A Randomized Double Blind Placebo Controlled Clinical Study to Assess Blood-Derived Autologous Angiogenic Cell Precursor Therapy in Patients with Critical Limb Ischemia (ACP-CLI)

CLINICAL PROTOCOL

Study No.: HS 12-01

Version: 5.5

Document	Version Date	Summary of Changes
Amendment 1	24-Nov-15	To amend the primary endpoint of the study to a combined endpoint of time to de novo gangrene, or doubling of wound size, or major amputation, or death, the following sections have been updated: Section 2, Section 20. To account for the updated statistical analysis plan, approximately 95 subjects will be randomized and treated in the study. To revise the secondary endpoints and exploratory endpoints, the following sections have been updated: Section 1, Section 7, Section 20 The following sections have been updated to conduct an interim analysis for futility: Section 1, Section, 10, Section 20. To provide clarity whilst enabling operational efficiency and maintaining the safety of subjects, the following sections have been updated: List of abbreviations, Section 1, Study Design Section 6, Section 7, Section 8, Section 14, Section 15, Section 16, Section 17, Section 18, Section 19, Section 20, Section 21, Section 22, Section 23 To correct typographical and language errors and to account for administrative updates, the following sections have been amended: Title, Protocol Signature Page, Table Of Contents, Section 2, Scientific Section 3, Section 4, Section 5, Section 6, Section 7, Section 8, Section 9, Section 10, Section 11, Section 12, Section 13, Section 14, Section 15, Section 16, Section 17, Section 18, Section 19, Section 20, Section 21, Section 22, Section 23
Amendment 2	20-Nov-17	Updated Protocol number and date Section 7.0 was amended to reflect the change in location of the cell manufacturing facility from Israel to the US. Sponsor approval signatures updated Sponsor contact information added
Amendment 3	8-May-18	Various typographical and language errors in the List of Abbreviations, section 7.2.1, 7.4 Section 7.1 and 7.4.1 Added Rutherford Assessment 7.2.1 typographical error licocaine changed to lidocaine Section 14 Clinical Studies Procedure Table – added Rutherford assessment at screening and visits 6-10. Window between screening visit 1 and visit 3 IMP injections was increased to 30 days total.

		7.4.5 Visit 10: Removed the requirement of 2 consecutive ABI assessments 1 week apart.
Amendment 4	10-Dec-18	Updated Protocol number and date Updated Sponsor contact and signatory information Removal of Section 2.3.1 that is duplicated in Protocol Synopsis and Section 8.1.1 and 8.1.2 Section 3 Introduction - added technical term *Thromboangiitis obliterans* to differentiate from Berger's disease IgA nephropathy. Section 8.1.1 clarifies revascularization history in C and adds an upper age limit in E Section 8.1.2 adds the wording “of the affected limb” for clarification and includes the weight less than 69.9kg as exclusion E (reordering subsequent exclusions F through Z Section 7.1, 7.2 and 7.4 – added Rutherford Assessment to visit descriptions, making the descriptions consistent with the table in Section 14. Section 7.1.2 – Added a second check of selected screening blood tests to confirm the patient continues to meet study eligibility criteria. Section 7.2 – Added recheck of wound size and photography to ensure the patient continues to meet study eligibility criteria. Section 7.2.1 includes clarification of the unblinded physician role and pain management Section 12.4 - The shipping temperature for the blood bag was revised to 2-8^{0}C to meet current regulations/standards for the shipping of whole blood. Section 14 Clinical Studies Procedure Table – added the following: • Rutherford assessment at visit 3. • Ulcer size measurement and photography (with scale bar/ruler in place) at visit 3. • changed lower bound of Consecutive visit day for screening Visit 1 to day -7, ensuring consistency with section 7.1.1 on page 25. • blood work to Visit 2 to confirm eligibility • note to table that all imaging be completed within the last six (6) months.

Amendment 5	27-Dec-18	Updated Protocol Number and date Section 7.1 Clarification and addition of blood tests Section 14 Deletion of historic inclusion procedures and blood testing duplication
Amendment 6	18-Oct-19	Updated Protocol Version Number and date Updated Sponsor representatives, Sponsor contacts and TOC Minor formatting changes Section 7: Clarified study periods, visit numbers and visit days Section 7 Visit 1: Added bilateral leg assessment to Visit 1 Section 7 Visit 4: Clarified vital signs and ECG performed at end of 2 hour observation. Section 7 All Visits: Clarified ankle and toe pressure measurements are bilateral, leg assessments are bilateral, and assessment of leg ulcers, ulcer measurement and ulcer photography are bilateral. Section 7 Visits 6-10: Added C-reactive protein to follow-up blood tests Section 8 Exclusion Criteria E: Changed to body weight less than 45 kg. Section 14: Clinical Study Procedure Table: Clarified ankle and toe pressure measurements bilateral, leg assessment bilateral, assessment of leg ulcers, ulcer measurement and ulcer photography bilateral. Section 16.2 Serious Adverse Event: Clarified requires in-patient hospitalization ≥ 24 hours. Section 20.2 Futility Analysis: Changed to Interim Analysis and specified interim analyses Section 20.4 Sample Size: Added Sample Size Re-estimation Section 20.7.1 Primary Endpoint: Removed subjects lost to follow-up considered as treatment failures Section 20.7.3 Exploratory Outcome Measures: Removed reduction in hospitalization time Section 20.8 Clinical Laboratory Tests, and Vital Signs and ECG: Clarified that each will be summarized using descriptive statistics and removed that criteria will be specified in more detail in SAP Section 23 References: Added statistical references

Hemostemix Representatives:

Reviewed and Approved by:

Function	Name	Signature	Date
Chief Medical Officer	Alan Jacobs, MD, PhD	Alan Jacobs	18 OCT 2019

SPONSOR CONTACTS:

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Protocol Signature Page

Protocol Title: A Randomized Double Blind Placebo Controlled Clinical Study to Assess Blood-Derived Autologous Angiogenic Cell Precursor Therapy in Patients with Critical Limb Ischemia (ACP-CLI)

Study No.: HS 12-01

Version: 5.5

Study Phase: 2

Sponsor: Hemostemix Inc.

Principal/ Qualified Investigator

By signing below, I, the Investigator, approve the protocol and agree to conduct the clinical trial according to all stipulations of the protocol as specified in both the clinical and administrative sections, Case Report Forms and any protocol-related documents (subject to any amendments agreed to in writing between the Sponsor and Principal/ Qualified Investigator). I agree to comply with the ICH-GCP, World Medical Association Declaration of Helsinki (and relevant updates) and applicable local regulations. I agree to ensure that confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Hemostemix Inc. I understand that the study may be terminated or enrollment suspended at any time by the Sponsor, or by me, at my center, if it becomes necessary in my opinion, to protect the best interests of the study subjects/participants.

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Name

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Signature

_____

Date

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1. LIST OF ABBREVIATIONS

ABI	Ankle Brachial Index
Ac-LDL	Acetylated Low Density Lipoprotein
ACP	Angiogenic Cell Precursor
ACP-01	Name of Product that contains ACPs
AE	Adverse Event
ANCOVA	Analysis of Covariance
CD	Cluster of Differentiation
CLI	Critical Limb Ischemia
DSMB	Data Safety Monitoring Board
GFR	Glomerular Filtration Rate
HbA1c	Hemoglobin A1c
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HTLV	Human T-Cell Lymphotropic Virus
IMP	Investigational Medicinal Product (includes Placebo and ACP-01)
INR	International Normalized Ratio
ITT	Intent-to-Treat
LOV	Last Observed Value
LSM	Least Squares Means
mITT	Modified Intent-to-Treat
PAD	Peripheral Arterial Disease
PP	Per-Protocol
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
PVR	Pulse Volume Recording
QoL	Quality of Life
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCP	Synergetic Cell Population
ST Analysis Set	Safety Analysis Set
TASC	Trans-Atlantic Inter-Society Consensus
TBI	Toe Brachial Index
VAS	Visual Analogue Scale

2. PROTOCOL SYNOPSIS

2.1. ACPs' role in the treatment of peripheral arterial disease

Peripheral arterial disease (PAD), which is caused by atherosclerotic occlusion of the arteries to the legs, is a manifestation of systemic atherosclerosis. Treatment of PAD is an unmet medical need presenting a highly prevalent worldwide condition (10-14 million people in the US) that affects men and women equally. It is estimated that worldwide, 1% of adults over 50 years of age suffer from critical limb ischemia (CLI) (approximately 1.5–2 million CLI patients in Europe and the US).

Hemostemix Inc. (Hemostemix) has developed a proprietary technology to generate lineage specific precursor cells from blood-derived adult mononuclear cells. A peripheral blood-derived progenitor cell population, named synergetic cell population (SCP), can be harvested and then induced to differentiate *in vitro*, under specific culture conditions, into angiogenic (angiogenic cell precursors – ACPs, see below), myocardial or neural lineages.

ACPs are characterized by similar features as endothelial progenitor cells. They secrete tissue survival and regeneration-supporting growth factors and cytokines, which also facilitate the mobilization of additional progenitor cells and other important elements involved in the healing process of ischemic tissues. ACPs facilitate vasculogenesis and angiogenesis. The potent combination of all these beneficial capabilities makes ACPs a potentially effective treatment for severe vascular disorders.

Positive evidence of the use of the ACP product arises from three clinical studies in which a total of forty subjects were treated. Two of the clinical studies demonstrated primarily the safety of the cellular therapy in critical limb ischemia (CLI), while the third showed a positive effect of the treatment in angina pectoris. Blood samples will be collected from subjects. A certain fraction of peripheral blood mononuclear cells will be isolated and then grown under conditions that induce differentiation of those cells into ACPs. Following this differentiation stage, the ACPs will be harvested, vialed, transported to the hospital and injected into the ischemic limb of the subjects.

2.2. Study design

This is a prospective, randomized, double-blind, placebo-controlled study to assess the efficacy and safety of autologous ACPs administered intramuscularly into the gastrocnemius and dorsal foot muscles of one affected limb in subjects with CLI with no surgical or endovascular revascularization options.

A total of approximately 95 subjects will be randomized to treatment with ACP-01 or placebo using a 2:1 randomization scheme, respectively, stratified by site. Study will be continued until all subjects treated with the IMP have been followed for 52 weeks. One futility analysis for potentially stopping enrolment

into the study will be performed when approximately 42 subjects have completed at least 26 weeks of follow-up.

2.3. Study population

Subjects diagnosed with critical limb ischemia (CLI) and hemodynamic indicators of severe peripheral arterial occlusive disease who are not a candidate for revascularization treatment.

3. INTRODUCTION

Peripheral arterial disease (PAD), which is caused by atherosclerotic occlusion of the arteries to the legs, is a manifestation of systemic atherosclerosis. Other manifestations of atherosclerosis are coronary and cerebral artery disease. Treatment of PAD is an unmet medical need, representing a highly prevalent worldwide condition (10-14 million people in the US) that affects men and women equally (Criqui et al., 1985, Hiatt et al., 1995). The major risk factors for peripheral arterial disease are old age, smoking, and diabetes mellitus. Hyperlipidemia and hypertension are also important risk factors (Hiatt et al.,1995, Newman et al.,1993). Other etiologies of PAD besides atherosclerosis are *Thromboangiitis obliterans* (Buerger's disease) and arteritis.

Approximately one third of patients with peripheral arterial disease typically suffer from pain in one or both legs during walking (primarily in the calves), which does not vanish with continued walking and is relieved by rest (Rose et al., 1968). This symptom is known as claudication. In a certain group of the patients with claudication, the severity of the condition increases gradually until it develops into critical limb ischemia (CLI); the end stage of the disease.

Approximately 15% to 30% of patients with lower extremity arterial disease will progress from intermittent claudication to CLI over the course of their disease (Dormandy et al., 1989, McDaniel et al., 1989). It is estimated that, worldwide, 1% of adults over 50 years of age suffer from CLI (McDaniel et al., 1989) (a total of 1.5–2 million CLI patients in Europe and the US) (McDaniel et al.,1989, Weitz et al.,1996)

According to the Trans-Atlantic Inter-Society Consensus (TASC) document on management of Peripheral Arterial Disease II (2007), the clinical definition of CLI should be used for all patients with chronic ischemic rest pain, ulcers or gangrene attributable to objectively proven arterial occlusive disease. The term CLI implies chronicity and is to be distinguished from acute limb ischemia (Norgren et al., 2007).

Patients with CLI may need prompt percutaneous transluminal or open surgical revascularization in order to avoid limb loss. It is estimated that 160,000 amputations are carried out in the US annually due to failure of revascularization (Wolfe et al., 1986, Fisher et al., 1999).

Hemostemix intends to use autologous Angiogenic Cell Precursors (ACPs) to treat patients suffering from CLI and who do not have surgical or endovascular revascularization options. The use of Angiogenic Cell Precursors aims at promoting natural revascularization in the limb and as a consequence, attenuating ischemia.

Blood samples will be collected from subjects. A certain fraction of peripheral blood mononuclear cells will be isolated and then grown under conditions that induce differentiation of those cells into ACPs. Following this augmentation stage the ACPs will be harvested, vialed, transported to the hospital and injected into the ischemic limb of the subjects.

4. SCIENTIFIC BACKGROUND

Hemostemix has developed a proprietary technology to generate lineage specific precursor cells from blood-derived adult mononuclear cells. This was previously considered impractical for therapeutic use because of the small number of adult progenitor cells in the peripheral circulation. Hemostemix has shown that a blood-derived progenitor cell population, named synergetic cell population (SCP), can be harvested from peripheral blood and then induced to differentiate *in vitro*, under specific culture conditions, into angiogenic (angiogenic cell precursors – ACPs, see below), myocardial or neural lineages (Porat et al., 2006).

ACPs are characterized by similar features as endothelial progenitor cells (EPCs). They secrete tissue survival and regeneration-supporting growth factors and cytokines, which also facilitate the mobilization of additional progenitor cells and other important elements involved in the healing process of ischemic tissues (Porat at al., 2007, Badorff et al., 2003, Urbich et al., 2004). ACPs facilitate the formation of new blood vessels *de novo* by vasculogenesis, or from pre-existing blood vessels by angiogenesis. The potent combination of all these beneficial capabilities makes ACPs a potentially effective treatment for severe vascular disorders. The first evidence indicating the presence of EPCs in the adult circulation was obtained when mononuclear blood cells from healthy human volunteers were shown to acquire an endothelial cell–like phenotype in vitro and to incorporate into capillaries in vivo (Asahara et al., 1997).

The fact that EPCs can take part in the formation of new blood vessels was first observed by Bhattacharya and colleagues who showed the formation of capillary-like structures from hematopoietic stem early progenitor cells or ex-vivo expanded EPCs (Bhattacharya et al., 2000, Kaushal et al., 2001). The contribution of bone marrow-derived EPCs, to neovascularization after ischemic injury in vivo, was shown in experiments using labeled populations of progenitor cells to reconstitute lethally irradiated mice. The cells or their progeny were shown to migrate into ischemic cardiac muscle and blood vessels, differentiate to cardiomyocytes and endothelial cells, and contribute to the formation of functional tissue (Jackson et al., 2001). Other work, involving a mouse retinopathy model, demonstrated the important role that the recruitment of endothelial precursors to sites of ischemic injury plays in neovascularization (Grant et al., 2002).

ACPs are a unique population of cells that stain $CD31^{Bright}$, demonstrate uptake of Ac-LDL and secrete IL-8 and angiogenin. Similar to EPCs, ACPs simultaneously express Ulex-lectin and uptake of Ac-LDL, a molecule rapidly internalized by endothelial and angiogenic cells. Other cells included in the cellular product express the hematopoietic stem cell (HSC) markers CD34, CD133, and CD117, as well as the angiogenic cell markers VEGFR2, Tie-2, CD144, and von Willebrand factor.

The majority of EPCs reside in the bone marrow and have been shown to mobilize (i.e. migrate in increased numbers from the bone marrow into circulation) in patients with vascular trauma or acute myocardial infarction (AMI) (Gill et al., 2001, Shintani et al., 2001) or in response to administration of VEGF via gene transfer (Kalka et al., 2000, Iwagaro et al., 2002).

Considerable work has been carried out over the last few years to elucidate the mechanisms behind EPC mobilization, localization and function. Progress has also been achieved by establishing therapeutic protocols for progenitor cells to treat a variety of conditions, such as peripheral limb ischemia, acute myocardial ischemia and infarction and congestive heart failure.

The source of autologous EPCs varies and includes bone marrow, peripheral blood and various mesenchymal organs. In this proposed trial, APCs will be harvested from the peripheral blood without pre-administration of G-CSF or AMD3100, or any other drug used to recruit progenitor cells from the bone marrow (Jamiolkowski et al., 2015).

Significant research has been carried out in humans during the last few years to examine the potential benefits of using progenitor cells to treat cardiovascular disease. Many studies that enrolled hundreds of subjects have been carried out with various types of progenitor cells to treat peripheral arterial disease or its late deleterious effects (Shimamura et al., 2013). In these trials the cell source was either autologous, harvested from bone marrow or peripheral blood, or allogeneic, from umbilical cord blood. In most cases, the cells were injected into the muscle of the affected limb. As early as 2002, researchers, especially in Japan, initiated clinical trials to assess the effect of autologous progenitor cells as a treatment for limb ischemia. Miyamoto et. al., for example, investigated the safety and efficacy of implantation of autologous bone marrow mononuclear cells in refractory chronic peripheral arterial disease of limbs (Miyamoto et. al. 2004). The treatment was effective in relieving severe pain, including rest pain in the legs in 11 of the 12 patients treated. Resting ABI in legs implanted with bone marrow mononuclear cells also improved. Significant perfusion improvement was demonstrated by 99mTc-tetrofosmin perfusion scintigraphy. No serious adverse events were reported.

Since then, the rationale of treating CLI patients with progenitor cells was welcomed by a large number of medical groups which translated into many clinical studies treating hundreds of patients (Shimamura et al., 2013). Although some of the studies were relatively small, open label with few control groups, the results were very encouraging.

The relatively low risk and early success of the various progenitor cell-based therapies tried in those studies led several sponsors to undertake larger, placebo-controlled studies. In the last few years, several large placebo-controlled Phase 1b and Phase 2 clinical trials were initiated to assess the effect of various types of adult progenitor cells in the treatment of peripheral arterial disease. Some of the therapies used progenitor cells harvested from the patients' bone marrow by using various devices and immediately administered to the target area, while in other studies, the cells were harvested, expanded and differentiated *ex vivo* for a certain period and only then given to the patient. Several examples are listed in Table 1.

Table 1: List of randomized, placebo-controlled clinical trials assessing progenitor cell therapies for CLI

Sponsor	Cell type	Number of patients	Study design	Outcome
Baxter (completed July 2011)	Autologous CD34+ cells (harvested from peripheral blood)	28	Randomized, placebo-controlled.	Results not yet published
Aastrom Biosciences (completed July 2011)	Autologous bone marrow cells harvested directly from the hip bone and then expanded *ex vivo*.	150	Phase 2 study Randomized, placebo-controlled.	Improved limb salvage and other vascular parameters
Aldagen (completed May 2011)	Autologous bone marrow-derived Aldehyde Dehydrogenase^{Bright} ($ALDH^{br}$) cells vs. unfractionated autologous mononuclear bone marrow	20	Randomized, controlled	Treatment well tolerated and improvement of treated limb
J.W. Goethe University Hospitals	Bone Marrow Mononuclear Cells	40	Randomized, placebo-controlled	Improved healing of ulcers
University of Wisconsin, Madison	autologous CD133+ cells	24	Randomized, placebo-controlled	Study ongoing
Leiden University Medical Center	bone marrow derived mononuclear cells	108	Randomized, placebo-controlled	Study ongoing
Harvest Technologies	Autologous Bone Marrow Aspirate Concentrate (using a bed-side device)	210	Randomized, placebo-controlled.	Study ongoing
Biomet Biologics, LLC	Bone Marrow concentrate (MarrowStim PAD Kit)	152	Randomized, placebo-controlled (sham bone marrow aspiration,	Study ongoing

Sponsor	Cell type	Number of patients	Study design	Outcome
			sham delivery of placebo	

5. HEMOSTEMIX'S CLINICAL EXPERIENCE WITH ACPS

Positive evidence of the use of Hemostemix's ACP-01 arises from three clinical studies and one animal study described below. Two of the clinical studies demonstrated primarily the safety of ACP-01 as well as encouraging results suggesting the possibility of its efficacy in the treatment of CLI, while the third study showed the effect of the treatment in subjects with angina pectoris. The animal work demonstrated the engraftment of ACPs into the myocardium of rats resulting in improved cardiac function.

5.1. Pilot clinical trial for CLI

A pilot non-randomized, open-label study was carried out in six patients with the permission of the Ethics Committee, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Mutirangura et al., 2009). The patients enrolled suffered from severe symptoms of CLI (Fontaine stage III and IV), such as ischemic rest pain, ischemic non-healing ulcers and digital gangrene. In addition, limb hemodynamic assessment indicated limb threatening ischemia with ankle brachial index (ABI) below 0.4, and/or toe brachial index (TBI) below 0.24. The goals of the study were to assess the safety and feasibility of the implantation of ACPs in patients with CLI who were poor candidates for standard revascularization treatment options.

Six patients with CLI due to infra-popliteal artery occlusive disease were recruited in the study and after signing the informed consent form underwent intramuscular injections of autologous ACPs into the ischemic limbs.

There was no evidence of local or systemic complications related to the procedure. Five of the six patients showed clinically significant improvement of the circulation in the distal limb. Four of them had complete healing of ischemic ulcers and stumps of toe amputation. However, one patient with adequate granulation tissue at the stump of the left first toe amputation subsequently suffered from severe foot infection originating from the other toes and eventually underwent below the knee amputation. One patient underwent a major amputation since no improvement of circulation at the distal limb could be induced after ACP administration. The result of this preliminary trial showed that ACP therapy is potentially safe and could provide benefits in the improvement of circulation in patients with CLI.

5.2. Phase 1b clinical trial for CLI

Hemostemix (formerly TheraVitae Ltd.) provided ACPs to Salus Ltd, the clinical development arm of Kelen Hospital in Hungary to conduct a larger controlled clinical study at two medical centers in Budapest, Hungary (Szabó et al, 2013). The local Sponsor obtained all the necessary approvals

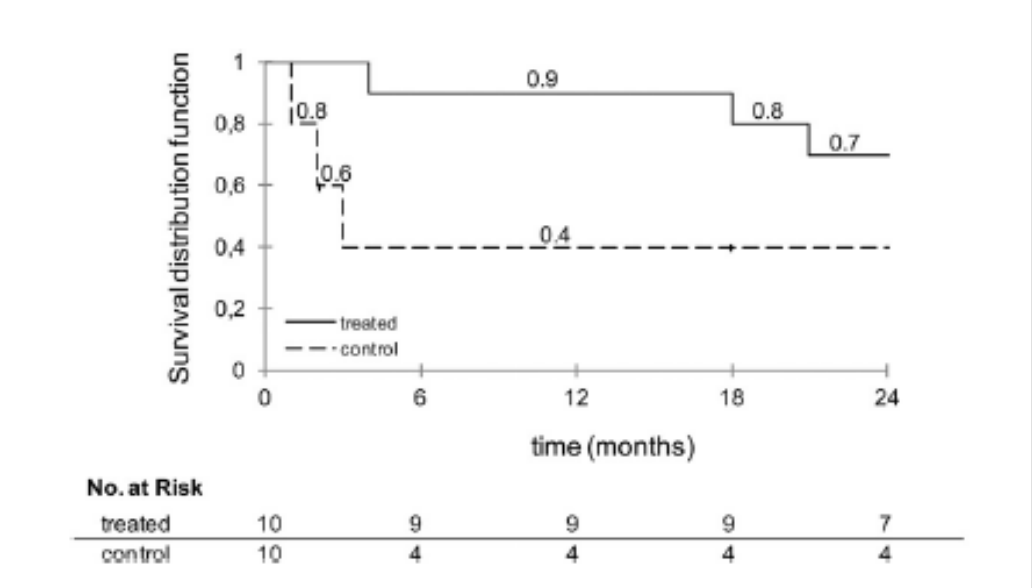
required by the authorities in order to perform the studies. Ominis Research was retained as the Contract Research Organizations (CRO) by the Hungarian Sponsor to monitor and ensure the quality of the study in accordance with the requirements of the local authorities.

The safety and feasibility of intramuscular administration of ACPs was assessed in 20 patients with severe CLI (Fontaine phase III-IV) with high risk of amputation of an affected limb. Half of the patients were treated with ACPs while the other half, the control patients, received standard of care medical therapy. The patients were followed for 3 months post treatment.

The primary endpoints of the study were to assess the safety of ACP administration as well as its effect in the prevention of limb loss. Secondary endpoints were ulcer healing, improvement of circulation in the affected limb as assessed by the ankle-brachial index and transcutaneous CO2 pressure (TCO2).

No adverse events related to the IMP were observed. During the 3 months follow-up in the control group, one death occurred due to septicemia and six major amputations were performed. In the treated group, there were no deaths or major amputations, one patient had an hallux amputation and another patient had a trans-metatarsal amputation (Figure 1). The difference of limb loss was statistically significant (p=0.01) between the two groups. In the treatment group, one amputation occurred at 4 months. At 2-year follow-up in the control group, two deaths and six major amputations occurred; in the treated group, there were three major amputations.

Figure 1: Amputation-free interval in treated and control Subjects



At 3-month follow-up, the change in hemodynamic parameters showed improvement in the ACP-treated group. Other efficacy data parameters are not reviewed due to the small number of patients left in the control group without major amputation or death. Improvements in pain score, wound healing and walk test were reported at 2 years

5.3. Clinical trial for angina pectoris

An open label clinical trial was conducted to assess ACP administration for severe angina pectoris at Siriraj Hospital in Bangkok, Thailand, the largest and most renowned academic hospital in Thailand (Tresukosol et al., 2006). The study was approved by the hospital's ethics committee, which is the regulatory body in charge with supervising clinical trials in Thailand.

Similar to the CLI pilot study, maximal efforts were made to ensure proper conduct and supervision of the clinical trials in compliance with Good Clinical Practices. As required by international standards of clinical trials, Hemostemix (formerly TheraVitae Ltd.) established a dedicated team of professionals to supervise and monitor both clinical trials in Thailand (CLI and angina pectoris). The team was led by a practicing Israeli-licensed physician with extensive clinical experience.

The objectives of the trial were to determine the safety and feasibility of intracoronary injection of blood-derived autologous ACPs in relieving symptoms of angina pectoris in patients on standard of care with no option for revascularization procedures (angioplasty or CABG).

Twenty-four patients with chronic stable angina pectoris on standard of care drug therapy were enrolled and signed the informed consent. ACPs ($32.3x10^6 \pm 4.2x10^6$) were injected via a catheter with proximal

balloon occlusion of the coronary artery supplying the ischemic myocardium. The patients were assessed prior to and 3 and 6 months after the treatment by using the Canadian Cardiovascular Scale (CCS), the six-minute walking test (6MW), their exercise capacity (METs), and perfusion defect measurements.

5.3.1. Safety data

All adverse events were recorded, monitored and reported throughout the study (Table 2). Safety results were analyzed and the PI determined if there was a relation to cell administration.

One patient died from a myocardial infarction that occurred one month after the administration of the cells, which according to coronary catheterization occurred in a region of the heart supplied by another stented coronary artery and not the one in which the cells were injected. The study PI concluded that the death was not related to the administration of the cells or to the cells themselves. There were no other deaths among the study population.

Three patients developed transient ventricular fibrillation, which is an expected occurrence as a result of catheterization. One of these events occurred during the catheterization procedure, which was treated successfully and the other two during angiography six months after the procedure. All three patients recovered. The PI determined that these events were unrelated to the cell administration, but rather to the catheterization procedures. Seven patients developed minor transient adverse events, which included: elevated erythrocyte sedimentation rate (ESR, 2 patients), transient chest pain during catheterization, hypoglycemia, leg pain, upper respiratory tract infection (URTI), oral paresthesia, urticaria (2 patients), and a hematoma at the groin puncture site. These findings subsided after a short period without affecting the patient's clinical condition.

Table 2: Summary of safety results of the clinical trial that tested the safety and efficacy of the administration of blood-derived autologous APCs to alleviate anginal symptoms and myocardial ischemia in patients with severe anginal syndrome.

Serious Adverse Events			**Adverse Events**
Number of patients: 24	**Deaths**	**Other Serious Adverse Events**	
Number of Adverse Events	1	3 in 3 patients	10 in 7 patients
	Myocardial infarction due to obstruction of a non-injected stented artery, 1month following procedure	Transient ventricular fibrillation during 6 month follow up catheterization (2) Transient ventricular fibrillation during procedure	Elevated Erythrocyte Sedimentation Rate (2) Chest pain during procedure Hypoglycemia Upper respiratory tract infection Oral paresthesia Urticaria (2) Hematoma at puncture site Leg Pain
Numbers in parenthesis ( ) indicate more than one event.			

5.3.2. Efficacy data

Symptoms and perfusion defects were significantly improved relative to baseline. CCS decreased from 2.17±0.18 at baseline to 1.04±0.04 at 3 and 1.13±0.10 at 6 months. Six minute walking ability increased from 346.7±27.3 meters to 409.7±25.8 meters and 427±25.6 meters at 3 and 6 months, respectively. METs values, analyzed in 20 patients, increased from 5.5±0.49 to 6.5±0.6 and 6.8±0.59 at 3 and 6 months. Perfusion defects in the territory of the target vessel decreased from 39.37%±5.65% to 24.38%±5.41% and 24.91±5.57% at 3 and 6 months. The results of this study support the assumption that intracoronary injection of autologous ACPs derived from non-mobilized peripheral blood into patients with chronic stable angina is safe, improves anginal symptoms, and alleviates myocardial ischemia at 3 and 6 months.

5.4. Conclusion

Although very encouraging efficacy was observed in comparison to best available care, the main reason to perform a Phase 2 trial is to continue to evaluate the safety of the therapy in parallel with its effectiveness in a larger cohort of patients. The data presented show that the therapy demonstrated a favourable safety profile in patients suffering from severe heart disease.

An extensive search of the literature was performed to find potential adverse effects of non-mobilised (by G-CSF) autologous blood-derived progenitor cells on patients. The search was performed on the database of the US National Library of Medicine and included articles published in the period 1998-2011. No articles were found that reported potential risks of such an approach. The use of autologous blood-derived progenitor cells (mainly bone-marrow derived) dates back decades; their main therapeutic use was for hematological malignancies. The potential positive effect that some of those cells may demonstrate in cardiovascular disorders was discovered in the last twenty years. However, regardless of the indication, the use of these cells does not raise safety concerns.

It is emphasized that in order to offer the patients the greatest chances of recovery, the best existing medical care will be provided to all subjects regardless of the group to which they are randomized.

6. ACPS IN A RAT MODEL OF ACUTE MYOCARDIAL INFARCTION

An animal study to evaluate the efficacy of intracoronary or intramyocardial human ACP administration in nude rats by examining cardiac function was carried out in collaboration with Dr. Ren-Ke Li and his team at the University of Toronto (Sun et al., 2008). Cells were prepared by the Company's scientists and shipped to Toronto for administration into the animals. The animal experiments, including the blind assessment of the results were performed by the scientists from Dr. Li's team in Toronto.

In order to examine ACP function in an *in vivo* paradigm, myocardial infarction was induced by ligation of left anterior descending (LAD) artery in nude rats. Seven days after myocardial infarction the rats were injected either intramyocardially ($1.5x10^6$ total number of cells, n=10) or intracoronarily ($1.5x10^6$ total number of cells, n=10) with human ACPs or culture medium (n=5). Cardiac function (ejection fraction, fractional shortening, functional area contraction and scar area at left ventricular free wall) was examined 14 and 28 days following cell or culture medium administration. ACP homing and engraftment into the scar border area was examined 28 days following cell administration.

Two weeks after implantation, ejection fraction, fraction shortening and functional area contraction decreased in the control groups but increased in the intramyocardial and intracoronary ACP groups (p=0.01 for both groups). Similarly, four weeks after implantation, ejection fraction and fraction shortening were lower in the control groups than those in the intramyocardial ACP and intracoronary ACP groups (p=0.01 for both). Dp/dt max (an index of systolic function) evaluated by pressure-volume catheter at 4 weeks after cell implantation was greater (p=0.01) in the intramyocardial ACP and intracoronary ACP. Dp/dt min (an index of diastolic function) evaluated by pressure-volume catheter

was better (p=0.01) in the two cell-implanted groups. Scar size of the intramyocardial ACP group was smaller (p=0.02) than the one in the control group. The scar size was smaller in the intracoronary ACP group than the control group, however, the difference was not statistically significant (p=0.06). The grafted cells were detected four weeks after implantation by immunohistochemistry: antibodies against myosin heavy chain and troponin-I demonstrated muscle-like cells in the scar tissue of the intramyocardial and intracoronary ACP groups suggesting possible cell differentiation and thus a contribution of the implanted cells to tissue repair.

Overall, this study demonstrates the engraftment of ACPs within the infarcted myocardium resulting in significant beneficial effects, including reduced infarct size and improved cardiac function in rats.

7. STUDY DESIGN

This is a prospective, randomized, double-blind, placebo controlled study to assess the efficacy and safety of *ex vivo* autologous ACP administered intramuscularly into the gastrocnemius and dorsal foot muscles of one limb in subjects with CLI.

Subjects treated at each investigative site will provide written informed consent prior to the conduct of any study-related procedures. Thereafter, they will be screened and those meeting the inclusion/exclusion criteria will be enrolled into the trial and undergo all the study procedures including intramuscular injection of autologous ACPs or placebo. The autologous ACPs or placebo will be administered in addition to any conventional treatment that the subject is receiving.

To enable blinding of the assessors and the subjects, the control group will undergo a similar procedure as the treatment group and receive injections into the gastrocnemius and dorsal interossei foot muscles.

Blood samples and cells that are not used in the treatment of subjects with ACPs, e.g., from the placebo group, will be de-identified and may be utilized for manufacturing and quality control testing such as for the development of potency and release assays. Results from the assays and tests may be used to retrospectively examine cell and product characteristics in CLI patients and/or the response to treatment with ACPs.

The placebo will consist of a growth medium, the same medium used in the ACP product suspension. The physician performing the administration of the cells or the placebo may become unblinded to the group to which the subject is randomized while performing the injections of the IMP. Therefore, he/she will not participate in the assessment of the subjects that he/she injected. In the event that the administrator becomes aware of the treatment assigned, every effort will be made to maintain appropriate blinding. All other participants in the study (PI, clinical investigators, other physicians, the subjects, study nurses, coordinators, etc.) will not be aware of the subjects' randomization and will therefore remain blinded to the study.

The study consists of four periods: *Screening, Treatment, safety monitoring* and *Long-term follow-up* periods. Subjects will be followed for one year post treatment.

7.1. Screening Period

Screening follows informed consent and begins with the evaluation of the subject at the investigative site to assess eligibility according to the inclusion/exclusion criteria and ends just prior to the administration of the IMP. After signing the informed consent form, the subject will have been formally recruited into the study. The PI or one of the clinical investigators (CI) will decide which leg needs to be treated according to the severity of the disease. In general, the more ischemic limb should be injected.

During this period the following clinical and laboratory examinations will be performed in order to obtain baseline values and verify inclusion/exclusion parameters:

7.1.1. Visit 1: Day –30 to -7

1) Evaluation according to the inclusion/exclusion criteria to determine eligibility.
2) Obtain and record medical history including current clinical condition, chronic disabilities and current medication/therapy, obtain and record demographical data, review angiography or color flow duplex ultrasound imaging results.
3) Assessment of pain on a visual analogue scale (VAS).
4) Type and dose of analgesic drug taken by subject.
5) Physical examination including Rutherford Assessment.
6) Measurement of blood pressure, heart rate, ECG, and temperature (to reduce the likelihood of infection prior to administration of the IMP).
7) Leg assessment, including skin color, swelling, limb temperature, presence of ulcers or gangrene, bilaterally.
8) Measurement of resting ankle blood pressure using an appropriate adult cuff, bilaterally
9) Measurement of toe blood pressure using an appropriate cuff, bilaterally.
10) Assessment of leg ulcers, measurement of ulcer size and ulcer photography, bilaterally.
11) Vascular quality of life (QoL) questionnaire (Morgan et al., 2008).
12) Blood tests:
    - *Hematology*: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT (or INR), and aPTT

- *Blood Chemistry*: Blood glucose; Blood urea nitrogen (BUN); Serum creatinine; C-reactive protein; Serum chloride; Serum potassium; Serum sodium; Serum magnesium; Serum albumin; Total serum protein; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, Creatine phosphokinase (CPK), HbA1c.
- Pregnancy test (serum Beta-hCG), if female of childbearing age.
- Screening for severe contagious infections: HIV-1, HIV-2, HTLV-1, HTLV-2, HBV, HCV.

7.1.2. Visit 2: Day -7 ± 1 day

The following activities should be performed under the supervision of the PI:

1) Blood tests:
   - *Hematology*: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT (or INR), and aPTT.
   - *Blood Chemistry*: Blood glucose, Blood urea nitrogen (BUN); Serum creatinine; C-reactive protein; Serum chloride; Serum potassium; Serum sodium; Serum magnesium; Serum albumin; Total serum protein; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, Creatine phosphokinase (CPK), HbA1c.
2) Obtain up to 500 ml of the subject's blood by venipuncture (equivalent to typical blood donation volume). The site should be swabbed with betadine solution followed by 70% alcohol swab. Allow to dry by evaporation. A 16g needle is to be used to obtain the blood that will be collected in a transfusion bag containing anticoagulant. It is strongly recommended to draw the entire volume of blood into only one transfusion bag.
3) Obtain and send an additional 10 ml blood for blood bacterial culture tests (tested locally at the investigative site's laboratory).
4) Complete forms, label blood bag and package for transport to the manufacturing facility.
5) Pack the blood and ship to the manufacturing facility.

7.2. Visit 3: Treatment (Day 0)

Preparation of the subject for the treatment procedure will be initiated upon communication by Hemostemix's representative of the estimated date of the investigational product delivery.

If the batch is not released due to failure to meet release specifications, the subject will undergo a repeat Visit 2 within 21 days of the first Visit 2.

If the batch of IMP produced from the second blood also fails to meet specified release criteria, the subject will be withdrawn from the study.

The following clinical assessments will be performed just prior to the administration of the IMP.

- Physical examination, including Rutherford Assessment.
- Standard preoperative monitoring of heart rate, blood pressure, ECG, and body temperature.
- Assessment of ulcers including measurement of area in cm^2 based on photography of lesions.

7.2.1. IMP administration

The subject will be taken to a treatment room.

The unblinded treating physician, at their discretion, may administer intravenous, intramuscular, and/or oral analgesic medications alone, or in combination with conscious sedation, sufficient to alleviate pain associated with the injection of the investigational product. Additionally, at the discretion of the unblinded treating physician, EMLA (lidocaine and prilocaine) Cream may be applied to the dorsal aspect of the foot to alleviate pain as needed, provided no contraindications for its use exist and the application will not interfere with standard sterilization procedures for the preparation of the injection site. Impaired kidney function may slightly prolong the recovery from conscious sedation.

Under sterile conditions, the subject will then receive a dose of not more than 200 million ACPs in total, containing at least $5x10^6$ $CD31^{Bright}$ x AcLDL cells and at least $1x10^6$ CD34 positive cells (which constitute at least 1.5% of the total number of cells injected). The cell suspension will be injected approximately 1.5 cm deep into a minimum of 24 locations in the gastrocnemius muscle of the affected limb and at 6 locations into the dorsal interossei foot muscles (extensor digitorum brevis and extensor hallucis brevis muscles). The 6 injections into the foot should be approximately in one line at the midline of the metatarsus.

The general goal should be to deliver ACPs to the entire ischemic tissue. Since the bulk of the ischemic tissue is in the leg, it is this part of the limb that should receive the bulk of cells. It is therefore required that at least 24 injections of approximately 1ml are administered into the leg.

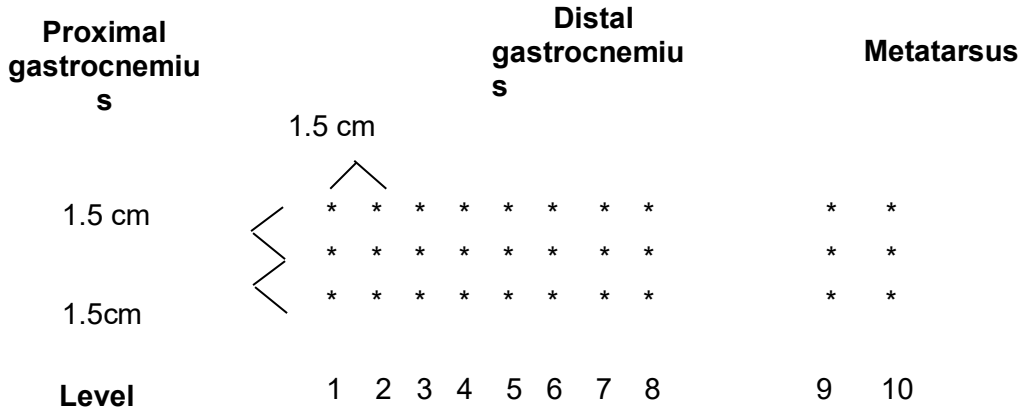
The following recommendations should also be followed:

- The unblinded investigator should try to avoid injections in the proximity of gangrene.
- The unblinded investigator must under no circumstances inject into areas that show signs of cellulitis.
- The unblinded investigator should try to cover the entire ischemic tissue and not inject specifically around chronic wounds.
- The unblinded investigator should start with the injections into the foot. The injections should be performed slowly in order to avoid spill out of liquid containing cells. If the investigator notices that some of the volume injected spills out, he/she should assume that there is not

sufficient space to inject the entire 1 ml into each location in the foot. Therefore, a smaller volume should be injected into the remaining spots in the foot and save the additional volume for the leg injections. This additional volume of cells/placebo should be injected into additional spots in the leg. Thus, in such cases, there may be 1-2 additional injections into the leg. A treatment diagram (example shown below) will be used to document the injections administered to each subject.

- The measurements in the diagram below are only suggestions and the investigator should keep in mind that the principle is to cover as much of the ischemic area as possible. Therefore, he/she may need to reduce or increase the distance between the injection sites depending on the length of the limb.

The administration of the IMP is expected to take no longer than 20-30 minutes, after which the subject will continue to be tightly monitored for a period of 2 hours. The total volume will be 30 ml and approximately 0.75 -1 ml of cell suspension will be implanted into each of 30 injection sites, using a grid. A 23–gauge needle will be used.



The unblinded investigator should ensure that there are no clinical concerns that may prevent the subject from being able to receive the injection on the intended injection date. If the subject is not fit on the planned injection day, he/she should not be injected with the IMP (ACPs/placebo). This subject should be discontinued and if his/her health condition improves he/she can be re-screened into the study. Reasons for being unfit on the injection day include, but are not limited to: acute ischemia, cellulitis or other severe infection in the intended injection leg, wet gangrene, significant deterioration of clinical status, suspected severe concurrent disease with fever > 38.4 ^{0}C, etc.

The rationale for the dose of cells and volume is based on several literature reports. Specifically, based on studies of human EPC transplantation into nude mice, Iwaguro et al., calculated that satisfactory reperfusion of the hind limb would require 0.5 to 2x10^{4} endothelial progenitor cells/gram of animal weight

(approximately 25 grams) (Iwaguro et al., 2002). Since the authors injected the cells systemically, it would be expected that a significantly lower number of cells reached the ischemic tissue. It is difficult to extrapolate the therapeutic equivalence of mouse EPCs and human ACPs. However, the general principle was used in the calculation of the putative dose. Therefore, the minimal dose of the CD31+ and Ac-LDL-uptaking subpopulation of ACPs to be used for subjects will be 5 million cells, which is approximately the average of 10,000 cells per gram of tissue.

A review of the literature was performed and the information was found to support the cell dose proposed in the present study. Elliott et al., 1997, report the volume of the gastrocnemius muscle in 7 patients. Assessments were done by MRI and the approximate volume of the gastrocnemius muscle (Lateral and medial gastrocnemius muscles taken together) was 435-475 cc. Since the density of mammalian skeletal muscle tissue is approximately 1.06 kg/liter, one can assume the approximate weight of the gastrocnemius muscle, with differences depending on the height of the patient, to be approximately 500 grams.

The release specification for ACPs used in the clinical trial for severe angina pectoris and for patients suffering from ischemic heart disease treated on a compassionate basis was 3 million ACPs. In those patients, the treatment with cells showed a good safety profile and encouraging efficacy. The weight of the human heart is roughly 300 grams. By applying the same rationale, since in this proposed study tcells will be injected intramuscularly into the gastrocnemius (which roughly weighs 500 grams), the release specification was set to a minimum of 5 million cells expressing the CD31 marker and uptake of Ac-LDL.

The rationale behind the administration of the treatment is to ensure that the cells can induce their therapeutic effect by being applied across the entire ischemic tissue. It is assumed that intra-muscular injections offer optimal delivery of the cells to reach the ischemic tissue and remain within it. The underlying principle of intramuscular injection is the creation of a cell depot with paracrine activity in the ischemic area that can induce neovascularisation and vasculogenesis most efficiently.

These assumptions are in line with other reports from the literature (Rutherford et al., 1997, Fadini et al., 2010). 33 of 37 reported trials using cells for critical limb ischemia utilized intramuscular rather than intra-arterial injections. Most of the studies used between 20 and 60 injections that were delivered into the gastrocnemius muscle along a symmetric grid. Furthermore, meta-analysis of the 37 studies showed that the intramuscular approach is effective in improving critical CLI endpoints, such as ulcer healing, ankle-brachial index and tissue O2 tension, while intra-arterial injections did not impact those parameters (Fadini et al., 2010).

If a subject is randomized into the control group, he/she will receive placebo which consists of X-Vivo™ 15 only, the same medium in which the ACPs are suspended.

7.3. Safety Monitoring after IMP administration

7.3.1. Visit 4: First 48 hours after IMP Administration

Acute monitoring will be performed after IMP administration, and will include monitoring of body temperature, heart rate, systolic and diastolic blood pressure, ECG, including blood tests as described below.

The first 5 subjects in the study will be hospitalized for 24 hours following the administration of the IMP. Unless a serious adverse event emerges, the subject will be released from the hospital after 24 hours from the administration of the IMP.

If no severe adverse events related to the IMP are recorded during the first 48 hours from the administration of the IMP in the first 5 subjects enrolled in the study, the rest of the subjects will not be hospitalized. These remaining subjects will be supervised for 2 hours after the administration of the IMP at the hospital and then released to their homes if the clinical investigator determines that their clinical condition permits it. Vital signs and ECG will be performed at the end of this 2-hour period. No additional blood tests will be required.

On the same day, before bed time, if applicable, the subject will be assessed in person or by telephone by a member of the clinical team. All the information reported by the subject will be noted and reported in the Case Report Forms (CRF).

Approximately 24 hours after the administration of the IMP another telephone (or in person) assessment will be done by a member of the clinical team.

All the subjects will return for a scheduled follow-up visit on the second day after injection.

The following physical and laboratory examinations will be performed on the first 5 subjects enrolled in the trial on Day 2 after administration of the IMP:

1) Monitoring of blood pressure, heart rate, ECG and body temperature.
2) Physical examination, including local examination of the injected leg.
3) Pain scale assessment (VAS).
4) Blood tests: *Hematology*: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT (or INR), and PTT (48 hours after administration of ACPs, just prior to release from hospital). *Blood Chemistry*: blood glucose level; Blood urea nitrogen (BUN); Serum creatinine; Serum chloride; Serum Potassium; Serum sodium; Serum magnesium; Serum albumin; Total serum proteins; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, Creatine phosphokinase (CPK), *Blood cultures*: To proactively detect any potential infection, approximately 48

hours after administration of the investigational medicinal product blood samples will be taken for aerobic and anaerobic bacterial cultures.

Following acute monitoring according to the procedures listed above, the subject may be released from the hospital. Continued hospitalization until completion of Visit 5 without any deterioration in the subject's clinical condition will not be considered an adverse event.

7.3.2. Visit 5: 48-72 hours after IMP administration

If not hospitalized, the subject will return to the hospital 48-72 hours after the administration of the IMP. The following examinations will be performed on all patients:

1) Monitoring of blood pressure, heart rate, ECG and body temperature.
2) Physical examination, including local examination of the injected leg.
3) Pain scale assessment (VAS)

7.3.3. Detection of possible infection

Infection will be suspected if either the subject develops fever, or if the bacteriological cultures of the product are found to be positive. Depending on subject's condition and other details of the case, a suitable treatment procedure will be followed (see Section 14).

7.4. Follow-up Period

The subjects will be followed, with general as well as specific safety and efficacy parameters according to the established routine for such cases.. Follow-up visits will take place at 1 month, 3 months , 6 months, and 12 months after administration of the IMP.

7.4.1. Visit 6: One Month follow-up visit (30 ± 5 days)

The following will be performed:

1) Assessment of pain on the visual analogue scale.
2) Assessment of analgesic drug types and doses.
3) Physical examination, including Rutherford assessment.
4) Blood pressure, heart rate, body temperature and ECG.
5) Leg assessment, including skin color, swelling, limb temperature, presence of ulcers or gangrene, bilaterally.
6) Measure resting ankle pressure using an appropriate adult cuff, bilaterally.
7) Measure resting toe pressure, bilaterally.
8) Assessment of leg ulcers, measurement of ulcer size and ulcer photography, bilaterally.
9) Vascular QoL questionnaire.
10) Blood tests:

- *Hematology*: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT, (or INR), and PTT.
- *Blood Chemistry*: blood glucose level; Blood urea nitrogen (BUN); Serum creatinine; C-reactive protein; Serum chloride; Serum Potassium; Serum sodium; Serum magnesium; Serum albumin; Total serum proteins; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, Creatine phosphokinase (CPK), HbA1c.

7.4.2. Visit 7: Three Month Follow-up (90 ±7 days)

The following will be performed:

1) Assessment of pain on the visual analogue scale.
2) Assessment of analgesic drug types and doses.
3) Physical examination, including Rutherford Assessment.
4) Blood pressure, heart rate, body temperature and ECG.
5) Leg assessment, including skin color, swelling, limb temperature, presence of ulcers or gangrene, bilaterally.

6) Measure resting ankle pressure using an appropriate adult cuff, bilaterally.
7) Measure resting toe pressure, bilaterally.
8) Assessment of leg ulcers, measurement of ulcer size and ulcer photography, bilaterally.
9) Vascular QoL questionnaire.
10) Blood tests:
    - *Hematology*: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT, INR.
    - *Blood Chemistry*: blood glucose level; Blood urea nitrogen (BUN); Serum creatinine; C-reactive protein Serum chloride; Serum Potassium; Serum sodium; Serum magnesium; Serum albumin; Total serum proteins; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, HbA1c.

7.4.3. Visit 8: Six Month Follow-up (182 ±14 days)

The following will be performed:

1) Assessment of pain on the visual analogue scale.
2) Assessment of analgesic drug types and doses.
3) Physical exam, including Rutherford Assessment.
4) Blood pressure, heart rate, body temperature and ECG.
5) Leg assessment, including skin color, swelling, limb temperature, presence of ulcers or gangrene, bilaterally.
6) Measure resting ankle pressure using an appropriate adult cuff, bilaterally.
7) Measure resting toe pressure, bilaterally.
8) Assessment of leg ulcers, measurement of ulcer size and ulcer photography, bilaterally.
9) Vascular QoL questionnaire.
10) Blood tests:
    - Hematology: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT, (or INR), and PTT.

- Blood Chemistry: blood glucose level; Blood urea nitrogen (BUN); Serum creatinine; C-reactive protein; Serum chloride; Serum magnesium; Serum Potassium; Serum sodium; Serum albumin; Total serum proteins; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, HbA1c.

7.4.4. Visit 9: Nine Month Follow-up (270 ±14 days)

The following will be performed:

1) Assessment of pain on the visual analogue scale.
2) Assessment of analgesic drug types and doses.
3) Physical exam, including Rutherford Assessment.
4) Blood pressure, heart rate, temperature and ECG.
5) Leg assessment, including skin color, swelling, limb temperature, presence of ulcers or gangrene, bilaterally.
6) Measure resting ankle pressure using an appropriate adult cuff, bilaterally.
7) Measure resting toe pressure, bilaterally.
8) Assessment of leg ulcers, measurement of ulcer size and ulcer photography, bilaterally.
9) Vascular QoL questionnaire.
10) Blood tests:

- Hematology: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT, (or INR), and PTT.
- Blood Chemistry: blood glucose level; Blood urea nitrogen (BUN); Serum creatinine; C-reactive protein; Serum chloride; Serum Potassium; Serum magnesium; Serum sodium; Serum albumin; Total serum proteins; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, HbA1c.

7.4.5. Visit 10: Twelve Month Follow-up (365 ±14 days)

The following will be performed:

1) Assessment of pain on the visual analogue scale.
2) Assessment of analgesic drug types and doses.
3) Physical exam, including Rutherford Assessment.

4) Blood pressure, heart rate, temperature and ECG.
5) Leg assessment, including skin color, swelling, limb temperature, presence of ulcers or gangrene, bilaterally.
6) Calculate resting ankle pressure using a standard adult cuff, bilaterally.
7) Calculate resting toe pressure, bilaterally.
8) Assessment of leg ulcers, measurement of ulcer size and ulcer photography, bilaterally.
9) Vascular QoL questionnaire.
10) Blood tests:
    - Hematology: RBC; Hemoglobin; Hematocrit; WBC; Neutrophils; Lymphocytes; Monocytes; Platelet count, PT, (or INR), and PTT.
    - Blood Chemistry: blood glucose level; Blood urea nitrogen (BUN); Serum creatinine; C-reactive protein; Serum chloride; Serum Potassium; Serum sodium; Serum magnesium; Serum albumin; Total serum proteins; SGOT/AST; SGPT/ALT; Alkaline phosphatase; Total bilirubin, HbA1c.

8. STUDY POPULATION

8.1. Criteria for subjects

8.1.1. Inclusion Criteria

A. Subject is diagnosed with critical limb ischemia according to the Trans-Atlantic Inter-Society Consensus (TASC) II definition (2007): “The term critical limb ischemia should be used for all [subjects] with chronic ischemic rest pain, ulcers or gangrene attributable to objectively proven arterial occlusive disease. The term CLI implies chronicity and is to be distinguished from acute limb ischemia” (Norgren et al., 2007).

B. Subject has one or more of the following hemodynamic indicators of severe peripheral arterial occlusive disease
    i. Systolic ankle pressure ≤ 70 mmHg,
    ii. Toe systolic pressure ≤ 50 mmHg (or absent palpable pedal pulse).

    In cases where the pulse volume recording (PVR) is flat or barely pulsatile, patients with higher systolic blood pressures may be included after Sponsor review with the investigator.

C. Subject is not a candidate for standard revascularization treatment options for peripheral

arterial disease because of no distal target as seen on angiography or by color flow duplex ultrasound imaging (completed within six (6) months of screening visit), without patent runoff vessels below the knee to enable a distal bypass or following a failed revascularization attempt. In addition, the subject will not be considered a candidate for revascularization treatment if open surgical or revascularization attempts pose a high risk to the subject's life. Examples include subjects suffering from very severe cardiac disease (as determined by a cardiologist), are bedridden, suffer from paralysis of at least one limb or are in poor general health, or suffer from any other severe condition that the PI considers as high-risk.

D. Subjects must be on standard of care medical therapy for peripheral vascular disease such as 1) control of hyperlipidemia with statins or other anti-hyperlipidemic drugs as indicated, 2) control of hypertension as indicated, 3) antiplatelet therapy with aspirin, clopidogrel (Plavix), or other anticoagulant therapy, and/or cilostazol (unless medically contraindicated, e.g., bleeding or allergy), 4) control of hyperglycemia as indicated.

E. Male or female age 18 to 86, inclusive.

F. Non-pregnant, non-lactating female.

G. Subject is able to understand and provide voluntary signed informed consent.

8.1.2. Exclusion Criteria

A. Subject having on angiography (or other appropriate imaging modality) uncorrected aorto-iliac occlusive disease down to the origin of the profonda-femoris artery of the affected limb.

B. Subjects who, in the opinion of the investigator, have a vascular disease prognosis that indicates they would require a major amputation (at or above the ankle) within approximately 4 weeks after administration of the IMP.

C. Advanced CLI presenting as severe ischemic ulcers > 10 cm2 or dry gangrene proximal to the metatarsophalangeal joint heads or lower extremity wet gangrene.

D. Lower extremity non-treated active infection, e.g., wet gangrene, purulent discharge, cellulitis, MRSA infection.

E. Body weight less than 45 kg (99 lbs).

F. Hypercoagulable state, which is already known from the documented history.

G. Subject received blood transfusion during the previous 4 weeks (to exclude the potential of non-autologous ACPs in the harvested blood).

H. Subject's condition precludes - in 2 consecutive attempts - the manufacturing of batches that pass release specifications.

I. Inability to communicate (that may interfere with the clinical evaluation of the subject).

J. Major non-vascular operation during the preceding 3 months.

K. Myocardial infarction or uncontrolled myocardial ischemia or persistent severe heart failure (New York Heart Association (NYHA) class IV) during the preceding 3 months.

L. Severe aortic stenosis

M. Renal failure where GFR is < 30 mL/min/1.73 m2.

N. Hepatic failure (Child-Pugh class C).

O. Anemia (Hemoglobin lower than 11g/dl).

P. Major stroke within the preceding 3 months resulting in debilitating hemiplegia.

Q. Diagnosis of malignancy within the preceding 3 years (excluding non-melanoma skin cancers and non-metastatic prostate cancer).

R. Concurrent chronic or acute infectious disease and uncontrolled infectious symptoms.

S. Severe concurrent disease, e.g., septicemia, evident severe osteomyelitis, HIV-1,2/HBV/HCV/HTLV infections, poorly controlled insulin-dependent diabetes mellitus (HbA1c >10%), systemic lupus erythematosus, multiple sclerosis, amyotrophic lateral sclerosis, nephrotic syndrome, connective tissue disorders or arteritis (e.g., Takayasu arteritis).

T. Bleeding diathesis.

U. Participation at the same time in another investigational medicinal product or device study.

V. Chronic immunomodulating or cytotoxic drug treatment.

W. Subjects who have a temperature of above 38.40C for 2 consecutive days during and immediately prior to the time the subject is about to receive the investigational medicinal product.

X. Life expectancy of less than 6 months.

Y. Subject unlikely to be available for follow-up.

Z. Acute worsening of CLI.

9. TRIAL OBJECTIVE

To determine the efficacy and safety of intramuscular injection of ACP-01, containing blood-derived autologous ACPs, in subjects with critical limb ischemia who are on standard of care therapy and who have no endovascular or surgical revascularization options.

10. ASSESSMENT OF FUNCTIONAL PARAMETERS

10.1. Primary endpoints

10.1.1. The primary efficacy endpoint for this study is the earlier time from treatment with IMP to either de-novo gangrene, or doubling of wound size, or major amputation, or death.

10.1.2. Safety of intramuscular injection of ACP-01.

10.2. Secondary endpoints

10.2.1. Change from Baseline in VAS pain score.

10.2.2. Change from Baseline in Ulcer Size.

10.3. Exploratory Outcome Measures

10.3.1. Change from baseline in the dose and quantity of analgesic drugs used by the subject.

10.3.2. Reduction in total hospitalization time of subjects treated with ACP-01 compared to subjects treated with placebo.

10.3.3. Change from Baseline in Quality of Life.

10.3.4. Change from Baseline in Ankle Pressure.

10.3.5. Change from Baseline in Toe Pressure.

11. AMENDMENTS TO THE PROTOCOL

The approval of the Ethics Committees/IRBs will be obtained before any changes are implemented.

12. TEST MATERIAL

12.1. Investigational Medicinal Product

The active substance being tested is a mixture of cells (ACPs) consisting of at least 5 million autologous $CD31^+$ and Ac-LDL^+ and at least 1 million $CD34^+$ cells (constituting at least 1.5% of the total number of cells in the product). In addition to $CD31^+$ and Ac-LDL^+ and $CD34^+$ cells, the product consists of other cellular subpopulations considered not to be the effector cells. The maximal number of cells injected will not exceed 200 million. The cells are isolated from the subject's peripheral blood and then cultured *ex vivo* under sterile conditions. The product will be provided in 6 separate syringes (5 ml each) to be administered by 30 injections.

The placebo is X-Vivo™ 15, the same medium for the production of the ACP product. It will be administered to the subjects in the same type and number of syringes and in the same volume as the cell-containing product. X-Vivo™ 15, is a chemically defined, serum-free growth medium used for the cultivation of a variety of cell types, including progenitor cells.

12.2. Biological activity analyses

Acceptable culture parameters as assessed by microscopy flow cytometry and ELISA will be in accordance with the following specifications:

- Cell viability – $\geq$ 75%
- Morphology – spindle-shaped, large cells forming long thread-like structures.
- Angiogenic capacity is assessed by flow cytometry. The following cell markers are evaluated: $CD31^{bright}$ and uptake of Acetylated-Low Density Lipoprotein (Ac-LDL).
- Stemness capacity is also assessed by flow cytometry and is defined by the percentage and number of cells expressing the CD34 marker.
- Physiological activity specific for ACPs is assessed by secretion of IL-8 by the cultured cells and is assessed by ELISA.
- Angiogenin, a potent angiogenic cytokine will also be assessed and potentially correlated with the clinical outcome of the subjects.
- Cell migration – the cells' ability to migrate towards angiogenic or stem cell factors will be assessed and potentially correlated with the clinical outcome of the subjects.

12.3. Safety analyses

12.3.1. Sterility

Sterility tests will be performed according to 21 CFR 610.12. Assessment of cell culture sterility will be performed on samples of the cell fraction supernatant following cell washing. Interim negative sterility results of all samples taken at different stages of the culture will be compulsory for the release of the final product.

12.3.2. Bacterial Endotoxin Test

The Bacterial Endotoxin test will be performed according to current USP 28 chapter <85>. The Lymulus Amebocyte Lysate (LAL) test will be performed on a sample of supernatant taken from the cells and the final washing medium of the cells before vialing. Endotoxin levels below the acceptable limits will be compulsory for the release of the final product.

12.3.3. Gram stain

Gram stain will be used as a rapid and qualitative method for assessing bacterial contamination of tissue culture samples. Negative results of the Gram stain performed on samples taken from the cells and the final washing medium of cells before vialing will be compulsory for the release of the final product.

12.3.4. Mycoplasma

Mycoplasma contamination will be tested and the results of the test will be obtained before the administration of the product, but after the shipment of the product to the clinical site. Immediately, upon receipt of the test results, they will be communicated to the clinical site.

12.4. Culture preparation

The isolation and incubation of autologous ACPs will be performed in a specially designed and dedicated clean room of Class 10,000, in a Class 100 laminar flow biological safety cabinet.

Approximately two hundred and fifty milliliters of peripheral blood will be collected from the subject in a standard sterile blood transfusion bag containing CPDA-1 as anticoagulant. In addition, approximately 10 ml blood will be collected and used for tests performed at the hospital (bacterial cultures and mycoplasma). The blood bag will be packed immediately at 2-8^{0}C and transported to the manufacturing facility.

Blood samples and cells that are not used in the treatment of subjects with ACPs, e.g., from the placebo group, will be de-identified and may be utilized for manufacturing and quality control testing such as for the development of potency and release assays. Results from the assays and tests may be used to

retrospectively examine cell and product characteristics in CLI patients and/or the response to treatment with ACPs.

The autologous ACP culture will be prepared and tested according to standard operating procedures. The cells will be incubated at 37°C, in a 5% CO_2 incubator for approximately 5 days. The final product will be obtained and the decision on whether the culture is suitable for administration to human subjects will be based on concordance between the analytical results of tests performed after incubation and the pre-defined release specifications.

Concurrently, Gram staining of a sample from the supernatant taken from the culture will be performed before sending the cells to the hospital. Negative results of the Gram staining will be compulsory for continuation of the procedure. Bacterial cultures started at blood harvesting, and at different stages of the cell manufacturing process will be assessed for evidence of bacterial/fungal contamination and negative sterility results will be compulsory for continuation of the procedure.

The final product will be transported back to the hospital for administration to the subject. The investigator will receive notice before the arrival of the final product, so that preparations for the administration can be completed.

13. CONCOMITANT TREATMENT

The investigators will not deviate from the standard treatment as practiced in their institution for subjects with CLI, except for the administration of the IMP (ACPs or placebo) and the other requirements of the protocol. The same principle will guide the management of the subjects after administration of IMP at the medical center and after hospitalization.

14. CLINICAL STUDY PROCEDURE

Visit type	**Screening**		**Adm. of IMP**	**Primary safety Follow-up**		**Long-term follow-up**				
Visit number	1	2	3	4	5	6	7	8	9	10
Consecutive visit day	-30 to -7	-7 ±1	0	1 (Phone f/u except for first 5 patients)	2-3	30 ±5	90 ±7	182 ±14	270 ±14	365 ±14
Demography	X									
History	X									
Physical exam	X		X	X (only first 5 subjects)	X	X	X	X	X	X
Rutherford Assessment	X		X			X	X	X	X	X
Leg assessment, bilaterally (skin, swelling, temperature, ulcers, gangrene, etc.)	X		X	X (only first 5 subjects)	X	X	X	X	X	X
Heart rate, BP, ECG & Temperature	X		X (before admin of IMP and 2 hours after)	X (only first 5 subjects)	X	X	X	X	X	X
Eligibility assessment	X									
Consent form signing	X									
Pain scale assessment (VAS)	X			X	X	X	X	X	X	X
Analgesia drugs and doses	X					X	X	X	X	X

Visit type	Screening		Adm. of IMP	Primary safety Follow-up		Long-term follow-up				
Visit number	1	2	3	4	5	6	7	8	9	10
Laboratory tests	X	X			X (only first 5 subjects)	X	X	X	X	X
Screening for HIV,HTLV, HVB,HVC	X									
Pregnancy test	X									
Ankle pressure, bilaterally	X					X	X	X	X	X
Toe pressure, bilaterally	X					X	X	X	X	X
Vascular Quality of Life Questionnaire	X					X	X	X	X	X
Ulcer assessment, size and photography, bilaterally.	X		X			X	X	X	X	X
Telephone follow-up				X (at night)						
Blood cultures		X			X (only first 5 subjects)					
Blood draw for manufacture of investigational medicinal product		X								
IMP administration			X							

15. PROTOCOL FOR CASES OF SUSPECTED INFECTION

Possible infection may be suspected in one of the following situations:

Possibility 1: The subject is newly febrile (>38.4°C) and cultures of samples taken from the ACP product are positive.

- Obtain blood sample and culture to assess potential bacterial growth and sensitivity, evaluate heart function.
- Treat according to culture sensitivities.

- Treatment guided by Infectious Diseases Expert (ID expert) from the beginning.
- Put recruitment of additional subjects on hold at that site until a report of the suspected infection is issued.

Possibility 2: The subject is newly febrile (>38.4°C) and cultures of samples taken from the ACP product are negative.

- Obtain 2 sets of blood cultures, urine culture and urinalysis, WBC-count + differential.
- Evaluate heart function.
- Rule-out all other infectious options.
- An ID expert should evaluate the subject and the laboratory data. The treatment will be decided upon by the ID expert.

Possibility 3: The subject is afebrile and cultures of samples taken from the ACP product are positive.

- Obtain blood sample(s).
- Do not start antibiotic therapy unless blood culture findings dictate it.
- Follow closely under the supervision of the ID expert.

16. ADVERSE EXPERIENCES

All adverse experiences (events) will be recorded at each visit throughout the duration of the trial or at any other occasion when they are diagnosed. The corresponding information should also be documented on the appropriate Case Report Form. The characterization of all adverse events will be performed according to the WHO dictionary. The following provides definitions of both adverse and serious adverse events that will be used in the trial.

16.1 Adverse event (AE)

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the IMP.

16.2. Serious Adverse Event (SAE)

Any untoward medical occurrence that at any dose:

- Results in death,

- Is life-threatening,
- Requires in-patient hospitalization (≥ 24 hours) or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

All fatalities and serious adverse experiences (SAE), including serious clinical laboratory abnormalities even those that the investigator considers not to be related to the leg condition or the administration of the IMP must be immediately reported by phone, by email, or by fax to Hemostemix or its designee.

All significant symptoms and signs, which were not present at the previous visits, must be recorded as potential adverse experiences to be evaluated by the clinical investigator. Any medical condition present at the previous visits that remains unchanged or improves should not be recorded as an adverse experience at subsequent visits. However, if there is deterioration of a medical condition that was present at the initial visit, then this should also be considered a new adverse experience and reported.

If an SAE occurs, it will be noted and reported starting from the time point of the subject signing the Informed Consent Form, even if causality cannot be attributed to the IMP. AEs will be reported from Visit 2 onwards (time of blood draw for manufacture of the IMP).

16.3. Relationship to the IMP

The investigator, using the following guidelines, should determine relationship between each adverse experience and treatment with the IMP.

***Not related*:** The experience is clearly related to other factors such as the subject's clinical state or other therapeutic intervention.

- ***Unlikely***: The experience was most likely produced by other factors such as the subject's clinical state, other therapeutic interventions, or concomitant drugs administered to the subject, and does not follow a known response pattern to the autologous ACP administration.
- ***Possible***: The experience follows a reasonable temporal sequence from the time of the IMP injection and/or follows a known response pattern to autologous ACP administration, but could have been produced by other factors such as the subject's clinical state, other therapeutic intervention, or concomitant drugs administered to the subject.
- ***Probable***: The experience follows a reasonable temporal sequence from the time of IMP injection and follows a known response pattern to the autologous ACP administration and cannot be reasonably explained by other factors such as the subject's clinical state, other therapeutic interventions, or concomitant drugs administered to the subject.

- ***Highly probable***: The experience either occurs immediately following the IMP injection or there is a positive reaction at the application site, follows a known response pattern to the autologous ACP therapy, and cannot be reasonably explained by other factors such as the subject's clinical state, other therapeutic interventions, or concomitant drugs administered to the subject.

16.4 Severity of adverse experience

Severity of an adverse experience is defined as a qualitative assessment of the degree of intensity of an adverse experience, as is determined by the investigator or reported to him/her by the subject. The assessment of severity is made irrespective of relationship to the administration of the IMP or seriousness of the experience and should be evaluated according to the following scale:

- 1= Mild
- 2= Moderate
- 3= Severe
- 4= Life threatening
- 5= Death

16.5 Actions to be undertaken by the PI

AEs/SAEs are captured using an EDC. The following lists the procedures and written documentation for reporting SAEs including deaths:

1) All deaths have to be reported immediately by telephone to the company and subsequently in writing via fax or email within 24 hours using the Serious Adverse Experience (SAE) Report form. The corresponding information should also be documented in the Adverse Experience (AE) column in the CRF.
2) SAEs, which the investigator considers are not related to the clinical condition under study, must be reported by telephone to the company immediately and subsequently in writing using the SAE Report form within 24 hours. This includes SAEs and/or serious abnormal laboratory findings occurring during the entire trial period. The corresponding information should also be documented on the Adverse Experience (AE) column in the CRF.
3) All adverse experiences will be followed until resolution and/or until the etiology is established as not related to the administration of the ACP products using the AE follow-up CRF for non-serious AEs or the SAE Report form. If a subject requires long-term follow up because of a suspected adverse reaction to the IMP, a complete medical summary

with pertinent laboratory report is to be submitted to the company at the end of the follow-up period.

The investigator must inform the Ethics Committee/REB/IRB of any life-threatening toxicity, and all deaths according to local regulatory requirements.

16.6. Expected adverse events

A full list of expected adverse events is presented in Appendix 1.

17. DATA SAFETY MONITORING BOARD (DSMB)

An independent DSMB, comprised of two experienced clinicians with relevant expertise (vascular surgery, cardiology, anesthesiology, trauma, stem cell technology) and a biostatistician serving the DSMB will blindly review the accumulated study data.

DSMB members and the unblinded biostatistician cannot be investigators involved in the conduct of the clinical trial and should have no vested financial interest in the company or in the outcome of the study and are not allowed to possess any shares or stock options in the company. DSMB members shall be reimbursed for their time and any out-of-pocket expenses incurred as part of their duties.

The company may designate one additional member to the DSMB, who will also coordinate the activity of the board.

The DSMB will be provided all study accumulated data. and schedule of meetings and role of DSMB will be outlined in a DSMB Charter to be signed and approved by all DSMB members.

18. WITHDRAWAL OF SUBJECTS FROM TRIAL

The following events are considered sufficient reasons for a subject to discontinue the trial:

- Whenever the subject decides that it is in his/her best interest.
- Whenever the investigator considers it advisable or in the subject's best interest.

It is agreed that, for reasonable cause given by either the investigator or the Sponsor, the company may terminate this trial, provided a written notice is submitted at a reasonable time in advance of the intended termination.

19. ETHICAL AND REGULATORY ISSUES

19.1. Informed consent

Informed consent for each subject will be obtained prior to initiating any trial procedures in accordance with the statement entitled "Requirements for Informed Consent." According to the existing laws and regulations, one copy of the informed consent form must be given to each subject and one signed copy must be retained in the investigator's trial records.

The “Declaration of Helsinki” recommends that consent be obtained from each potential subject in biomedical research trials after the aims, methods, anticipated benefits, and potential hazards of the trial, and discomfort it may entail, are explained to the individual by the physician. The subject should also be informed of his or her right not to participate or to withdraw from the trial at any time. If the subject is in a dependent relationship to the physician or given consent under duress, the informed consent should be obtained by an independent physician. If the subject is legally incompetent (i.e., a minor, or mentally incompetent), informed consent must be obtained from the parent, legal guardian, or legal representative in accordance with the law of the country in which the trial takes place. By signing this protocol, the investigator agrees to conduct the trial in accordance with the “Declaration of Helsinki.”

19.2. Ethical principles

This trial complies with the principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments laid down by the 18th World Medical Assemblies, and the ICH guidelines for Good Clinical Practice.

19.3. Laws and regulations

This Clinical Trial will be conducted in compliance with the International and National laws and regulations of the country(ies) in which this Clinical Trial is performed, as well as any applicable guidelines.

19.4 Research Ethics Board/Institutional Review Board/Independent Ethics Committee (REB/IRB/IEC)

The Investigator must submit this protocol to the appropriate Ethics Committee (REB/IRB/IEC), and is required to forward to the Sponsor a copy of the written and dated approval signed by the Chairperson of such committee (REB/IRB/IEC).

20. STATISTICAL METHODOLOGY

A total of approximately 95 subjects will be randomized to treatment with ACP-01 or placebo using a 2:1 randomization scheme, respectively, stratified by site. Study will be continued until all subjects treated with the IMP are followed-up for 52 weeks.

One futility analysis for potentially stopping enrolment into the study will be performed when approximately 42 subjects have completed at least 26 weeks of follow-up but no more than 52 weeks from IMP administration or experienced before that time point the study event.

20.1. Randomization Procedure

After a subject meets the eligibility criteria, he/she will be randomized to one of the two treatment groups, based on a randomization procedure employing a 2:1 assignment ratio, i.e., treatment with ACP-01 or placebo, respectively, using permuted blocks stratified by centers. #

20.2. Interim Analysis

Interim analyses will be conducted according to the Lan & Demets (1983) maximum information group sequential test, in which the Type I error spent at each analysis k is a function of the observed information level, using the error spending function family

$$f(t)= \min\{\alpha t^{\rho}, \alpha\},$$

where t = fraction of the maximum anticipated information obtained at the time of analysis k. To maintain the blind, interim analyses will be conducted by an independent party without unblinding either the Sponsor or Investigators to the treatment assignments.

20.3. Significance Level

The overall significance level for this study will be 5% using two-tailed tests. The study will have one primary endpoint and 2 secondary end-points ordered in a hierarchy, and analyses will utilize the gatekeeping approach to maintain the experiment-wise type-I error of 5%. The order of the hierarchy of the secondary endpoints testing following the primary end-point testing is as follows:

- Change from Baseline in VAS pain score
- Change from Baseline in Ulcer Size

20.4. Sample Size

Sample size calculations were performed under the following assumptions:

- The primary study endpoint is the earlier time from treatment with study IMP to either de-novo gangrene, or doubling of wound size, or major amputation, or death.
- Randomization will allocate subjects to autologous ACPs or placebo using a 2:1 assignment ratio, respectively, stratified by site.
- The expected median time to study event as defined by the primary efficacy endpoint, assuming exponentially distributed time to event, is 3 months for placebo-treated subjects and 7 months for the ACP-treated arm.

- The anticipated monthly randomization and study IMP administration rate is 6 subjects per month.
- The minimal follow-up period of a subject will be 26 weeks if the primary endpoint is not reached, and the maximal follow-up period will be 52 weeks.
- Final analysis, under these assumptions is planned to be conducted at approximately at 22 months from first subject IMP treatment including a total of 95 subjects.
- As the effect of the early futility analysis is marginal, the final analysis will use a two-sided alpha level of 0.05

Under the above assumptions, a total of 95 subjects treated with the study IMP will provide 94% power to detect a statistically significant result at a two-sided alpha level as specified above.

Sample Size Re-estimation

Periodic, blinded review of the overall, pooled event rate will be conducted using the method of Gould and Shih(1992). If the observed overall event rate is lower than anticipated at study initiation, this procedure will allow for adjusting the sample size without altering the significance level. Consistent with FDA guidance (2018), Adaptive Designs for Clinical Trials of Drugs and Biologics Guidance for Industry, such sample size adjustments require no adjustment of Type I error:

> In general, adequately prespecified adaptations based on non-comparative data have a negligible effect on the Type I error probability.

20.5. Analysis Sets

- Intent-to-Treat (ITT) Analysis Set: The intent to treat (ITT) analysis set will consist of all subjects who have been randomized to the study. In accordance with the ITT principle, all subjects randomized will be kept in their originally assigned treatment group. This analysis set will serve as the primary analysis set for efficacy inference.
- Modified Intent-to-Treat (mITT) Analysis Set: The mITT analysis set is a subset of the ITT analysis set. This set will consist of data from all subjects who have been randomized to the study and administered the IMP and had at least one treatment visit post IMP administration.
- Per-Protocol (PP) Analysis Set: The per protocol (PP) analysis set is a subset of the mITT analysis set and will consist of all subjects with no major protocol violations as will be defined in a more detailed Statistical Analysis Plan (SAP) to be written prior to revealing of the blind and having follow up assessments available.

- Safety (ST) Analysis Set: The safety analysis set (ST) will consist of all subjects who have been randomized and received the Study IMP. This analysis set will be used as the primary set for safety inference.

20.6. Demographics and Baseline Characteristics

Demographics and baseline data will be described for the ITT analysis set. Subject demographics and baseline characteristics, including underlying disease history, medical history and prior medications will be compared between the study groups to assess baseline comparability. Continuous variables (e.g., subject age, weight, height, and body mass index (BMI)) will be summarized using descriptive statistics (number [n], mean, standard deviation, and standard error, median, minimum, and maximum) Categorical variables will be summarized using subject counts and percentages. Categories for missing data will be presented if necessary.

20.7. Efficacy Assessment

The following trial end-points will be calculated and statistically analyzed for the ITT cohort which will serve as the primary analysis set for efficacy inference.

20.7.1. Primary End-Point and Principal Analysis

The primary efficacy end-point for this study is the erlier time from treatment with IMP to either de-novo gangrene, or doubling of wound size, or major amputation, or death. The time to the primary end-point will be presented by Kaplan-Meier curves stratified by treatment group.

The time form day of treatment with the IMP to the earlier time of either de-novo gangrene, or doubling of wound size, or major amputation, or death will be right censored by the subject's last follow-up date in the study.

The primary analysis for the between study groups comparison will be conducted utilizing the baseline adjusted Cox's proportional hazards (PH) model (SAS® PROC PHREG). In addition to treatment group and study site, baseline prognostic factors as will be defined in a more detailed SAP written prior to revealing of the blind will be included as covariates in the model.

The adequacy of the proportional hazards (PH) assumption will be confirmed by including a time dependent covariate for the active treatment group (as dummy variable) by log (time) interaction in the primary analysis model and testing it using 5% significance level. In case the PH assumption will be rejected the log rank test (SAS® PROC LIFTEST) will be used for statistical inference. A detailed sensitivity analysis of the principal analysis of the primary endpoint will be provided in a more detailed SAP to be sent to the Division prior to revealing of the blind and conduct of the pre-defined futility analysis.

20.7.2. Key Secondary Endpoint Analyses

20.7.2.a. Change from Baseline in VAS pain score

The change from baseline in VAS pain score is defined at the difference between the Last Observed Value (LOV) of an individual subject substracted from the last measurments taken prior to study IMP administration. Subjects who early terminated from the study or met the study event will be imputed a value according to the worst-case scenario; the worst recorded measurement of change of the entier study population.

The model baseline adjusted Least Squares Means (LSM) of the change from baseline to LOV of this endpoint will be compared the two study groups by applying an ANCOVA model (SAS® MIXED procedure). The model will include treatment group, study site and baseline pain measurement.

20.7.2.b. Change from Baseline in Ulcer Size

The change from baseline in Ulcer Size (cm^2) is defined at the difference between the Last Observed Value (LOV) of an individual subject substracted from the last measurments taken prior to study IMP administration. Subjects who early terminated from the study or met the study event will be imputed a value according to the worst-case scenario; the worst recorded measurement of change of the entier study population.

The model baseline adjusted Least Squares Means (LSM) of the change from baseline to LOV of this endpoint will be compared the two study groups by applying an ANCOVA model (SAS® MIXED procedure). The model will include treatment group, study site baseline ulcer size measurement.

The model adjusted Least Squares Means (LSM) of the change from baseline to LOV of this endpoint will be compared by applying an ANCOVA model (SAS® MIXED procedure). The model will include treatment group, study site and baseline toe pressure measurement.

20.7.3. Exploratory Outcome Measures

Additional exploratory endpoints as described below will be analyzed for further exploration of the ACP treatment effect with no multiplicity adjustment. Assessments will be done for Study Week 13 and Week 26 and will be described in a more detailed SAP to be sent to the Division prior to revealing of the blind and conduct of the pre-defined futility analysis. The pre-defined exploratory outcome measures are:

- Change from baseline in the dose and quantity of analgesic drugs used by the subject.
- Change from Baseline in Quality of Life
- Change from Baseline in Ankle Pressure
- Change from Baseline in Toe Pressure

- Proportion of subjects with an improvement in VAS pain score
- Change from baseline in Ulcer Size.

20.8. Safety Assessment

Safety assessments, which will be performed for the Safety Analysis set only, will include the following:

20.8.1. Adverse Events

All adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Summaries will be presented for all adverse events (overall and by severity), adverse events determined by the investigator to be treatment-related (overall and by severity), serious adverse events, adverse events causing early termination and non-serious adverse events. The incidence of adverse events will be summarized using descriptive statistics by system organ class and preferred term. Subjects are counted only once in each system organ class category, and only once in each preferred term category. Treatment-related adverse event summaries will include adverse events with missing relationship to study drug. For the summaries by severity, subjects are counted at the greatest severity. Adverse events missing the flag indicating serious will be excluded from the summary of serious adverse events but included in the summary of non serious adverse events. Listings for deaths, serious adverse events, adverse events leading to discontinuation, MedDRA dictionary terms for adverse event descriptions, and adverse event preferred terms by subject number will be presented.

20.8.2. Clinical Laboratory Tests

Summary statistics for laboratory tests will be presented at baseline and consecutive scheduled treatment visits. Laboratory tests results and changes from baseline to each visit and endpoint will be summarized using descriptive statistics. Shifts (below, within, and above the normal range) from baseline to each visit and endpoint will be summarized using subject counts. The incidence of clinically significant abnormal results will also be summarized for laboratory data using descriptive statistics.

20.8.3. Vital Signs and ECG

Summary statistics for vital signs and ECG will be presented at baseline and consecutive scheduled treatment visits. Vital signs values and changes from baseline to each visit and endpoint will be summarized using descriptive statistics. The incidence of clinically significant abnormal values will be summarized for selected vital signs using descriptive statistics..

20.8.4. Statistical Analysis Plan (SAP)

A more detailed SAP will be developed while the study is still ongoing, prior to revealing of the study blind and the conduct of the futility analysis and will be submitted for the review of the Division. This

SAP will also outline the sensitivity analyses to be performed for the primary study endpoint using other set of methods accounting for the missingness mechanism as well as assessment of other analysis sets allowing to evaluate the robustness and internal consistency of primary results and conclusions.

20.8.5. Statistical Software

The data will be analyzed using the SAS® version 9.4 or higher (SAS Institute, Cary North Carolina).

21. STUDY MONITORING

21.1. Responsibilities of the Investigator(s)

The Investigator(s) undertake(s) to perform the study in accordance with this protocol, Good Clinical Practices and the applicable regulatory requirements.

The Investigator is required to ensure compliance with the schedule and procedures required by the clinical protocol. The Investigator agrees to provide all information requested in the Case Report Form in an accurate and legible manner according to the instructions provided and to ensure direct access to source documents to Sponsor representatives.

21.2. Responsibilities of the Sponsor

The Sponsor of this study is responsible to Health and Regulatory Authorities for taking all reasonable steps to ensure the proper conduct of the study in regards to ethics, protocol compliance, integrity and validity of the data recorded on the Case Report Forms. Thus, the main duty of the Monitoring Team is to help the Investigator and the Sponsor maintain a high level of ethical, scientific, technical and regulatory quality in all aspects of the study.

22. CONFIDENTIALITY

All materials, information (oral or written) and unpublished documentation provided to the Investigators (or any company/institution acting on their behalf), inclusive of this protocol, the subject Case Report Form and the Investigator's Brochure, are the exclusive property of the Sponsor and may not be given or disclosed, either in part or in whole, by the Investigator or by any person under his/her authority to any third party without the prior express consent of the Sponsor.

However, the submission of this protocol and other necessary documentation to the REB/IRB/IEC is expressly permitted, the REB/IRB/IEC members having the same obligation of confidentiality.

The sub-investigators shall be bound by the same obligation as the Investigator. The Investigator shall inform the sub-investigators of the confidential nature of the clinical trial.

The Investigator and the sub-investigators shall use the information solely for the purposes of the clinical trial.

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24. APPENDIX 1- LIST OF EXPECTED ADVERSE EVENTS.

24.1. Expected adverse events related to the medical procedure

a. Anemia - probability – low

Due to blood draw - subjects with severe anemia will not be included in the trial and the volume of blood drawn is not expected to cause severe anemia.

b. Local infection – probability - low

Strict antiseptic procedures will be carried out.

c. Systemic infection - probability – low

Due to injection of infected product – cGMP with strict sterile production will be implemented including sterility testing to ensure the sterility of the product.

d. Muscular compartment syndrome - probability – low

Due to multiple injections into the partially ischemic gastrocnemius muscle – the total volume of injections is restricted to 30 ml.

e. Deep vein thrombosis - probability – low

Due to general thrombogenic diathesis – subjects will continue to receive anticoagulant therapy.

f. Adverse events due to conscious sedation - probability – low

Adverse events related to conscious sedation may include hypoxemia (reversible to profound), respiratory arrest, aspiration pneumonia, hypotension, or death. The risk of these events is minimized since sedation is administered by a trained medical professional.

g. Discomfort/pain at injection site - probability – high

Transient pain/discomfort at the injection site will be minimized through the adherence to standard phlebotomy and injection techniques, performed by trained medical personnel.

h. Nerve damage – probability – low

Blood collection and injection may cause nerve damage secondary to trauma from puncture needles or compression secondary to hematoma; however, this risk will be minimized by adhering to standard phlebotomy and injection techniques carried out by a trained health care professional.

i. Embolism - probability - low

Due to the risk of inadvertent injection into a vessel, there is a risk of causing an embolism, e.g., air, cellular material. This risk will be minimized through adherence to standard injection techniques by a trained health care professional.

j. Bleeding/Hematoma - probability - low

There is a risk of inadvertent perforation of a blood vessel with or without the formation of a hematoma. This risk is minimized through use of a small gauge needle (23 gauge) and careful consideration of the injection sites.

k. Elevation of skeletal muscle enzymes/proteins - probability - low

The injection into the muscular tissue may cause transient increase in skeletal muscle proteins including but not limited to total serum creatinine kinase.

24.2. Expected adverse events related to the administration of the IMP

a. Immunological reaction - probability – low

In order to avoid rejection reactions, subjects who had blood transfusions within the previous 3 months, are excluded from the trial and minimizing the possibility of immune reactions. Further, through the ex vivo culture of the cellular product a potential risk of immunological reaction exists; however, the investigational medicinal product has never been reported to elicit adverse clinical immunological reactions.

b. Ectopic Tissue Formation - probability - low

Due to the injection of a progenitor cell containing product, a theoretical risk of ectopic tissue formation exists. However, there are no known reports suggesting ectopic tissue formation in prior clinical or preclinical experience with the investigational medicinal product.

c. Long term complications including tumor formation - probability - low

Due to the injection of a cell containing product, a theoretical risk of tumor formation exists, however this event has never been reported with the investigational medicinal product.

Based on the worldwide experience of over 11 years with autologous EPC therapies there are no long-term complications that have been reported.