Eur J Vasc Endovasc Surg (2019) 57, 538—545

PLX-PAD Cell Treatment of Critical Limb Ischaemia: Rationale and Design of the PACE Trial

Lars Norgren [a,*], Norbert Weiss [b], Sigrid Nikol [c], Robert J. Hinchliffe [d], John C. Lantis [e], Manesh R. Patel [f], Holger Reinecke [g], Racheli Ofir [h], Yael Rosen [h], Dan Peres [h], Zami Aberman [h]

[a] Department of Surgery, Faculty of Medicine and Health, Örebro University, Sweden
[b] University Centre for Vascular Medicine and Department of Medicine - Section Angiology, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Germany
[c] Asklepios, Klinik St Georg, Hamburg, Germany
[d] Bristol Centre for Surgical Research, Bristol NIHR Biomedical Research Centre, University of Bristol, Bristol, UK
[e] Icahn School of Medicine, New York, NY, USA
[f] Duke University, Durham, NC, USA
[g] Department of Cardiology I — Coronary and Peripheral Vascular Disease, Heart Failure, University Hospital of Muenster, Muenster, Germany
[h] Pluristem Ltd., Haifa, Israel

WHAT THIS PAPER ADDS

Placebo controlled trials of cell therapy to reduce major amputations in patients with critical limb ischaemia and no option for revascularisation have so far been unsuccessful. PLX-PAD cell treatment (placenta derived adherent stromal cells) has in small studies shown promising results, and the phase III PACE trial is designed to evaluate the efficacy and safety of two sessions of intramuscular injections, eight weeks apart with follow up of 12—36 months. The study will provide long-term outcome and will collect parameters to assess the potential economic benefit of this kind of treatment.

Background: Critical limb ischaemia (CLI) is a life threatening condition with a considerable risk of major amputation and death. Besides revascularisation, no treatment has been proven to reduce the risks. Therapeutic angiogenesis by gene or cell therapy has not demonstrated definitive evidence in randomised controlled trials. PLX-PAD is an "off the shelf" allogeneic placental derived, mesenchymal like cell therapy, which, in preclinical studies, has shown pro-angiogenic, anti-inflammatory, and regenerative properties. Favourable one year amputation free survival (AFS), and trends in reduction of pain scores and increase of tissue perfusion have been shown in two small, open label, phase I trials.

Methods: The PACE study is a phase III randomised, double blind, multicentre, multinational placebo controlled, parallel group study to evaluate the efficacy, tolerability, and safety of intramuscular injections of PLX-PAD cells to treat patients with atherosclerotic CLI with minor tissue loss (Rutherford Category 5) up to the ankle level, who are unsuitable for revascularisation or carry an unfavourable risk benefit for that treatment. The study will enroll 246 patients, who after screening are randomised in a ratio of 2:1 to treatment with intramuscular injections of PLX-PAD 300 $\times$ 10^6 cells or placebo on two occasions, eight weeks apart. The primary efficacy endpoint is time to major amputation or death (amputation free survival), which will be assessed in follow up of at least 12 months and up to 36 months.

Conclusions: Based on favourable pre-clinical and initial clinical study results, the PACE phase III randomised controlled trial will evaluate placenta derived PLX-PAD cell treatment in patients with critical limb ischaemia, with an unfavourable risk benefit for revascularisation. Clinicaltrials.gov: NCT03006770.

Keywords: Cell therapy, Critical limb ischaemia, Trial design
Article history: Received 16 August 2018, Accepted 7 November 2018, Available online 25 January 2019

INTRODUCTION

Critical limb ischaemia (CLI) constitutes the most advanced stage of chronic peripheral arterial disease (PAD) and includes rest pain and ischaemic foot lesions. The condition affects 1—5% of all PAD patients, which corresponds to an incidence of 500—1000/million population per year.[1]

* Corresponding author. Department of Surgery, University Hospital, S-70185, Örebro, Sweden.
E-mail address: lars.norgren@regionorebrolan.se (Lars Norgren).

https://doi.org/10.1016/j.ejvs.2018.11.008

Overall, the prevalence of PAD is increasing worldwide, most remarkably in low and middle income countries.[2] Major amputation and death are the ultimate consequences of CLI, and a one year amputation rate of 15—25% is commonly reported, while the amputation and mortality rate ranges 30—40%. The single evidence based recommendation for treatment is revascularisation.[1,3,4] However, as a result of comorbidities at greater risk when performing an interventional procedure, or based on anatomical or technical issues, it is not reasonable to revascularise or to re-revascularise after a failed procedure in a proportion of CLI patients. Few treatments exist for such "poor option" cases. Prostanoid therapy has been reasonably well studied in randomised controlled trials (RCT), but does not have evidence of an effect, and is not recommended in current guidelines.[1,3] Therapeutic angiogenesis has been studied for about 20 years, based on either gene or cell therapy.

Gene therapy

Gene therapy using growth factors, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF), has been investigated in mostly smaller clinical trials, with varying success with regard to the major efficacy endpoint, amputation free survival (AFS). Only NV1FGF has been investigated in a larger randomised placebo controlled trial, TAMARIS,[5] which did not show any better outcome regarding survival or major amputation in the treatment group compared with placebo, despite the fact that a former, smaller trial, TALISMAN,[6] showed that major amputation, as a secondary endpoint, was significantly less common among NV1FGF treated subjects. Injections of the HGF plasmid have yet to prove efficacy with regard to major events (e.g amputation) although smaller randomised placebo controlled trials have shown reduced rest pain[7] and increased toe brachial index (TBI)[8] at follow up. More has to be learned both from basic and clinical research to possibly adopt effective gene therapy for PAD,[9] although Iver and Annex[10] discuss a conceivable end of gene therapy trials, based on the lack of an evident breakthrough.

Cell therapy

The potential benefit of cell therapy is that cell secretion is multifactorial and therefore not based solely on a single growth factor. Initiated by a Japanese study[11] comparing bone marrow mononuclear cells (BM-MNC) and peripheral blood mononuclear cells (PBMC) injected into the limb muscles of patients with PAD, several cell based studies have been performed, specifically in CLI patients with no revascularisation option. The Japanese study[11] showed improved ankle brachial index (ABI) and transcutaneous tissue oxygen pressure ($TcPO_2$) and reduced rest pain in the BM-MNC treated group. Although the majority of studies used intramuscular injections of the growth factor, the largest trial, Juventas, treated 160 patients with intra-arterial infusions of BM-MNC compared with placebo.[12] At six months there was no difference in the rate of major amputations.

In a meta-analysis by Teraa et al.,[13] including 12 RCT in autologous cell therapy for CLI, major amputations were significantly reduced. Most importantly, when only placebo controlled RCTs were included, the major amputations were no longer significantly reduced, indicating the importance of placebo controlled trials in cell therapy. In a later meta-analysis by the same author group,[14] including only placebo controlled RCTs, this outcome was verified. Recently this finding was also verified in another meta-analysis on CD34 + mononuclear cell therapy (CD34 + MCT), including 10 trials.[15] Total amputations and ulcer healing were reduced in comparison with findings in the placebo treated groups. Major amputation and survival were, however, not significantly reduced. This publication also concluded the beneficial value of a high CD34 + cell content.

Autologous or allogeneic cell use

From an immunological point of view, autologous cell treatment may theoretically provide an advantage. Nevertheless, it has been shown that cells harvested from older individuals, and in particular those with cardiovascular risk factors or critical limb ischaemia, are reduced in number and functionality.[16,17] Furthermore, harvesting autologous cells from bone marrow involves an invasive procedure, whereas peripheral blood use requires granulocyte colony stimulation factor (G-CSF) treatment that potentially may cause harm because of the high white blood cell content that is developed.[18] Allogeneic MSCs have been shown to exhibit low immunogenicity,[19] thus, using allogeneic younger, more potent cells, rather than treatment with cells harvested from the diseased patients themselves, should be of benefit. In this respect PLX-PAD cells from young healthy placental tissue have the potential for higher efficacy than previously seen with autologous cell products.

PLX-PAD: allogeneic cell therapy

PLX-PAD is a cell therapy product, composed of placental expanded adherent stromal cells. While PLX-PAD cells exhibit membrane marker expression typical of classical mesenchymal stromal cells,[20] they have minimal ability to differentiate *in vitro* into cells of mesodermal lineage. Therefore, their proposed mechanism of action is a timely secretion of various proteins which induce angiogenesis, immunomodulatory activities, and promotion of regeneration of muscle tissue.

Angiogenesis, the formation of new vessels, is induced by a variety of factors released from ischaemic tissues, and is a critical physiological mechanism for alleviation of PAD or for recovery of muscle tissue functionality after injury. The angiogenic process involves migration of endothelial progenitors and pericytes towards the site of interest. *In vitro* studies have shown the capacity of PLX-PAD cells to promote endothelial cell proliferation.[20] The cells secrete pro-angiogenic proteins including VEGF, angiopoietin-1,

osteopontin, MMP-1, MMP-2, HGF, and angiogenin, all of which are upregulated under hypoxic culture conditions[20,21], and unpublished data. Angiogenin further interacts with endothelial and smooth muscle cells, resulting in cell migration, invasion, proliferation, and formation of tubular structures[22] (Fig. 1, Table 1).

PAD is associated with an inflammatory process that leads to tissue damage and precludes active repair. Oxidative stress, because of endothelial dysfunction, is evident in PAD and leads to persistent inflammation. Pro-inflammatory cytokines, for example TNF-α, IL-6, and IL-1β, play a key role in the inflammatory process, and PLX-PAD cells mitigate this process by releasing anti-inflammatory and immunomodulating cytokines (i.e. GDF-15, CXCL12, TGF-β). Following exposure to pro-inflammatory cytokines (such as TNF-α and IFN-γ), PLX-PAD cells further upregulate some of the anti-inflammatory secretions (i.e. IDO, PD-L1, HGF, IL-11, CCL5). Furthermore, when cultured with activated PBMCs, PLX-PAD induce upregulation of PBMC secreted anti-inflammatory cytokines such as IL-10 and IL-1RA, also indirectly affecting endothelial dysfunction and protecting endothelial cell viability[20] and unpublished data.

As ischaemic conditions lead to muscle degeneration, muscle regeneration is of potential therapeutic benefit in PAD. PLX-PAD cells have been shown to promote migration of skeletal muscle cells *in vitro* and improve muscle function and accelerate muscle regeneration *in vivo* (manuscript in preparation).

To summarise, PLX-PAD cells secrete proteins that are known to be involved in promoting angiogenesis, down-regulating inflammation, and inducing regeneration of muscle tissue.

In vivo, in the mouse hind limb ischaemia (HLI) model in which the femoral artery of one hindlimb is cut and ligated thus inducing complete ischaemia in the operated limb,[21,23] PLX-PAD cells have been shown to restore blood flow to the ischaemic limb. Furthermore, it has been shown that PLX-PAD cells exert a systemic effect, as injection of the cells to the contralateral limb exerted an almost similar restoration of blood flow, but required a larger dose of cells. A second administered dose of PLX-PAD cells 21 days after the first dose afforded additional efficacy in re-establishing blood flow in case the effect was declining (Fig. 2). This study and others have also shown that PLX-PAD cells injected intramuscularly do not migrate from the injection site to other tissues and do not

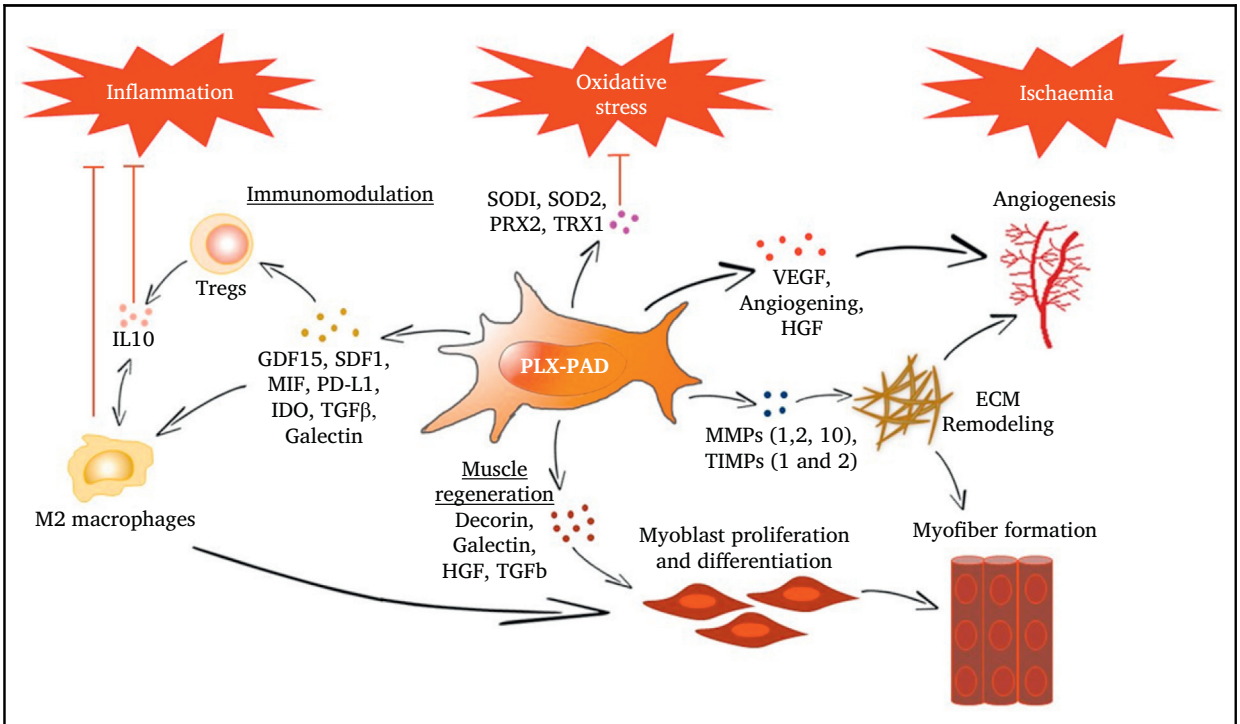

Figure 1. Suggested mechanism of placenta derived adherent stromal cells (PLX-PAD) effect in critical limb ischaemia (CLI), reducing inflammation and oxidative stress, and supporting angiogenesis. PLX-PAD secretions can mitigate CLI pathology by simultaneously affecting several disease associated pathways. PLX-PAD secrete immunomodulatory cytokines which support the induction of M2 macrophages and elevate the level of circulating regulatory T cells, leading to elevation in interleukin (IL)-10 and resolution of inflammation. In addition, PLX-PAD secrete factors which directly support angiogenesis and muscle regeneration. These processes are further supported by the PLX-PAD secretion of enzymes with antioxidant activity, which can protect blood vessels from oxidative damage, and the secretion of extracellular matrix (ECM) remodelling enzymes, which enable regeneration. VEGF = vascular endothelial growth factor; FGF = fibroblast growth factor; HGF = hepatocyte growth factor; TGFb = transforming growth factor b; MIF = macrophage migration inhibition factor; IDO = indoleamine 2, 3 - dioxygenase; PD-L1 = programmed death ligand 1.

Table 1. Cytokines secreted by PLX-PAD and their function

Angiogenesis	VEGF (vascular endothelial growth factor)
	Angiogenin
	Angiopoietin 1
	HGF (hepatocyte growth factor)
	Osteopontin
	MMP-1
	MMP-2
Immunomodulation	Osteopontin
	CXCL12/SDF 1 (stromal cell derived factor 1)
	GDF 15 (growth differentiation factor 15)
	MIF (macrophage migration inhibition factor)
	IDO (indoleamine 2,3-dioxygenase)
	TGF-β (transforming growth factor β)
	PD-L1 (programmed death ligand 1)
	HGF
	IL-11 (interleukin 11)
	CCL5 (RANTES, regulated on activation, normal T cell expressed and secreted)
Muscle regeneration	Decorin
	MMP 1
	HGF
	TGF β
	Galectin 1
	IGFBP-3 (insulin growth factor binding protein 3)
	FLRG (FSTL3, follistatin related protein 3)
	Osteopontin
	CXCL12/SDF 1

FSTL3 = follistatin related protein 3; GDF = growth differentiation factor; HGF = hepatocyte growth factor; IDO = indoleamine 2,3-dioxygenase; IGFBP-3 = insulin growth factor binding protein 3; IL-11 = interleukin 11; MIF = macrophage migration inhibition factor; MMP = matrix metalloproteinase; PD-L1 = programmed death ligand 1; PLX-PAD = placenta derived adherent stromal cells; RANTES = regulated on activation, normal T cell expressed and secreted; SDF one = stromal cell-derived factor 1; TGF-β = transforming growth factor β; VEGF = vascular endothelial growth factor.

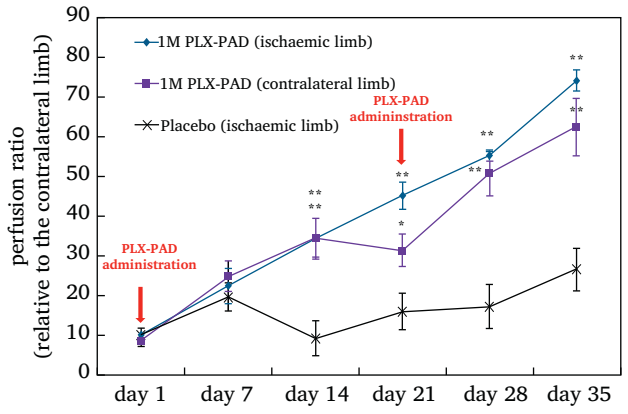

Figure 2. Placenta derived adherent stromal (PLX-PAD) cells are effective in re-establishing blood flow in the hind limb ischaemia (HLI) mouse model. Intramuscular (IM) administrations to the ischaemic or contralateral limb, were effective in rescuing blood flow to the ischaemic limb compared with placebo control. PLX-PAD were administered one and 21 days (depicted by arrows on graph) following induction of HLI. $N = 10$ for each PLX-PAD treated group and $n = 5$ for placebo group. F (39,70) = 30.82, $p < .0001$. Blood flow is measured as perfusion ratio relative to the contralateral limb. *$p < .05$; ***$p < .0001$, compared with placebo control.

differentiate in culture, further supporting the suggested mode of action of PLX-PAD cells through secretion of proteins.

METHODS

Clinical studies in PAD

Two phase I open label, dose escalation studies were conducted to assess the safety of intramuscular injections of PLX-PAD cells in 27 CLI subjects (Rutherford Categories 4 and 5), who were not candidates for revascularisation. A phase II study in patients with intermittent claudication has recently been performed (to be published).

Study 1202-1 was conducted in Germany and assessed three single doses of 175 million cells (low dose, $n = 3$), 315 million cells (intermediate dose, $n = 6$), and 595 million cells (high dose, $n = 6$). Study 1202-2 was conducted in the United States (US) and assessed a single vs. two doses (2 weeks apart) of 280 million cells; the first group included seven patients, the second group included five patients. PLX-PAD cells were administered intramuscularly into the affected leg via 30—50 injections.

Overall, the safety of this process in CLI subjects was found to be acceptable, and it was confirmed that HLA matching is not required. Adverse events included mostly injection site reactions such as pain, muscle contractions/fasciculations, pruritus, haematoma, etc. (mostly transient and of mild/moderate intensity), transient allergic reactions, and bad breath because of the DMSO (dimethyl sulfoxide) content.

These phase I studies were not powered to demonstrate clinical efficacy; however, some parameters have indicated a positive clinical effect. The pooled amputation free survival rate at six months and one year across the two studies was 96% and 85%, respectively, which is higher than the rates described in similar patient populations.[24,25] Pain scores, as assessed by the Visual Analog Scale (VAS), showed a decreasing trend after treatment with PLX-PAD in all dose groups, up to a decrease of 2.5 units in the patients treated at the dose of 315 million cells. $TcPO_2$, which is considered an indicator of tissue perfusion, demonstrated an increasing trend over time in all study groups with the greatest increase of up to 15 mmHg in the repeated dose group (Fig. 3) (data on file).

In summary, based on the pro-angiogenic, immunomodulatory, and muscle regeneration capacities of PLX-PAD, as well as the results from animal experiments and outcome of the clinical studies in PAD patients, a phase III trial was designed.

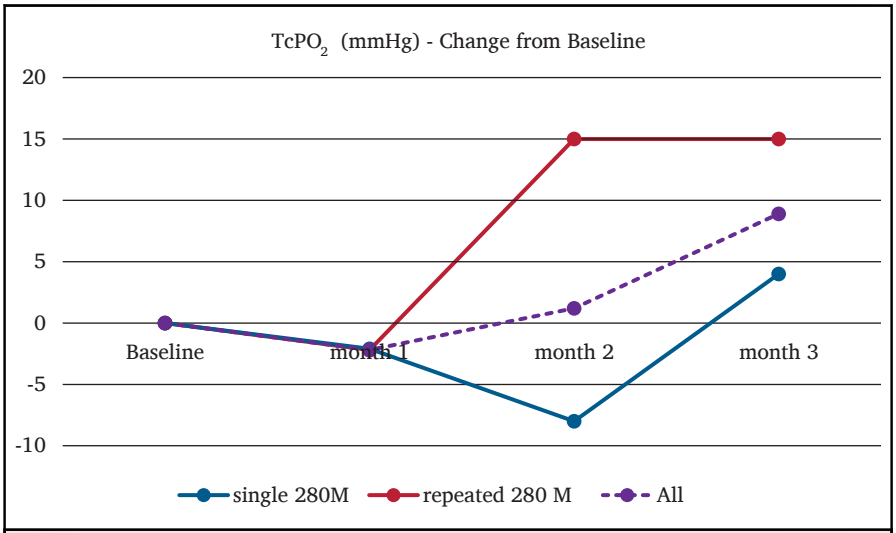

Figure 3. Change of $TcPO_2$ in study no. 1202-2. M = million cells; TcPO = transcutaneousc tissue oxygen pressure.

PACE trial design

The PACE study (a randomised, double blind, multicentre, placebo controlled, parallel group phase III study to evaluate the efficacy, tolerability, and safety of intramuscular injections of PLX-PAD for the treatment of subjects with CLI with minor tissue loss who are unsuitable for revascularisation) was designed to investigate time to major amputation or death (AFS) up to 36 months. It is planned that the study will enroll a total of 246 patients with minor foot lesions (Rutherford Category 5) up to ankle level. Patients should be unsuitable for revascularisation or carry an unfavourable risk benefit to revascularisation. Ineligibility for revascularisation is determined by severe comorbidity, anatomical, or technical challenges (e.g. lack of vein for a bypass or inadequate target vessels for an endovascular procedure) or failed revascularisation procedures with

Table 2. Main inclusion and exclusion criteria

Main inclusion criteria:
• Age 45–99 years
• CLI caused by atherosclerosis with minor tissue loss (Rutherford 5) up to the ankle level
• Ankle pressure $\leq$ 70 mmHg or toe pressure $\leq$ 50 mmHg
• Subject unsuitable for revascularisation (by any method) in the index leg, based on unfavourable risk benefit assessment
• Ischaemic lesions neither healing, nor significantly worsening (within two weeks of screening)
• Ischaemic lesions without tendon or bone exposure (unless secondary to a minor amputation)
Main exclusion criteria:
• Non-atherosclerotic PAD (e.g. Buerger's disease)
• CLI with major tissue loss (Rutherford 6) in either leg
• Evidence of active infection (e.g., cellulitis, osteomyelitis)
• Subject having undergone surgical revascularisation < 1 month prior to study, or endovascular revascularisation/minor amputation < 2 weeks prior to study
• Planned or potential need for major/minor amputation or revascularisation within one month of study entry
• Aorto-iliac stenosis or common femoral artery stenosis ≥70%
• Use of hyperbaric oxygen therapy, prostanoids, spinal cord stimulation, lumbar sympathectomy, wound dressing containing cells or growth factors, or topical platelet derived growth factor
• Stroke or acute myocardial infarction/unstable angina within three months before screening
• Severe congestive heart failure symptoms (New York Heart Association [NYHA] Stage IV)
• Uncontrolled severe hypertension
• Diabetes mellitus with HbA1c > 10%
• Subject on renal replacement therapy or with eGFR < 15 mL/min/1.73m^2
• Pulmonary disease requiring supplemental oxygen treatment on a daily basis
• Active malignancy or history of malignancy within five years prior to study entry

CLI = critical limb ischaemia; eGFR = estimated glomerular filtration rate; NYHA = New York Heart Association; PAD = peripheral arterial disease.

persistence of CLI after the procedure. Only patients with atherosclerotic disease are included, while those with thrombangitis obliterans (Buerger's disease) are excluded. Table 2 shows the main inclusion and exclusion criteria.

Subjects are screened up to five weeks before randomisation. If found eligible, patients are randomised in a ratio of 2:1 to treatment with PLX-PAD 300 $\times$ 10^6 cells or placebo. Treatment is administered at two time points, eight weeks apart. On each occasion, 30 intramuscular injections, 0.5 mL each, are administered in the index leg along its length, anteriorly and posteriorly, according to a standard injection site scheme. A strict procedure is applied for cell preparation and administration to maintain study blinding. Dosage and timing of injections are based on preclinical and accumulated clinical data.

Each subject will be followed up for at least 12 months post randomisation or until the 12 months visit of the last patient randomised. Maximum follow up allowed by protocol is 36 months post randomisation, hence all subjects will be followed up for 12—36 months. The study design is presented in Fig. 4.

The primary efficacy endpoint of the study is time to occurrence of major amputation or death, that is amputation free survival up to 36 months after randomisation. Safety and tolerability are to be evaluated as well as other secondary and exploratory endpoints (Table 3). The study will also assess a potential economic benefit of this regenerative treatment approach by applying a health economic evaluation, taking into account relevant parameters as days of hospitalisation and patient reported quality of life.

The study will be performed in 50 sites in Europe and the USA.

Table 3. Endpoints

Primary efficacy endpoint:
- Time to occurrence of major amputation or death (amputation free survival)

Main secondary and exploratory endpoints:
- Time to first occurrence of any of the following single events:
  - Major amputation of the index leg
  - Revascularisation caused by worsening of CLI in the index leg
  - Doubling of total ulcer area from baseline in the index leg
  - De novo necrosis in the index leg
  - All cause mortality
- Time to major amputation of the index leg
- Complete healing of all ischaemic lesions at 12 months
- Change from baseline in ischaemic pain (numerical rating scale [NRS]) at six months
- Time to death or major amputation or adjudicated major amputation of the index leg
- Time to all cause death
- Decrease of 50% or more in total ulcer area at six months
- Complete healing of all ischaemic lesions in the contralateral leg
- Time to occurrence of major amputation of the contralateral leg
- Change in health and disease related Quality of Life at 12 months
- Changes in TcPO2, ankle brachial index (ABI), toe brachial index (TBI)
- Revascularisation procedure in the index leg within 12 months from treatment
- Hospitalisation days
- Change from baseline in plasma cytokine levels after PLX-PAD administration
- Change from baseline in mRNA expression profile after PLX-PAD administration

ABI = ankle brachial index; CLI = critical limb ischaemia; NRS = numerical rating scale; PLX-PAD = placenta derived adherent stromal cells; TBI = toe brachial index; TcPO = transcutaneous tissue oxygen pressure.

Statistical considerations

The sample size of 246 subjects provides a power of 89.7%, and is based on the 2:1 ratio randomisation to treatment, an estimated AFS of 65% in the placebo group at the end of the first year, and a risk reduction of approximately 50% for the PLX-PAD group during the first year, using the log rank test. The primary endpoint will be analysed using the Cox Proportional Hazards model. The study randomisation is stratified for the presence of diabetes mellitus, for the extent of ischaemic lesions, and for geographical region, which will be covariables in the statistical model.

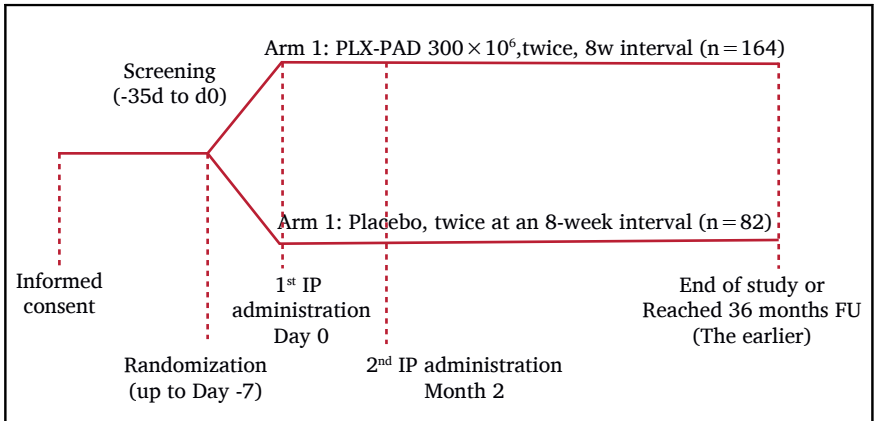

Figure 4. Study design, timing of injections, and follow up. PLX-PAD = placenta derived adherent stromal cells; FU = follow up; d = day.

DISCUSSION

Although CLI affects a small proportion of patients with PAD, and an increasing number of them are offered revascularisation,[26] other treatments are required for some patients to possibly increase survival and reduce major amputations. The fact that trials have had problems with slow recruitment of no option patients, for example the TAMARIS trial[5] and the AGILITY HGF trial, which had to be cancelled for that reason,[10] might be interpreted in a way that few patients do require alternative treatments. However, in addition to no option cases, revascularisations may fail or only partly reduce CLI symptoms, and poor option subjects for revascularisation because of comorbidity or for technical reasons will still be a reality. In a recent paper, Martinez et al.[27] discussed predictive factors of poor short-term outcome (mortality and major amputation) following revascularisation, including age, low haemoglobin, acute myocardial infarction, ischaemic ulcers, and infrapopliteal revascularisation. For such groups of fragile CLI patients, therapeutic angiogenesis may be an alternative.

As larger gene therapy trials have failed, although there is still an interest in the evaluation of HGF,[9] and doubts exist with regard to cell therapy,[14,15] no such treatment has yet been approved for clinical use. It could be interpreted that single growth factor trials may not be able to provide the complete array of factors that the patients in this population require. Therefore, precursor cell therapy would potentially provide a more complete array of factors. It is reasonable to assume that the age and condition of cells, harvested from the potential patients, are crucial. It has been shown that CLI patients produce lower levels of progenitor cells,[17] and an increasing cardiovascular risk is also related to a lower number of progenitor cells.[16] In addition, cells harvested and injected at the point of service, are not by their nature able to be characterised or quantified before being injected, therefore bringing into question their very nature. Furthermore, it has been shown that growth of isolated mesenchymal stem cells is significantly related to the age of the donor,[28] and thus young allogeneic placental cells may be most relevant for the purpose of treatment as they come from a young healthy donor.

Most importantly, PLX-PAD cells, being of a placental source, known for their immune privileged characteristics, have been shown not to exert an immunological effect, neither *in vitro* nor *in vivo* in animal models and humans, requiring no immunosuppression prior to PLX-PAD administration.[29]

The PACE trial includes only patients with ischaemic lesions and does not enroll Rutherford 4 cases with rest pain alone, because of the lack of objectivity of evaluating pain. In practice, CLI patients with rest pain may also be those who are most often offered revascularisation. Hence, pain is not included in the composite primary efficacy endpoint in Rutherford 5 patients. Furthermore, these patients are at higher risk of major amputation, thus providing the best evidence on the effect on AFS.

The trial design takes into account the greater efficacy of two cell administrations rather than one as shown in both animal models and human subjects, and therefore a second administration session is given two months after the first. Some patients will be followed up to 36 months, which will enable collection of highly important information on long-term effects of the treatment, and will also increase knowledge on the natural course of severe CLI. The primary efficacy endpoint, amputation free survival is selected as the strictest endpoint to be evaluated. Disease progression, wound healing, ischaemic pain, quality of life, $TcPO_2$, ABI/TBI measures, and hospitalisation day data are included as secondary and exploratory endpoints.

The term "therapeutic angiogenesis" may be interpreted as the mode of action by which new vessels are formed, thus potentially increasing perfusion. In human studies, however, present imaging technology is only occasionally able to show newly developed vessels despite the fact that subjects may be improved. It is evident that other pathophysiological events are affected as well, primarily the inflammatory process. PLX-PAD cells exert effects not only on both angiogenesis and tissue inflammation, but also on regeneration of muscle cells. Whether the latter is a mechanism of value for improvement of function and symptoms in CLI patients should be investigated further.

In summary, cell therapy works in a multifactorial way, PLX-PAD cells are young and potent, they secrete relevant factors, are easily accessible in the required quantity without requiring harvesting from fragile patients putting them at additional risk, and have shown preclinical and initial clinical evidence of efficacy. The design of the PACE trial, including only patients with ischaemic foot lesions, dual injections along the whole limb, follow up to 36 months, and with a primary efficacy endpoint based on long-term time to event regarding amputation free survival may allow for better understanding of perfusion enhancement and change of inflammatory response and improved outcome for patients with severe critical limb ischaemia.

CONFLICT OF INTEREST

L Norgren, N Weiss, S Nikol, RJ Hinchliffe, JC Lantis, MR Patel, and H Reinecke are members of the Steering Committee for the PACE trial. R Ofir, Y Rosen, D Peres, and Z Aberman are employees of Pluristem Ltd. L Norgren: Consultations, advisory boards and/or research grants: AnGes, AstraZeneca, Bayer, CESCA, Mitsubishi, Pluristem. N Weiss: Consultations, advisory boards and/or research grants: Amgen, Bard, Bayer, Fresenius, Merck, Pfizer, Pluristem, Terumo. S Nikol: Consultations: Pluristem. RJ Hinchliffe: Nothing to declare. JC Lantis: Consultations: Pluristem. MR Patel: Advisory boards: Bayer, Jansen. Research grants: Pluristem, Bayer, Jansen, AstraZenca. H Reinecke: Consultations: BMS, MedUpdate, NephroUpdate, Pfizer, Pluristem. Research grants: German Federal Ministry for Education and Research, Bard, Bayer, Biotronic.

FUNDING

The PACE trial has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No: 733006.

REFERENCES

1 Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. Inter-society consensus for the management of peripheral arterial disease (TASC II). *Eur J Vasc Endovasc Surg* 2007;**33**:S1–75.

2 Fowkes FG, Rudan D, Rudan I, Aboyans V, Denenberg JO, McDermott MM, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. *Lancet* 2013;**382**:1329–40.

3 Gerhard-Herman MD, Gornik HL, Barrett C, Barshes NR, Corriere MA, Drachman DE, et al. 2016 AHA/ACC guideline on the management of patients with lower extremity peripheral artery disease: a report of the American college of cardiology/American Heart association task force on clinical practice guidelines. *Circulation* 2017;**135**:e726–79.

4 Aboyans V, Ricco J-B, Bartelink EL, Bjorck M, Brodmann M, Cohnert T, et al. 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg* 2017;**55**:305–68.

5 Belch J, Hiatt WR, Baumgarnter I, Driver IV, Nikol S, Norgren L, et al. Effect of fibroblast growth factor NV1FGF on amputation and death: a randomized placebo-controlled trial of gene therapy in critical limb ischemia. *Lancet* 2011;**377**:1929–37.

6 Nikol S, Baumgartner I, Van Belle E, Diehm C, Visona A, Capogrossi MC, et al. Therapeutic angiogenesis with intramuscular NV1FGF improves amputation-free survival in patients with critrical limb ischemia. *Mol Ther* 2008;**16**:972–8.

7 Shigematsu H, Yasuda K, Iwai T, Sasajima T, Ishimaru S, Ohashi Y, et al. Randomized, double-blind, placebo-controlled clinical trial of hepatocyte growth factor plasmid for critical limb ischemia. *Gene Ther* 2010;**17**:1152–61.

8 Powell RJ, Goodney P, Mendelsohn FO, Moen EK, Annex BH, HGF-205 Trial Investigators. Safety and efficacy of patient specific intramuscular injection of HGF plasmid gene therapy on limb perfusion and wound healing in patients with ischemic lowere extremity ulceration: results of the HGF-0205 trial. *J Vasc Surg* 2010;**52**:1525–30.

9 Sanada F, Taniyama Y, Muratsu J, Otsu R, Shimizu H, Rakugi H, et al. Gene-therapeutic strategies targeting angiogenesis in peripheral artery disease. *Medicines (Basel)* 2018;**30**:5.

10 Iver SR, Annex BH. Therapeutic angiogenesis for peripheral artery disease. *JACC Basic Trans Sci* 2017;**2**:503–12.

11 Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al. Theraputic angiogenesis for patients with critical limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002;**360**:427–35.

12 Teraa M, Sprengers RW, Schutgens RE, Slaper-Cortenbach I, van der Graaf Y, Algra A, et al. Effect of repetetive intra-arterial infusion of bone marrow mononuclear cells in patients with no-option limb ischemia. The randomized, double-blind, placebo-controlled rejuvenating endothelial progenitor cells via transcutaneous intra-arterial supplementation. *Circulation* 2015;**131**:851–60.

13 Teraa M, Sprengers RW, van der Graaf Y, Peters CE, Moll FL, Verhaar MC. Autologous bone marrow derived cell therapy in patients with critical limb ischemia: a meta-analysis of randomized controlled clinical trials. *Ann Surg* 2013;**258**:922–9.

14 Peeters Weem SM, Teraa M, De Borst GJ, Verhaar MC, Moll FL. Bone marrow derived cell therapy in critical limb ischemia: a meta-analysis of randomized placebo controlled trials. *Eur J Vasc Endovasc Surg* 2015;**50**:775–83.

15 Pan T, Wei Z, Fang Y, Dong Z, Fu W. Therapeutic efficacy of CD34+ cell-involved mononuclear cell therapy for no-option critical limb ischemia: a meta-analysis of randomized controlled clinical trials. *Vasc Med* 2018;**23**:219–31.

16 Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al. Circulating endothelial progenitor cells, vascular function and cardiovascular risk. *New Eng J Med* 2003;**348**:593–600.

17 Teraa M, Sprengers RW, Westerweel PE, Gremmels H, Goumans MJ, Teerlink T, et al. Bone marrow alteration and lower endothelial progenitor cell numbers in critical limb ischemia patients. *PLoS One* 2013;**8**:e55592. Epub 2013.

18 Jonsson TB, Larzon T, Arfvidsson B, Tidefeldt U, Axelsson CG, Jurstrand M, et al. Adverse events during treatment of critical limb ischemia with autologous peripheral blood mononuclear cell implant. *Int Angiol* 2012;**31**:77–84.

19 Zhang J, Huang X, Wang H, Liu X, Zhang T, Wang Y, et al. The challenges and promises of allogeneic mesenchymal stem cells for use as a cell-based therapy. *Stem Cell Res Ther* 2015;**6**:234.

20 Roy R, Brodarac A, Kukucka M, Kurtz A, Becher PM, Jülke K, et al. Cardioprotection by placenta-derived stromal cells in a murine myocardial infarction model. *J Surg Res* 2013;**185**:70–83.

21 Zahavi-Goldstein E, Blumenfeld M, Fuchs-Telem D, Pinzur L, Rubin S, Aberman Z, et al. Placenta-derived PLX-PAD mesenchymal-like stromal cells are efficacious in rescuing blood flow in hind limb ischemia mouse model by a dose- and site-dependent mechanism of action. *Cytotherapy* 2017;**19**:1438–46.

22 Gao X, Xu Z. Mechanisms of action of angiogenin. *Acta Biochim Biophys Sin(shanghai)* 2008;**40**:619–24.

23 Prather WR, Toren A, Meiron M, Ofir R, Tschope C, Horwitz EM. The role of placenta derived adherent stromal cell (PLX-PAD) in the treatment of critical limb ischemia. *Cytotherapy* 2009;**11**:427–34.

24 Van Belle E, Nikol S, Norgren L, Baumgartner I, Driver V, Hiatt WR, et al. Insights on the role of diabetes and geographic variation in patients with critical limb ischaemia. *Eur J Vasc Endovasc Surg* 2011;**42**:365–73.

25 Reinecke H, Unrath M, Freisinger E, Bunzemeier H, Meyborg M, Lüders F, et al. Peripheral arterial disease and critical limb ischaemia: still poor outcomes and lack of guideline adherence. *Eur Heart J* 2015;**36**:932–8.

26 Hong MS, Beck AW, Nelson PR. Emerging national trends in the management and outcomes of lower extremity peripheral arterial disease. *Ann Vasc Surg* 2011;**25**:44–54.

27 Martinez M, Sosa C, Velescu A, Llort C, Elosua R, Clara A. Predicitve factors of a poor outcome following revascularization for critical limb ischemia: implications for practice. *Int Angiol* 2018;**37**:370–6.

28 Beane OS, Fonseca VC, Cooper LL, Koren G, Darling EM. Impact of aging on the regenerative properties of bone marrow-, muscle-, and adipose-derived mesenchymal stem/stromal cells. *PLoS One* 2014;**9**:e115963.

29 Consentius C, Akyüz L, Schmidt-Lucke JA, Tschöpe C, Pinzur L, Ofir R, et al. Mesenchymal stromal cells prevent allostimulation in vivo and control checkpoints of Th1 priming: migration of human DC to lymph nodes and NK cell activation. *Stem Cells* 2015;**33**:3087–99.