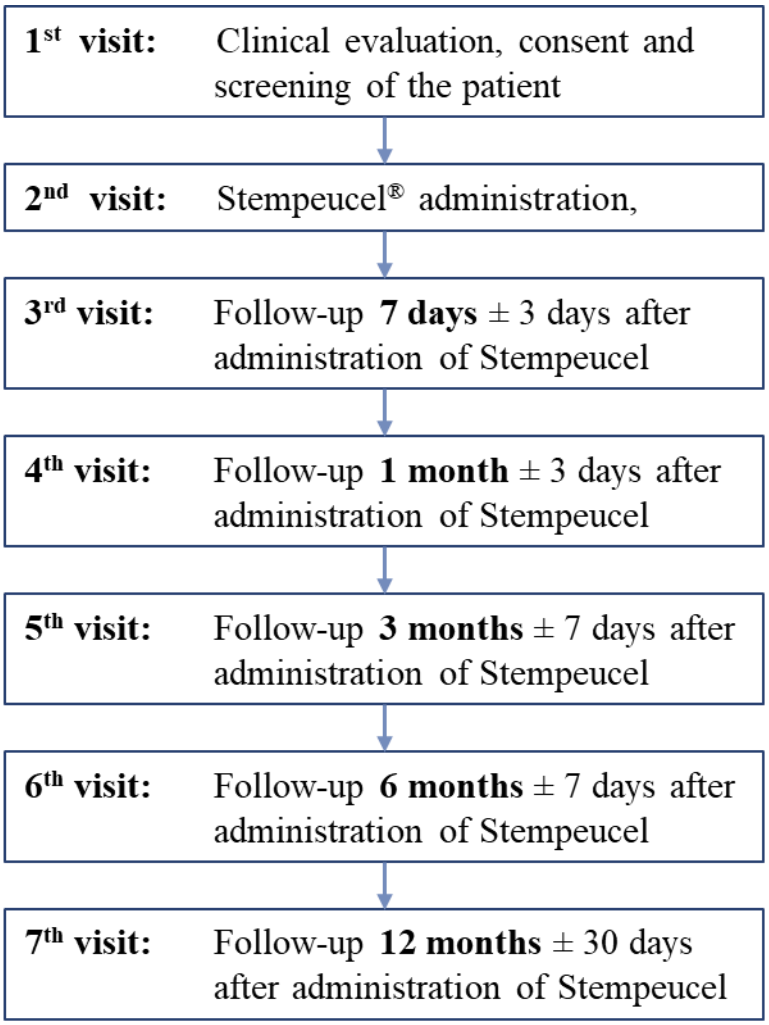

Figure 1. Overview of Study Design

5.3 Methods and Procedure

Visit -1: Clinical evaluation and screening of the patient (Day -14 to Day -1)

The following procedures will be performed during the screening process.

- Written informed consent.
- Inclusion / Exclusion criteria
- Demographic data along with medical history and concomitant medication.
- AE(s) will be monitored and recorded as voluntarily disclosed by the patients and as observed by the Investigator throughout the study.

- Physical examination with vital signs such as blood pressure, heart rate, respiratory rate, temperature.
- Clinical/diagnostic laboratory tests (as per Table 1) including TNF-α
- 12 lead ECG recording with long Lead II, chest X-ray, Two-Dimensional Echocardiography (2D ECHO; if needed)
- Peripheral Venous Doppler to rule out deep vein thrombosis (DVT)
- Assessment of rest pain by – Rest pain scale (0 to10)
- Total walking distance
- Ankle pressure – measured by Doppler
- ABPI – measured by Doppler
- Assessment of ulcerations (Ulcer area measurement using WoundZoom camera or any other suitable and validated measurement instrument / software)
- Digital subtraction angiogenesis (DSA)
- Amputation details
- Urine pregnancy test (for female patient with child bearing potential)

Visit-2: Stempeucel® administration (within 15 days of screening Visit 1) (Day 0)

The following procedures will be performed on the day of Stempeucel® administration.

- Patients will be enrolled into the study as soon as the results of the screening laboratory tests become available
- Physical examination including vital signs such as blood pressure, heart rate, respiratory rate and temperature.
- Review of concomitant medication
- AE(s) will be monitored and recorded as voluntarily disclosed by the patients and as observed by the Investigator throughout the study.
- 12 lead ECG with long Lead II will be done pre and post Stempeucel® administration.
- On Stempeucel® administration day, premedication of 100 mg of hydrocortisone IV and 45.5 mg pheniramine maleate IV / IM (based on investigator / anaesthetist discretion) will be administered within 1 hour prior to IM Stempeucel® injection.
- Stempeucel® will be administered at 40-60 multiple sites to the ischemic limb and also around the ulcer (2 to 3 ml). The procedure will be performed preferably under IV sedation with cardio-respiratory monitoring. Spinal, epidural or short general anesthesia as per the investigators/anesthetist's advice is also acceptable; however, the requirement should be justified for individual patient.
- Monitoring of oxygen saturation by pulse oximeter will be started 30 ± 10 min prior and continued till 2 hours ± 10 min post injection of Stempeucel®
- The patients will remain in the clinical facility under supervision for at least 24 hours post Stempeucel® administration.

Follow up Visit-3: 7 days ± 3 days safety visit (Day 7)

The following procedures will be performed at follow-up visit 3:

- Physical examination, vital signs and 12 lead ECG with long lead II.
- Review of concomitant medication
- AE(s), will be monitored and recorded as voluntarily disclosed by the patients and as observed by the investigator throughout the study
- Blood tests- hematology, serum chemistry, liver function tests and TNF-α.
- Qualitative D-dimer test for all the patients to be done.
- Peripheral venous Doppler if the D-dimer test is positive

Follow up Visit-4: One month ± 3 days after administration of Stempeucel® (Day 30)

The following procedures will be performed at follow-up visit 4:

- Review of concomitant medication
- Physical examination, vital signs and 12 lead ECG with long lead II
- AE(s), will be monitored and recorded as voluntarily disclosed by the patients and as observed by the Investigator throughout the study
- Blood tests- hematology, serum chemistry, liver function tests and TNF-α.
- Assessment of rest pain
- Total walking distance
- Ankle pressure
- ABPI
- Assessment of ulcerations
- Amputation details

Follow up Visit-5: 3 months ± 7 days after administration of Stempeucel® (Day 90)

The following procedures will be performed at follow-up visit 5:

- Review of concomitant medication
- Physical examination, vital signs and 12 lead ECG with long lead II
- AE(s) will be monitored and recorded as voluntarily disclosed by the patients and as observed by the Investigator throughout the study
- Blood tests- hematology, serum chemistry and liver function tests
- Assessment of rest pain

- Total walking distance
- Ankle pressure
- ABPI
- Assessment of ulcerations
- Amputation details

Follow up Visit-6: 6 month ± 7 days after administration of Stempeucel® (Day 180)

The following procedures will be performed at follow-up visit 6:

- Review of concomitant medication
- Physical examination, vital signs, 12 lead ECG with long lead II and chest X ray
- Blood tests- hematology, serum chemistry and liver function tests
- AE(s), will be monitored and recorded as voluntarily disclosed by the patients and as observed by the Investigator throughout the study
- Assessment of rest pain
- Total walking distance
- ABPI
- Assessment of ulcerations
- DSA
- Amputation details
- Urine pregnancy test (for female patient with child bearing potential)

Additional follow up Visit-7: 12 month ± 7 days after administration of Stempeucel® (Day 360)

The following procedures will be performed at additional follow-up visit 7:

- Review of concomitant medication
- Physical examination, vital signs and 12 lead ECG with long lead II
- AE(s) will be monitored and recorded as voluntarily disclosed by the patients and as observed by the Investigator throughout the study.
- Blood tests- hematology, serum chemistry and liver function tests.
- Assessment of rest pain
- Total walking distance
- Ankle pressure
- ABPI
- Assessment of ulcerations
- Amputation details

5.4 Research Procedure and Evaluation

	Screening	Treatment	Follow-up				
Week	**Day -14 to -1**	**Day 0**	**Day 7**	**Day 30**	**Day 90**	**Day 180**	**Day 360**
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Demographics	X						
Physical/Clinical Examination	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X
Medical History	X						
Concomitant Medication	X	X	X	X	X	X	X
Haematology	X		X	X	X	X	X
Serum Chemistry	X		X	X	X	X	X
Liver Function Tests	X		X	X	X		X
Urine Analysis	X					X	
Serology	X						
TNF-α	X		X	X			
Oxygen saturation monitoring		X					
Pre-Medication /Sedation		X					
IM injection of Stempeucel®		X					
12 Lead-ECG with Long Lead II	X	X	X	X	X	X	X
Peripheral Venous Doppler	X		X				
Chest X ray (PA view)	X					X	
Rest Pain Assessment	X			X	X	X	X
Ulcer healing assessment	X			X	X	X	X
Total walking distance	X			X	X	X	X
Ankle pressure	X			X	X	X	X
ABPI	X			X	X	X	X
DSA	X					X	

5.5 Clinical/Diagnostic Laboratory Tests

Table 1: Clinical / Diagnostic Laboratory Tests

<table>
<tr><th>SERUM CHEMISTRY</th><th>HAEMATOLOGY</th><th>LIPID PROFILE</th></tr>
<tr><td>• RBS
• Sodium
• Potassium
• BUN
• Serum creatinine
• Glycosylated haemoglobin (HbA1c)
• D- Dimer Test</td><td>• Haemoglobin
• Haematocrit
• Total and differential leukocyte count
• Red blood cell count
• Mean corpuscular volume (MCV)
• Platelet count
• Erythrocyte Sedimentation Rate (ESR)</td><td>• Total cholesterol
• Triglycerides
• High density lipoproteins (HDL)
• Low density lipoproteins (LDL)
• Very low-density lipoprotein (VLDL)</td></tr>
<tr><th>LIVER FUNCTION TESTS</th><th>SEROLOGY</th><th>URINE ANALYSIS</th></tr>
<tr><td>• Total bilirubin
• Direct bilirubin
• Total proteins
• Serum albumin
• Serum globulin
• A:G ratio
• Alanine Aminotransferase (ALT)
• Aspartate Aminotransferase (AST)
• Alkaline Phosphatase (ALP)</td><td>• HIV-1 & 2 (Anti-HIV 1 & 2)
• Hepatitis B (HBsAg)
• Hepatitis C (Anti HCV)
• Cytomegalo virus (CMV) (Anti CMV IgM)
• Rapid Plasma Reagin (RPR)
• High sensitivity C Reactive protein (hs-CRP)</td><td rowspan="3">• Colour
• Transparency
• pH
• Specific gravity
• Protein
• Glucose
• Ketone bodies
• Bilirubin
• Blood
• Nitrite
• Urobilinogen
• Microscopic examination
• Urine pregnancy test</td></tr>
<tr><td>TNF-α</td><td colspan="2">12-Lead ECG with long lead II
Chest X-ray (PA view)</td></tr>
<tr><td>DSA</td><td colspan="2">2D ECHO (when needed)
Peripheral Venous Doppler</td></tr>
</table>

5.6 Early Termination of the Study

Sponsor may terminate this study, after informing Investigators, at any time. Investigators will be notified by Sponsor if the study is placed on hold, completed or closed. The DSMB will be informed about all SAEs including life-threatening event, disabling event, or death. Available data will be provided to DSMB for review. Based on the DSMB opinion, the study may be continued or halted.

6. Study Treatment

6.1 Definition of Investigational Medicinal Product

For the purpose of this trial, investigational medicinal product (IMP) refers to:

- Stempeucel® (Ex-vivo cultured MSCs) supplied in 15 ml cryo bags consisting of 200

million or 150 million MSCs, 85% PlasmaLyte-A, 5% HSA and 10% DMSO in a total volume of 15 ml. Following thawing, 35 ml of PlasmaLyte A will be added to the Stempeucel® to make a total volume of 50 ml (Refer section 6.6 IMP Preparation and Designation). Final concentration of components will be 1.5% HSA and 3% DMSO.

6.2 Dosage Selection

Acute infusion toxicity studies in animals have shown that MSCs up to a single dose of 20 million cells/kg was well tolerated without either infusional or other toxicities. Based on this data 1/10th of the tolerated dose in animals i.e., 2 million cells/kg body weight were selected for dosing in humans.

In the phase I/II study conducted by Stempeutics (SRPL/CLI/07-08/001) the 2 million cells/kg body weight dose was well tolerated. As based on the preclinical and recent toxicology studies where 20 million cells/kg body weight human equivalent dosage (HED) was tested and based on phase I/II data and phase II study which was a dose finding study in two doses of 1 and 2 million cells/kg body weight, the present study will be conducted with 2 million cells/kg body weight.

6.3 Pre-Treatment and Monitoring

The administration visit will include monitoring of oxygen saturation 30 ± 10 min prior to injection of Stempeucel® and 2 hours ± 10 min post-injection. Premedication of hydrocortisone 100 mg IV and pheniramine maleate 45.5 mg IV / IM (based on investigator or anaesthetist discretion) will be administered within one hour prior to the injection of Stempeucel®.

6.4 IMP Administration

The procedure will be performed preferably under IV sedation and cardio-respiratory monitoring. Spinal, epidural or short general anesthesia as per the investigators/anesthetist's advice is also acceptable; however, the requirement should be justified for individual patient.

Since the migratory capacity of stem cells is dependent on natural growth factors such as VEGF which is highly unregulated in the hypoxic tissue. IM route of administration was selected so as to facilitate the MSCs in homing to the target site.

The patients will receive a single IM dose of allogeneic MSCs suspended in PlasmaLyte A at 0.6 ml/kg (200 million bag) or 0.8 ml/kg (150 million bag) of cell suspension/kg body weight

into 40-60 multiple sites to the ischemic limb (Refer section 6.7 IM Injection). Additional injections of 2 ml (200 million bag) or 3 ml (150 million bag) administered around the ulcer (Refer section 6.8 Local Injection Around the Ulcer).

Timing of dose administration will be such that the patients will receive the assigned Stempeucel® within 14 days of screening visit. The patients will remain in the clinical facility under supervision at least 24 hours post Stempeucel® administration.

6.5 Treatment Compliance

The PI or other trained clinical study site personnel will administer the Stempeucel® according to the procedures outlined above. The complete process of Stempeucel® dosing administration will be monitored by the Site Coordinator to ensure by visual inspection that the patient has been administered with the dose and the IM injection of Stempeucel® is completed.

6.6 IMP Preparation and Designation

Supplied Stempeucel® must be reconstituted immediately before giving an IM injection. Stempeucel® in the cryo bag will be thawed and re-suspended in 35 mL of Plasmalyte-A resulting in 50 mL of suspension for IM injection.

Based on the Certificate of Analysis (COA), the viability of cells is presumed to be ≥85%*. Hence actual number of viable cells in bags is described in Table 2.

Table 2. Number of viable cells in each bag of Stempeucel®

CELL BAG	VIABILITY OF CELLS	ACTUAL NUMBER OF VIABLE CELLS
200 million	85%	170 million
150 million	85%	128 million

*Based on COA. On site viability count will be done in as many patients as possible

Selection of the cell bag designated to each patient will be based on body weight as described in Table 3.

Table 3. Designation of bag size according to patient's body weight

BODY WEIGHT	BAG SUPPLIED	COMMENTS
≤ 62 Kg	150 million bag	1 bag supplied
63-83 Kg	200 million bag	1 bag supplied
≥ 84 Kg	200 million bag	2 bags supplied

After thawing and re-suspending the bag, the volume to be injected is aspirated from the bag in 3 ml syringes supplied and injected IM and locally around the ulcer.

Remaining volume of Stempeucel® is transferred to a centrifuge tube and sent back to the sponsor.

6.7 IM Injection

Based on the cell bag designation, the volume of the Stempeucel® suspension required to achieve the desired 2 million cells/kg body weight dose is illustrated in Table 4.

Table 4. Calculation of volume of Stempeucel® to be injected intramuscularly

PACK SIZE	DOSE	VOLUME OF IMP INJECTED
150 million cells	2 million cells/kg body weight	0.8 ml/kg
200 million cells	2 million cells/kg body weight	0.6 ml/kg

Each injection will be 0.5 ml or 1 ml of Stempeucel®. The PI will record the actual number of 0.5 and 1 ml injections administered in the source document and in the CRF. The number of injections must be at least 40 for each patient and not more than 60 injections. Depending on the weight of the patient the last injection volume may vary between 0.5 and 1.0 ml.

Depending on number of injections to be administered, a map will be drawn using 10 points at a distance of one cm each in the longitudinal axis on the posterior aspect of study limb (gastrocnemius muscle). The length of the horizontal axis will depend on the number of

injections as described in Table 5. Using an indelible marker (e.g. gentian violet), each cm mark on the scale will be marked on the skin both on the longitudinal and horizontal axis. Each of the marked dots can be site of injections. Thus, the size of the mapped area is illustrated in Figure 2.

Table 5. Determination of Stempeucel® site of injections

NO. OF INJECTIONS	LENGTH OF LONGITUDINAL AXIS (CM)	LENGTH OF HORIZONTAL AXIS (CM)	NO. OF INJECTION POINTS IN LONGITUDINAL AXIS	NO. OF INJECTION POINTS IN HORIZONTAL AXIS
40	9	3	10	4
41 – 50	9	4	10	5
51 – 60	9	5	10	6

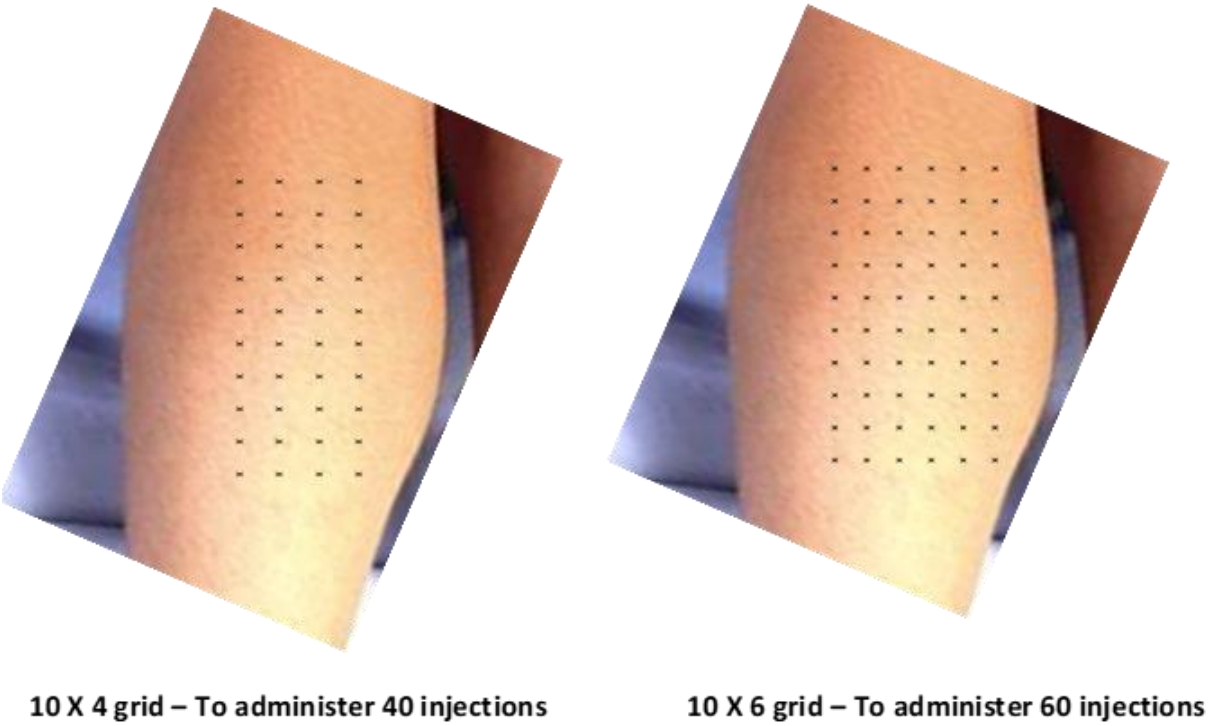

Figure 2. Distribution of injections site marked according to the cell bag designated

In brief, the number of injections in the longitudinal axis will be 10 for any number of injections, while the number of injections in the horizontal axis will vary based on the number of injections. The mapping will be done in such a way that it encompasses the maximum bulk of the gastrocnemius muscle. The required volume of Stempeucel® will be aspirated in 3 ml

syringes. Each syringe will be used to administer a maximum of 6 consecutive injections. With all aseptic precautions, under sedation Stempeucel® should be injected intramuscularly (at a depth of 1 – 1.5 cm depending on the muscle bulk) using a 22- or 23-gauge needle, at required number of marked sites into the calf muscle (gastrocnemius muscle) of the ischemic limb (as shown in the Figure 1). The injections will start at the top left dot and proceed horizontally until all the dots in the top row have been injected. Subsequent injections will start at the first dot on the left of subsequent rows and proceed similarly. This procedure will continue till the entire Stempeucel® is injected. Each site should be separately injected i.e., 40 – 60 separate injections are to be made.

6.8 Local Injection Around the Ulcer

Out of the calculated volume of IMP to be administered to the patient, the volume to be administered around the ulcer is described in Table 6.

Table 6. Calculation of volume of Stempeucel® to be injected around the ulcer

PACK SIZE	DOSE	VOLUME OF IMP TO BE ADMINISTERED (ULCER)
150 million cells	7.5 million cells/kg	3 ml
200 million cells	8 million cells/kg	2 ml

Two ml or three ml of Stempeucel® should be injected around the ulcer (single or multiple). This 2 ml or 3 ml (depending on the cell bags used – 200 million or 150 million) should be taken from the calculated volume of Stempeucel® to be administered for the patient.

E.g. Patient weighing 70 kg will have to receive a total volume of 42 ml of Stempeucel® (based on the formula: Volume = body weight multiplied by 0.6 in a 200 million cell bag)

Out of this 42 ml of Stempeucel®, 40 ml of Stempeucel® should be given by IM route and 2 ml should be given locally around the ulcer.

6.9 Precautions

1. Monitoring of oxygen saturation will be started 30 ± 10 minutes prior to IM injection and continued till 2 hours ± 10 minutes following IM injection.
2. The procedure will be performed preferably under IV sedation and cardio-respiratory monitoring. Spinal, epidural or short general anesthesia as per the

investigators/anesthetist's advice is also acceptable however the requirement should be justified for individual patient.

3. Intramuscular injection should be injected immediately (within 60 minutes) after preparing the suspension and it should not be stored.
4. Administration of Stempeucel® should be stopped if the patient shows tachypnea, cyanosis, breathlessness, or oxygen saturation decreases as per Investigator's discretion and can be restarted once the patient shows clinical improvement as per Investigator's discretion.
5. The whole procedure of Stempeucel® administration should be completed within 60 minutes of re-suspension of Stempeucel®.
6. Investigator/Co-investigator should administer the Stempeucel®.
7. Site coordinator should be available during administration of Stempeucel®, record the number of injections and monitor the AEs if any.
8. Injection of Stempeucel® should not be carried out if-the bag was kept in the cryoshipper for longer than 7 days. The site should inform the Sponsors for reshipment of Stempeucel® immediately.
9. Injection of the Stempeucel® should not be carried out if the bag is found to be damaged or leaking. The site should inform the Sponsors for reshipment of Stempeucel® immediately.

Any changes in the method will be communicated in writing by the Clinical Trial Manager to the Investigator.

6.10 Stability Data

Stempeucel® (200 million bag) is stable under liquid nitrogen for 18 months and Stempeucel® (150 million bag) is stable under liquid nitrogen for 24 months under laboratory conditions. However, it will remain stable at the site for 7 days (including time of transit) in the dry shipper.

The study treatment supplies will be stored in a secure location. The Stempeucel® supplied in the dry shipper, should be stored in the same at the site and Stempeucel® will remain stable at the site for 7 days (including time of transit) in the dry shipper.

6.11 IMP Shipment and Handling

Upon receipt of the study treatment supplies, an inventory must be performed and a Stempeucel® receipt log filled out and signed by the designated site staff accepting the shipment. It is important that the designated site staff will verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable Stempeucel® in a given shipment will be documented in the study files. The Investigator must notify Sponsor if study treatments that were received at the Investigator's site were damaged or unusable.

Supply of the cryo bag containing the Stempeucel® to the study center will be delivered under liquid nitrogen vapour phase in a Dry Shipper upon receiving Stempeucel® request for a trial subject from the site. The cryo bags will be labelled as per labelling requirements of Good Manufacturing Practice (GMP) Annex 13 Manufacture of Investigational Medicinal Products.

Sample label for 200 million cell bag:

<table>
<tr><td colspan="2">FOR CLINICAL TRIAL USE ONLY</td></tr>
<tr><td colspan="2">Protocol No.: CBR-BD-22-003</td></tr>
<tr><td colspan="2">Stempeucel®
The cryobags containing frozen Stempeucel® – Mesenchymal Stem Cells (MSC) (15ml) contains:
Mesenchymal Stem Cells (200 million cells), Human Serum Albumin 5%, PlasmaLyte A 85%, DMSO 10%
Directions of use
Before administering to the patient, each bag (Stempeucel®) is thawed and diluted with PlasmaLyte A, supplied along with the Cryobags to make up the volume to 50 ml. Thus, each bag in the final suspension contains 2 million cells per 0.6 ml. Shake gently before use. Do not add any other medicines to the contents. Administer through disposable sterile syringes. Use within 60 minutes after reconstitution. Refer to IMP Management Manual for procedure for reconstitution and more details.</td></tr>
<tr><td colspan="2">Batch No:</td></tr>
<tr><td colspan="2">Manufacturing Date:</td></tr>
<tr><td colspan="2">Expiry Date: Two years from date of manufacture</td></tr>
<tr><td>Stempeucel® Bag No.</td><td>Pack size: 200 million MSCs</td></tr>
<tr><td colspan="2">Storage Condition: -185 ^{O}C TO -196 ^{O}C</td></tr>
<tr><td colspan="2">Route of Administration: Intramuscular</td></tr>
<tr><td colspan="2">Instruction to use: Refer to IMP Management Manual</td></tr>
</table>

Manufacturer: Stempeutics Research Pvt. Ltd 4th floor, Shirdi Sai Baba Cancer Hospital Manipal – 576104, India Sponsor: Cell Biopeutics Resources Sdn. Bhd., 27, Level G3, Block C5, Jalan Dutamas 1, Publika Shopping Gallery, 50480 Kuala Lumpur Phone – +60 12 258 8779
DO NOT USE THE PRODUCT IF FOUND IN THAWED CONDITION UPON REMOVAL FROM DRY SHIPPER

Sample label on the cryo shipper:

"FOR CLINICAL TRIAL USE ONLY" DRY SHIPPER CONTAINING STEMPEUCEL® BAG (S) TRIAL ID: **CBR/CLI-BD/21-22/003** SUBJECT ID: SITE ID: DATE OF SHIPMENT: USE WITHIN 7 DAYS FROM THE DATE OF SHIPMENT OF STEMPEUCEL® **STORAGE CONDITIONS:** DRY SHIPPER CONTAINING STEMPEUCEL® BAG SHOULD BE STORED IN A SECURE PLACE STEMPEUCEL® SHOULD BE STORED IN THE DRY SHIPPER UNTIL USE. **DIRECTIONS FOR USE:** OPEN THE DRY SHIPPER ONLY JUST BEFORE USE OF THE BAG. SPONSOR NAME & ADDRESS: CELL BIOPEUTICS RESOURCES SDN. BHD., 27, LEVEL G3, BLOCK C5, JALAN DUTAMAS 1, PUBLIKA SHOPPING GALLERY, 50480 KUALA LUMPUR PHONE – +60 12 258 8779

6.12 Patient Identification Code Numbering System

The investigators involved in conducting the study and performing the evaluation and the patients participating in the trial will not be blinded. All patients who sign an IRB approved informed consent form will be assigned a unique patient identity code (PIC). The patient initials will be captured separately as an additional identity.

6.13 Concomitant Therapy

Concomitant therapy is permitted based on the standards of care for patients with CLI due to Buerger's disease, who are not eligible for or have failed traditional revascularization treatment and as per the investigators discretion so as not to jeopardize the study and its outcome. Patients should be advised not to take therapies from other systems of medicine like Ayurveda, Homeopathy. Any other stem cell-based products (other than Stempeucel®) should not be administered – either systemically or locally for treatment of ulcers.

Detailed history of concomitant medications at the baseline will be recorded in the CRF. All concomitant therapy taken by the patient during the study period will be recorded in the CRF in the concomitant medication section in detail.

Patients will be instructed to report to the Investigator any medication used over the course of the study. The Investigator will address the significance of the reported medication use on study integrity. At the discretion of the Investigator, these patients may continue study participation if the medication is not anticipated to alter study integrity.

6.14 Study Treatment Follow-up

Study patients will be assessed by the PI before administering the Stempeucel® for any other organ with inflammation. The study patients will also be followed up to duration of 1 year after study treatment administration for safety and efficacy assessment.

Since the MSCs are known to differentiate and home to the inflammatory site with potential of neo-vascularization, the PI will follow-up such patients on their medical condition as per his/her best clinical judgement and at intervals of his/her discretion.

7. Efficacy Assessments

7.1 Efficacy Analysis

Efficacy evaluation will include assessment of the following parameters at 1, 6 and 12 months.

- Rest pain score (VAS scale of 0 to 10) (refer Appendix II)
- Ankle pressure (Higher of systolic pressure at posterior tibial or anterior tibial artery of the affected limb (Measured by doppler)

- Ankle Brachial Pressure Index (Ratio of ankle pressure (as above) to brachial pressure. Brachial pressure is calculated as follows: Higher of brachial pressure of both arms (Measured by Doppler)
- Ulcer status (Ulcer present (Yes / No). If present, healed/improved/not healed at follow-up)

8. Safety Evaluation Criteria, Methods, and Reporting

8.1 Safety Analysis

Evaluate the frequency and severity of adverse events (AEs) recorded on CRF.

8.2 Definitions

8.2.1 Adverse Events (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

8.2.2 Serious Adverse Events (SAE)

A SAE (experience) or reaction is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,

(NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)

- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or

- is a congenital anomaly/birth defect.

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious.

8.2.3 Adverse Event Reporting Period

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. Abnormal clinically significant medical conditions found during screening will be recorded in medical history.

8.2.4 Preexisting Condition

A pre-existing condition is one that is present at the start of the study. A pre-existing condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

8.2.5 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

8.2.6 Post-Study Adverse Event

All unresolved AEs should be followed by the Investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the Investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The Investigator should notify the Sponsor of any death or AE occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The Sponsor should also be notified if the Investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

8.2.7 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- Clinically significant laboratory abnormality as opined by the investigator.
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

8.2.8 Hospitalization, Prolonged Hospitalization or Surgery

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a SAE. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a pre-existing condition. Surgery should not be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical Investigator.
- Hospitalization or prolonged hospitalization required for social reasons

8.3 Adverse Events Classifications

8.3.1 Relationship of Events to Stempeucel®

The relationship of the AE to the Stempeucel® will be described as one of the following:

- **Unrelated**: There was no relationship of the adverse experience to the use of the drug or biologic. This may include, but is not limited to, the adverse experience being an expected symptom of disease under study, expected outcome of a previously existing or concurrent disease, concomitant medication or procedure the patients experienced during their treatment period.
- **Remote/Unlikely**: Adverse experiences which are judged probably not related to the drug or biologic.
- **Possible**: There was no clear relationship of the adverse experience to the use of the drug or biologic; however, one cannot definitively conclude that there was no relationship.
- **Probable**: While a clear relationship to the drug or biologic cannot be established, the experience is associated with an expected adverse experience or there is no other medical condition or intervention which could explain the occurrence of such and experience.
- **Definite**: The relationship of the use of the drug or biologic to the experience is considered definitively established.

8.3.2 Severity of the Events

- **Mild:** Symptom(s) barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief or symptom(s) but may be given because of personality of subject.
- **Moderate:** Symptom(s) of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptom(s) may be needed.
- **Severe:** Symptom(s) cause severe discomfort; symptoms cause incapacitation or significant impact on subject's daily life; severity may cause cessation of treatment with study treatment; treatment for symptom(s) may be given and/or subject hospitalization.

The AEs will be classified as described above by the PI and appropriately summarized.

8.4 Expected Adverse Events

Hypersensitivity and mild pyrexia along with headache, myalgia, nausea/vomiting, chills and rigors following the administration of Stempeucel® may be expected. The investigational medicinal product contains DMSO which can cause allergic reactions, such as skin rash, chest tightness or breathing problems. It may also cause bladder discomfort, garlic breath odour, garlic odour on the skin for as long as 72 hours after treatment. Very rarely, DMSO has resulted in eye pain, eye burning, temporary changes in vision, haemolysis or encephalopathy. MSCs may cause immunomodulation which can lead to infections.

It may be noted that there can be few expected AEs, due to progression of underlying disease and / or due to any treatment interventions as per the hospital standard of care or its complications. Any AE as a result of underlying disease condition will be categorised as expected.

Listed below are the AEs observed in the stem cell therapy arm of the phase I/II study (SRPL/CLI/07-08/001) conducted by Stempeutics using the adult bone marrow derived allogeneic MSCs. The AEs observed in the Phase I/II trials were opined by the PI and DSMB to be not related to the IMP but due to the disease process.

- Increase in rest pain score
- Ulcer of the amputated stump
- Presence of ulcer on skin of left lower limb worsening of pre-existing ulcer
- Progression of CLI with ischemic gangrene
- Hospitalization for infected gangrene
- Death
- High ALT and high AST
- Epigastric pain / dyspepsia
- Pulmonary tuberculosis

AE related data of CLI II study (SRPL/CLI/10-11/001)

- Severe rest pain

Planned amputation for dry gangrene patients will not be considered an AE. Gangrene will be recorded as medical history and if amputation takes place during the study, it will be considered as outcome of medical history.

During the study, if there is worsening of CLI/gangrene it will be recorded as an AE and outcome as amputation if the limb needs to be amputated.

8.5 Reporting Requirements for Serious Adverse Events

8.5.1 Study Sponsor Notification by Investigator

Initial Reports

In the event of SAE, the Investigator will report the initial information of the event to sponsor, regulatory authority and respective ethics committee within 24 hours of occurrence of SAE.

Report all SAEs by phone and/ or email to:

Dr. Abid Nordin, Cell Biopeutics Resources Sdn. Bhd., 27, Level G3, Block C5, Jalan Dutamas 1, Publika Shopping Gallery, 50480 Kuala Lumpur. Phone: +60 12 258 8779 Email: abid@cellbiopeutics.com

The investigator will keep a copy of the SAE form in file at the study site. The patient identity should not be disclosed by the investigator while reporting SAE, unless specifically asked by the regulatory body or ethics committee.

Follow-up Reports

The investigator will forward all follow-up information on the SAE to the sponsor whenever available.

Post analysis of the event, the follow-up information has to be reported by the investigator within 14 calendar days of the occurrence of the event to the regulatory authority, head of institution and chairman of ethics committee.

Sponsor will submit the follow-up information on behalf of sponsor after due analysis to regulatory authority, head of institution and chairman of ethics committee within 14 calendar days of the occurrence of the event.

The SAE reports must be submitted with proper binding, indexing and page number. Without indexing of page number, no SAE report will be accepted by NPRA.

a) The reports of SAEs of deaths should be prepared and submitted in red cover

b) The reports of SAE of injury other than deaths should be prepared and submitted in blue cover.

c) The SAE report other than that mentioned at (a) & (b) above is to be prepared and submitted in white cover.

For SAE (fatal/non-fatal)

TYPE OF SAE	REPORTING TO	TIMELINES
Initial Information by investigator	Competent authority Sponsor Ethics committee	Within 24 hours of the occurrence of the event
After due analysis by investigator and Sponsor	Competent authority Chairman of ethics committee Head of institution	Within 14 calendar days of the occurrence of the event

The timelines and standards defined are as per applicable regulatory requirements. The above sections with respect to SAE reporting will be considered automatically updated whenever there is change in regulatory requirements to ensure compliance.

At the time of the initial report, the following information should be provided:

• Patient Details • Patient number • A description of the suspected Adverse Drug Reaction – Date of onset – Current status – De-challenge and re-challenge information – Setting – Outcome • Investigator assessment of the association between the event and IMP	• Suspected IMP details: – Generic name – Indications – Dosage of the IMP administered to the patient – Route of administration – Start and stop date and time. • Other treatments • Details of the Investigator

8.5.2 UKMREC Notification by Investigator

Reports of all SAEs (including follow-up information) must be submitted to the Universiti Kebangsaan Malaysia Research Ethics Committee (UKMREC) within 14 working days. Copies of each report and documentation of UKMREC notification and receipt will be kept in the PI's binder.

8.5.3 Notification by Sponsor

Any SAE (as defined in GCP Guidelines) occurring during a clinical trial will be communicated promptly (within 14 calendar days) by the CBR to NPRA, UKMREC, DSMB and to the other Investigator(s) participating in the study. Expectedness of the SAE will be determined by the investigators.

9. Ethical Consideration

This study is to be conducted according to the Malaysian Control of Drugs and Cosmetics Regulations 1984, applicable government regulations; NPRA CGTP revised 2016 guidelines, ICH GCP guidelines and Institutional research policies and procedures.

9.1.1 UKMREC Review and Communications

This protocol and any amendments will be submitted to UKMREC in agreement with local regulations and National Stem Cell Ethics and Research Sub-Committee (NCERT), for formal approval of the study to be conducted. The opinion of the UKMREC/NCERT concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of this study.

9.1.2 Informed Consent Process

Written informed consent will be obtained from the patients before screening for participation in the clinical trial. During this process, the purpose of the study, the procedures to be carried out and the possible potential hazards will be described to the patients, in a language which the patient comprehends. The patient will be required to read and sign the informed consent form (ICF) prior to any study related activity. A copy of the ICF will be given to the patient prior to admission which describes the study procedures and the possible potential hazards in non-technical terms in conformity with regulatory requirements. By signing the consent form, the patient attests that the information in the consent form and any other written information was accurately explained to and understood by him/her and that the informed consent was freely given. The ICF will be signed and dated by the patient and the Principal Investigator / Co-Investigators and then the patient will be admitted into the clinical study facility. The patients will have to undergo the laboratory investigations as mentioned in Table 1.

9.1.3 Statement of Patient Confidentiality Including Ownership of Data

The records of the patient's medical history, physical examination, laboratory results and any other information or data generated during the study will be made available to the Sponsor or its designees (auditors, monitors), ethics committee, and will be made available to drug regulatory bodies in Malaysia and possibly other countries, formulary committees of hospitals, at the opinion of the Sponsor. A pre-condition for entry into this study will be patient's agreement to release all of the above-mentioned documentation and data for any

lawful purpose. In such cases, the patients name will be removed from all documentation to ensure anonymity.

10. Study Monitoring and Supervision

10.1 Study Monitoring Plan

Before the first patient is recruited into the study, the Clinical Trial Manager or monitor(s) will visit the selected clinical study facilities to:

- Discuss with the Investigator(s) (and other personnel involved with the study) their responsibilities with regard to protocol adherence and other responsibilities

During the progress of the study, the Clinical Trial Manager or monitor(s) will have regular contacts with the clinical study facility, including visits at regular intervals to:

- Provide information and support to Investigator(s)
- Confirm the continued adequacy of the clinical study facility
- Confirm that the investigational team is adhering to the protocol, Good Clinical Practice (GCP), and applicable regulatory requirements
- Perform source data verification (a comparison of the data in the CRF's with the patient's medical records, and other records relevant to the study).
- Check that the data are being accurately and completely recorded in the CRF's, and that accountability checks for Stempeucel® are being performed
- Verify the protection of the rights and well-being of the patients

During these visits, CRFs and supporting documentation related to the study will be reviewed and any discrepancies or omissions or any other issues will be resolved.

The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Case Record Form

Remote data capture (RDC) e-CRF will be used as primary data collection instrument for the study. All data requested on the e-CRF must be entered. All missing data must be explained. If the procedure was not done or the question was not asked, "ND" has to be entered. If the item is not applicable to the individual case, "NA" has to be entered. Any changes done to the data in the e-CRF after save complete, has to be justified in the audit trail. The e-CRF data will be verified with the source data by the CRA and it would be confirmed by source data verification via electronic signature. After e-CRF entry completion, the investigator needs to confirm the data through approval via electronic signature. RDC provides a data entry interface, allowing the site personnel with on-the-spot data collection along with online edit checks, instantaneously cleaning the data in the data entry environment, invoking spontaneous discrepancy management.

10.3 Data Safety Monitoring Board (DSMB)

An independent DSMB will be formed to review the trial data. All SAEs will be notified to the DSMB.

Scheduled DSMB meetings

The first face-to-face meeting will be held prior to the start of the study. The DSMB will regularly meet by conference calls as agreed within the DSMB. It is expected that during the course of the study these calls will be held periodically.

The second meeting will be scheduled to review one-week follow-up data collected from the first 3 patients at visit 3 after administration of the Stempeucel®.

The third meeting will be scheduled to review 6 months follow-up data of 3 patients.

Ad-hoc meeting may be scheduled to discuss any safety issue brought to the notice of the DSMB. However, recruitment of the subjects into this single arm, open label study will not be stopped.

11. Data Management

Data management will be handled by Data Manager. Data Manager shall ensure that clinical study data collected throughout the trial are complete, accurate and of the highest quality and shall be performed in accordance with applicable ICH guidelines and regulations.

Data Management Plan (DMP) shall include all general and study-specific data management processes and will identify the applicable processes, the people responsible for performing it, all relevant SOPs to be used and what is expected as output/ documentation.

CRFs shall be made available to the data management site in the electronic format (e-CRF). Clinical data discrepancies will be identified and data queries shall be resolved depending on the type of query as described in the DMP. Types of data to be coded will include, but may not be limited to, AEs and medications. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) adverse experience coding dictionary. Medications will be coded using the World Health Organization Drug Dictionary (WHO – Drug).

Periodic reviews of the safety database and the clinical database will occur to ensure consistency between the databases. The protocol and/or CRFs may be amended after the database and DMP have been approved. If changes to the database structure, database contents, or DMP are needed, appropriate documentation, re-validation and approval will occur as necessary.

All data management documentation collected and stored by the Data Manager during the course of study will be sent to Sponsor at study closeout as determined by the study team. This documentation will include, but is not limited to, patient folders, database specifications, the DMP with any amendments, etc.

12. Data Analysis

Data analysis will be handled by Biostatistician. Biostatistician shall ensure that the final analysis of the clinical study data is complete, accurate and of the highest quality and shall be performed in accordance with applicable ICH guidelines and regulations.

Statistical Analysis Plan (DMP) shall include all general and study-specific data analysis processes and will identify the applicable processes, the people responsible for performing it, all relevant SOPs to be used and what is expected as output/ documentation. The following

information provides an overview of the scope related to the analysis of data collected during the study.

12.1 Statistical Analysis

The SPSS® package (IBM Inc., USA, version 22) will be used for statistical evaluation. All patients in the study with relevant efficacy and safety data will be considered for the analysis.

12.2 Analysis of Baseline Characteristics

The following demographic and baseline characteristics will be summarized as follows: age, height, weight, medical and medication histories, general physical examination, 12-Lead ECG with long lead II, chest X-ray (PA view), haematology, serum chemistry, serology, and urine analysis.

Continuous data such as age will be presented in the form of descriptive statistics, as number of patients, median, mean, SD, SE, min-max, 95% CI. Categorical data such as gender will be presented using contingency tables with absolute and relative frequencies.

12.3 Analysis of Efficacy Measurements

For the efficacy endpoints, the relief of rest pain and the percentage of healing in ulcerations in the target limb will be computed at the end of 6 months and 1 year. Change from baseline to follow-up visit (6 months and 1 year) will be analysed using an appropriate normal/non-normal test based on the normality of the data for the Malaysian population. If the two-sided p-value is less than 5%, then the change from baseline to follow-up visit (6 months and 1 year) will be considered statistically significant.

All other secondary endpoints will be analysed by appropriate statistical method.

12.4 Analysis of Safety Measurements

Safety evaluation will include assessment of physical examination, periodic monitoring of vital signs (heart rate, respiratory rate, blood pressure and temperature), clinical laboratory investigations (including haematology, serum chemistry, LFT, and urine-analysis), 12-lead ECG with long lead II recording and chest X-ray (PA view). The safety parameters will be summarized. Clinically significant changes in vital signs, laboratory parameters, physical

examination findings, and ECG abnormalities will be reported as appropriate and summarized.

12.5 Analysis of Tolerability Measurements

AEs monitored using information voluntarily disclosed by the patients and as observed by the PI will be summarized descriptively by total number of AE(s). AE's will be categorized as: All AEs, all TEAEs, all severe AE's, treatment-related AEs and severe treatment-related AEs. These events will be reported as appropriate and summarized.

13. Special Considerations

13.1 Medical Emergency

In a medical emergency requiring immediate attention, study staff will apply appropriate medical intervention, according to current standards of care. Investigators / site coordinators will be available at the site during the injection of the Stempeucel®. Regulatory authorities and UKMREC will be notified of the event(s) when applicable.

13.2 Investigators Responsibilities

The Investigators responsibilities involves the following-

- Monitor and record all AE(s), which includes SAE(s), regardless of the severity or relationship to Stempeucel®.
- Determine the seriousness, relationship, and severity of each event.
- Determine the onset and resolution dates of each event.
- Complete an SAE form for each SAE and fax it to the Sponsor within 24 hours of the study site staff becoming aware of the event and to the IEC within appropriate timelines.
- Pursue SAE follow-up information actively and persistently. Follow-up information must be reported to the Sponsor within 24 hours of the study site staff becoming aware of the information and to the IEC within 14 calendar days.
- Ensure all AE and SAE reports are supported by documentation in the patient's medical records.

- Notification of the Ethics Committee must be sent to the Sponsor in a timely manner.
- During and following the patient's participation in the trial, the investigator should ensure that adequate medical care is provided to the participant for any AEs.

14. Publication Policy

Sponsor has ownership of all data and results collected during this study. In consequence, Sponsor shall reserve the right to use the data generated in this study. No part of this study shall be used by anyone else in any form without the prior written approval of Cell Biopeutics Resources Sdn. Bhd.

Following completion of the study, Sponsor may choose to publish a part or entire result obtained in this study in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as NMRR. Other biomedical journals are considering adopting similar policies. It is the responsibility of Sponsor to register this trial in an acceptable registry.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome.

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