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PLX-PAD Cell Treatment of Critical Limb Ischemia – Rationale and Design of the PACE trial

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48 ***What this paper adds*:**

49 Placebo controlled trials of cell therapy to reduce major amputations in patients with critical

50 limb ischemia and no-option for revascularization have so far not been successful. PLX-PAD

51 cell treatment (placenta derived adherent stromal cells) has in small studies shown

52 promising results, and the phase III PACE trial is designed to evaluate the efficacy and safety

53 of two sessions of intramuscular injections, 8 weeks apart in follow up of 12-36 months.

54 Thus, the study will provide long term outcome and will collect parameters to assess

55 potential ecomomic benefit for this kind of treatment.

56

57 **Abstract**

58 *Background*: Critical limb ischemia (CLI) is a life threatening condition with a considerable

59 risk for death and major amputation. Besides revascularization, no treatment has been

60 proven to reduce the risks. Therapeutic angiogenesis by gene or cell therapy has not

61 demonstrated definitive evidence in randomized controlled trials. PLX-PAD is an 'off-the-

62 shelf' allogeneic placental derived, mesenchymal-like cell therapy that in preclinical studies

63 has shown pro-angiogenic, anti-inflammatory and regenerative properties. Favorable 1-year

64 amputation free survival (AFS), and trends in reduction of pain scores and in increase of

65 tissue perfusion have been shown in two small, open-label, phase I trials.

66

67 *Study design:* The PACE study is a phase III randomized, double-blind, multicenter,

68 multinational placebo-controlled, parallel-group study to evaluate the efficacy, tolerability

69 and safety of intramuscular injections of PLX-PAD cells to treat patients with atherosclerotic

70 CLI with minor tissue loss (Rutherford Category 5) up to the ankle level, who are unsuitable

71 for revascularization or carry an unfavorable risk-benefit for that treatment. The study will

72   enroll 246 patients, who after screening are randomized in a ratio of 2:1 to treatment with

73   intramuscular injections of PLX-PAD $300X10^6$ cells or placebo at two occasions, 8 weeks

74   apart. The primary efficacy endpoint is time to major amputation or death (amputation free

75   survival), which will be assessed in follow-up of after at least 12 months and up to 36

76   months.

77

78   *Conclusions:* Based on favorable pre-clinical and initial clinical study results, the PACE phase

79   III randomized controlled trial will evaluate placenta-derived PLX-PAD cell treatment in

80   patients with critical limb ischemia, carrying an unfavorable risk-benefit for

81   revascularization.

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Introduction

Critical limb ischemia (CLI) constitutes the most advanced stage of chronic peripheral arterial disease (PAD) and includes rest pain and ischemic foot lesions. The condition affects 1-5 % of all PAD patients, which corresponds to an incidence of 500-1000 / million population per year (1). Overall, the prevalence of PAD increases worldwide, most remarkably in low- and middle income countries (2). Major amputation and death are the ultimate consequences of CLI, and a 1-year amputation rate of 15-25% is commonly reported, while amputation & mortality rate ranges 30-40%. The single evidence-based recommendation for treatment is revascularization (1,3,4). Due to co-morbidities with greater risk to perform an interventional procedure, or based on anatomical or technical issues, a proportion of CLI patients is not reasonable to revascularize or to re-revascularize after a failed procedure. Few treatments exist for such "poor-option" cases. Prostanoid therapy has been reasonably well studied in randomized controlled trials, but does not carry evident effect, and is not recommended in present guidelines (1,3). Since about 20 years, therapeutic angiogenesis has been studied, based on either gene or cell therapy.

Gene therapy

Gene therapy utilizing 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 randomized placebo controlled trial, TAMARIS (5) that did not show any better outcome regarding survival or major amputation in the treatment group compared to placebo, despite the fact that a former, smaller trial, TALISMAN (6), showed that major amputation, as a secondary

120   endpoint, was significantly less frequent among NV1FGF treated subjects. Injections of the

121   HGF plasmid have yet to prove efficacy with regard to major events, though smaller

122   randomized placebo controlled trials have shown reduced rest pain (7) and increased toe

123   brachial index (TBI) (8) at follow up. More has to be learnt both from basic and clinical

124   research to possibly adopt effective gene therapy for PAD (9), though Iver and Annex (10)

125   discuss a conceivable end of gene therapy trials, based on the lack of an evident break-

126   through.

127

128   **Cell therapy**

129   The potential benefit of cell therapy is that cell secretion is multifactorial and therefore not

130   based solely on a single growth factor. Initiated by a Japanese study (11) comparing bone-

131   marrow- and peripheral blood mononuclear cells (PBMC) injected into the limb muscles of

132   patients with PAD, several cell-based studies have been performed, specifically in CLI

133   patients with no option for revascularization. The Japanese study (11) showed improved

134   ankle-brachial index (ABI) and transcutaneous tissue oxygen pressure ($TcPO_2$) and reduced

135   rest pain in the bone-marrow mononuclear cell treated group. Though the majority of

136   studies have utilized intramuscular injections of the growth factor, the largest trial, Juventas,

137   treated 160 patients with intra-arterial infusions of bone-marrow mononuclear cells (BM-

138   MNC) compared to placebo (12). At 6 months there was no difference in the rate of major

139   amputations.

140   In a meta-analysis by Teraa et al (13), including 12 randomized controlled trials (RCT) in

141   autologous cell therapy for CLI, major amputations were significantly reduced. Most

142   importantly, when only placebo controlled RCTs were included, the major amputations were

143   no longer significantly reduced, indicating the importance of placebo controlled trials in cell

144 therapy. In a later meta-analysis by the same author group (14) only including placebo-

145 controlled RCTs, this outcome was verified. Recently this finding was also verified in another

146 meta-analysis on CD34+ mononuclear cell therapy (CD34+MCT), including 10 trials (15). Total

147 amputations and ulcer healing were reduced in comparison with findings in the placebo

148 treated groups. Major amputation and survival were, however, not significantly reduced.

149 This publication also concluded the beneficial value of a high CD34+ cell content.

150

151 **Autologous or allogeneic cell utilization**

152 From an immunological point of view, autologous cell treatment may theoretically provide

153 an immunological advantage. Nevertheless, it has been shown that cells harvested from

154 older individuals, and in particular those with cardiovascular risk factors or critical limb

155 ischemia, are reduced in number and functionality (16, 17). Furthermore, harvesting

156 autologous cells from bone marrow involves an invasive procedure, while peripheral blood

157 utilization requires granulocyte colony stimulation factor (G-CSF) treatment that potentially

158 may cause harm due to the high white blood cell content that is developed (18). Allogeneic

159 MSCs have been shown to exhibit low immunogenicity (19), thus, utilizing allogeneic

160 younger, more potent cells, rather than treatment with cells harvested from the diseased

161 patients themselves, therefore should be of benefit. In this respect PLX-PAD cells from young

162 healthy placental tissue have the potential for higher efficacy than previously seen with

163 autologous cell products.

164

165 **PLX-PAD: Allogeneic Cell Therapy**

166 PLX-PAD is a cell therapy product, composed of placental expanded adherent stromal cells.

167 While PLX-PAD cells exhibit membrane marker expression typical of classical mesenchymal

168 stromal cells (20), they have a minimal ability to differentiate in vitro into cells of

169 mesodermal lineage. Therefore, their proposed mechanism of action is a timely secretion of

170 various proteins which induce angiogenesis, immunomodulatory activities, and promotion

171 of regeneration of muscle tissue.

172 Angiogenesis, the formation of new vessels, is induced by a variety of factors released from

173 ischemic tissues, and is a critical physiological mechanism for alleviation of PAD or for

174 recovery of muscle tissue functionality after injury. The angiogenic process involves

175 migration of endothelial progenitors and pericytes towards the site of interest. *In vitro*

176 studies have shown the capacity of PLX-PAD cells to promote endothelial cell proliferation

177 (20). The cells secrete pro-angiogenic proteins including VEGF, angiopoietin-1, osteopontin,

178 MMP-1, MMP-2, HGF and angiogenin, all of which are up-regulated under hypoxic culture

179 conditions (20, 21 and unpublished data). Angiogenin further interacts with endothelial and

180 smooth muscle cells, resulting in cell migration, invasion, proliferation and formation of

181 tubular structures (22). (Fig 1, Table 1).

182 PAD is associated with an inflammatory process that leads to tissue damage and precludes

183 active repair. Oxidative stress due to endothelial dysfunction is evident in PAD and leads to

184 persistent inflammation. Proinflammatory cytokines, e.g. TNF-α, IL-6 , IL-1β , play a key role

185 in the inflammatory process, and PLX-PAD cells mitigate this process by releasing anti-

186 inflammatory and immunomodulating cytokines (i.e. GDF-15, CXCL12,TGF-β). Following

187 exposure to pro-inflammatory cytokines (such as TNF-α and IFN-γ) PLX-PAD cells further

188 upregulate some of the anti-inflammatory secretions (i.e. IDO, PD-L1, HGF, IL-11, CCL5).

189 Furthermore, when cultured with activated PBMCs, PLX-PAD induce upregulation of PBMC

190 secreted anti-inflammatory cytokines such as IL-10, and IL-1RA), also indirectly affecting

191 endothelial dysfunction and protecting endothelial cell viability (20 and unpublished data).

192 As ischemic conditions lead to muscle degeneration, muscle regeneration is of potential

193 therapeutic benefit in PAD. PLX-PAD cells have been shown to promote migration of skeletal

194 muscle cells *in vitro* and improve muscle function and accelerate muscle regeneration in vivo

195 (manuscript in preparation).

196 To summarize, PLX-PAD cells secrete proteins that are known to be involved in promoting

197 angiogenesis, downregulating inflammation and inducing regeneration of muscle tissue.

198

199 *In vivo*, in the mouse hind limb ischemia (HLI) model in which the femoral artery of one

200 hindlimb is cut and ligated thus inducing complete ischemia in the operated limb, (21, 23),

201 PLX-PAD cells have been shown to restore blood flow to the ischemic limb . Furthermore, it

202 was shown that PLX-PAD cells exert a systemic effect, since injection of the cells to the

203 contralateral limb exerted an almost similar restoration of blood flow, but required a larger

204 dose of cells. A second administered dose of PLX-PAD cells 21 days after the first dose

205 afforded additional efficacy in re-establishing blood flow in case the effect was declining (Fig

206 2). This study and others have also shown that PLX-PAD cells injected intramuscularly do

207 not migrate from the injection site to other tissues and do not differentiate in culture,

208 further supporting the suggested mode of action of PLX-PAD cells through secretion of

209 proteins.

210

211 **Clinical studies in PAD**

212 Two phase I open-label, dose escalation studies were conducted to assess the safety of

213 intramuscular injections of PLX-PAD cells in 27 CLI subjects (Rutherford Categories 4 and 5),

214 who were not candidates for revascularization.

215 Study 1202-1 was conducted in Germany and assessed three single doses of 175 million cells

216 (low dose, n=3), 315 million cells (intermediate dose, n=6) and 595 million cells (high dose,

217 n=6). Study 1202-2 was conducted in the United States (US) and assessed a single versus 2

218 doses (2 weeks apart) of 280 million cells, the first group included 7 patients, the latter

219 included 5 patients. PLX-PAD cells were administered intramuscularly into the affected leg

220 via 30 to 50 injections.

221 Overall, the safety of this process in CLI subjects was found to be acceptable, and it was

222 confirmed that HLA–matching is not required. Adverse events included mostly injection-sites

223 reactions such as pain, muscle contractions/fasciculations, pruritus, hematoma, etc. (mostly

224 transient and of mild/moderate intensity), transient allergic reactions, and bad breath due to

225 the DMSO (dimethyl sulfoxide) content.

226 These phase I studies were not powered to demonstrate clinical efficacy, however, some

227 parameters have indicated a positive clinical effect. The pooled amputation free survival rate

228 at 6 months and 1 year across the two studies was 96% and 85% respectively, which is

229 higher than the rates described in similar patient populations (24, 25). Pain scores, as

230 assessed by the Visual Analog Scale (VAS), showed a trend of decrease after treatment with

231 PLX-PAD in all dose groups, up to a decrease of 2.5 units in the patients treated at the dose

232 of 315 million cells. $TcPO_2$, which is considered an indicator of tissue perfusion,

233 demonstrated a trend of increase over time in all study groups with the greatest increase of

234 up to 15 mmHg in the repeated-dose group (Fig 3). (data on file)

235 In summary, based on the pro-angiogenic, immunomodulatory, and muscle regeneration

236 capacities of PLX-PAD, as well as the results from animal experiments and outcome of the

237 clinical studies in PAD patients, a phase III trial was designed.

238

239   *PACE trial design*

240   The PACE study (*A randomized, double-blind, multicenter, placebo-controlled, parallel-group*

241   *Phase III study to evaluate the efficacy, tolerability and safety of intramuscular injections of*

242   *PLX-PAD for the treatment of subjects with critical limb ischemia (CLI) with minor tissue loss*

243   *who are unsuitable for revascularization*) was designed to investigate time to major

244   amputation or death (AFS) after up to 36 months. The study is planned to enroll a total of

245   246 patients with minor foot lesions (Rutherford Category 5) up to the ankle level. Patients

246   should be unsuitable for revascularization or carry an unfavorable risk- benefit to

247   revascularization. Ineligibility for revascularization is determined by either severe co-

248   morbidity, anatomical or technical challenges (e.g. lack of vein for a bypass or inadequate

249   target vessels for an endovascular procedure) or failed revascularization procedures with

250   persistence of CLI after the procedure. Only patients with atherosclerotic disease are

251   included, those with thrombangitis obliterans (Buerger´s disease) are excluded. Table 2

252   shows the main inclusion and exclusion criteria.

253   Subjects are screened up to 5 weeks before randomization. If found eligible, patients are

254   randomized in a ratio of 2:1 to treatment with PLX-PAD $300x10^6$ cells or with placebo.

255   Treatment is administered at two time points, 8 weeks apart. At each occasion, thirty

256   intramuscular injections, 0.5 mL each, are administered in the index leg along its length,

257   anteriorly and posteriorly, according to a standard injection-sites scheme. A strict procedure

258   is applied for cell preparation and administration in order to maintain study blinding. Dosage

259   and timing of injections are based on preclinical and accumulated clinical data.

260   Each subject will be followed-up for at least 12 months post randomization or until the 12

261   months visit of the last patient randomized. Maximal follow up allowed by protocol is 36

262   months post randomization, hence all subjects will be followed-up for 12-36 months. The

263   study design is presented in Fig. 4.

264   The primary efficacy endpoint of the study is time-to occurrence of major amputation or

265   death, i.e. amputation-free survival up to 36 months after randomization. Safety and

266   tolerability are to be evaluated as well as other secondary and exploratory endpoints (Table

267   3). The study will also assess a potential economic benefit of this regenerative treatment

268   approach by applying a health-economic evaluation, taking into account relevant parameters

269   as days of hospitalization and patient reported quality of life.

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

271

272   ***Statistical considerations***

273   The sample size of 246 subjects provides a power of 89.7%, and is based on the 2:1 ratio

274   randomization to treatment, an estimated AFS of 65% in the placebo group at the end of the

275   first year, and a risk reduction of approximately 50% for the PLX-PAD group during the first

276   year, using the the log-rank test. The primary endpoint will be analyzed using the Cox

277   Proportional Hazards model. The study randomization is stratified for the presence of

278   diabetes mellitus, for the extent of ischemic lesions, and for geographical region, which will

279   be covariates in the statistical model.

280

281   ***Discussion***

282   Although critical limb ischemia affects a small proportion of patients with PAD, and an

283   increasing part of them are offered revascularization (26), other treatments are required for

284   some patients in order to possibly increase survival and reduce major amputations. The fact

285   that trials have had problems with slow recruitment of no-option patients, e.g. the TAMARIS

286 trial (5) and the AGILITY HGF trial, that had to be canceled for that reason (10) might be

287 interpreted in a way that few patients do require alternative treatments. However, in

288 addition to no-option cases, revascularizations may fail or only partly reduce CLI symptoms,

289 and poor option subjects for revascularization due to co-morbidity or for technical reasons

290 will still be a reality. In a recent paper, Martinez et al (27) discussed predictive factors of

291 poor short-term outcome (mortality and major amputation) following revascularization,

292 including age, low hemoglobin values, acute myocardial infarction, ischemic ulcers and

293 infrapopliteal revascularization. For such groups of fragile CLI patients, therapeutic

294 angiogenesis may be an alternative.

295 As larger gene therapy trials have failed, although there is still an interest in the evaluation

296 of HGF (9), and doubts exist with regard to cell therapy (14,15), no such treatment has yet

297 been approved for clinical use. It could be interpreted that single growth factor trials may

298 not be able to provide the complete array of factors that the patients in this population

299 require. Therefore, precursor cell therapy would potentially provide a more complete array

300 of factors. It is reasonable to assume that the age and condition of cells, harvested from the

301 potential patients, are crucial. It has been shown that CLI patients produce lower levels of

302 progenitor cells (17) and an increasing cardiovascular risk is also related to a lower number

303 of progenitor cells (16). In addition, cells harvested and injected at the point of service, are

304 not by their nature able to be characterized nor quantified before being injected, therefore

305 bringing into question their very nature. Furthermore, it has been shown that growth of

306 isolated mesenchymal stem cells is significantly related to the age of the donor (28), and

307 thus young allogeneic placental cells may be most relevant for the purpose of treatment as

308 they come from a young healthy donor.

309   Most importantly, PLX-PAD cells, being of a placental source, known for its immune-

310   priviledged characteristics, have been shown to not exert an immunological effect neither in

311   vitro nor in vivo in animal models and humans, requiring no immunesupression prior to PLX-

312   PAD administration (29).

313   The PACE trial only includes patients with ischemic lesions and does not enroll Rutherford

314   Category 4 cases with just rest pain, due to the inobjectivity of evaluating pain. In practice,

315   CLI patients with rest pain may also be those who most frequently will be offered

316   revascularization. Hence, pain is not included in the composite primary efficacy endpoint in

317   Rutherford Category 5 patients. Furthermore, these patients are at higher risk of major

318   amputation, thus providing the best evidence on the effect on AFS.

319   The trial design takes into account the greater efficacy of two cell administrations rather

320   than one as shown in both animal models and human subjects, and therefore a second

321   administration session is given two months after the first session. Some patients will be

322   followed up to 36 months, which will enable collection of highly important information on

323   long-term effects of the treatment. and will also increase knowledge on the natural course

324   of severe CLI . The primary efficacy endpoint, amputation free survival is selected as the

325   strictest endpoint to be evaluated. Disease progression, wound healing, ischemic pain,

326   quality of life, $TcPO_2$, ABI/TBI measures and hospitalization days data are included as

327   secondary and exploratory endpoints.

328   The term therapeutic angiogenesis may be interpreted as the mode of action by which new

329   vessels are formed, thus potentially increasing perfusion. In human studies, however,

330   present imaging technology is only occasionally able to show newly developed vessels

331   despite the fact that subjects may be improved. It is evident that other pathophysiological

332   events are affected as well, primarily the inflammatory process. PLX-PAD cells exert effects

333 on both angiogenesis and tissue inflammation, but also on regeneration of muscle cells.

334 Whether the latter is a mechanism of value for improvement of function and symptoms in

335 CLI patients should be further investigated.

336 In summary, cell therapy works in a multifactorial way, PLX-PAD cells are young and potent,

337 they secrete relevant factors, are easily accessible in required quantity without harvesting

338 from fragile patients putting those at additional risk and have shown pre-clinical and initial

339 clinical evidence of efficacy. The design of the PACE trial, including only patients with

340 ischemic foot lesions, dual injections along the whole limb, follow-up up to 36 months, and

341 with a primary efficacy endpoint based on long term time-to-event regarding amputation-

342 free survival may allow for better understanding of perfusion enhancement and change of

343 inflammatory response and improved outcome for patients with severe critical limb

344 ischemia.

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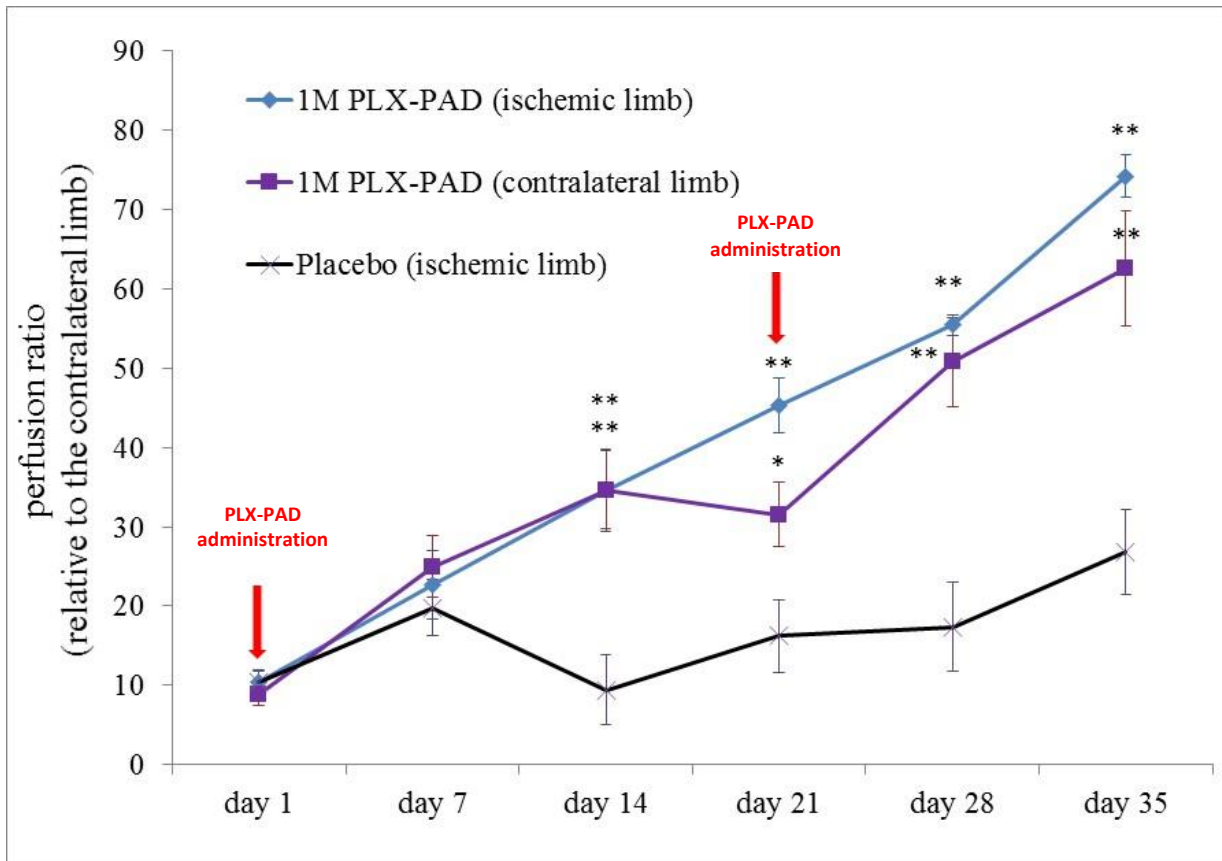472 stem/stromal cells. PLoS One. 2014 Dec 26;9(12):e115963.

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478   Figure 1
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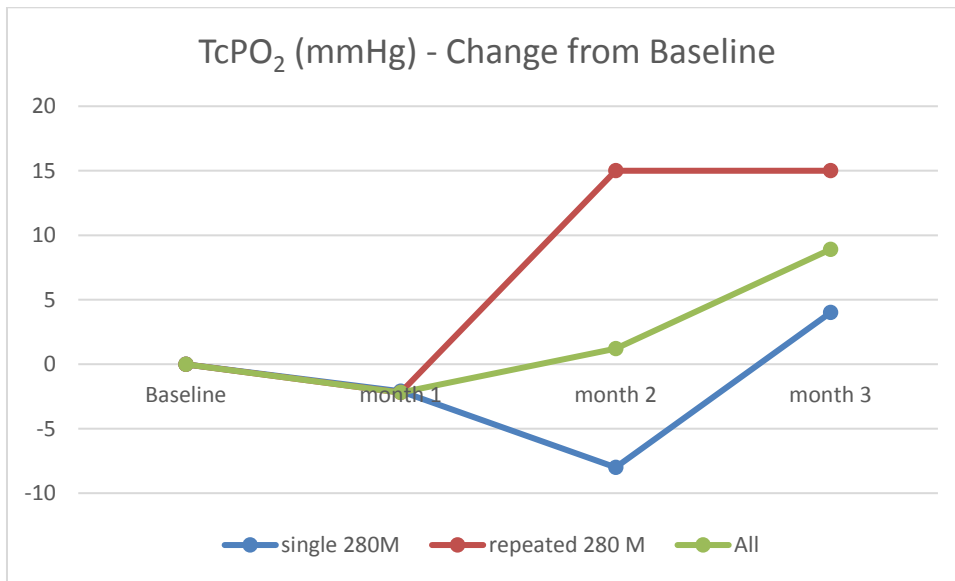480
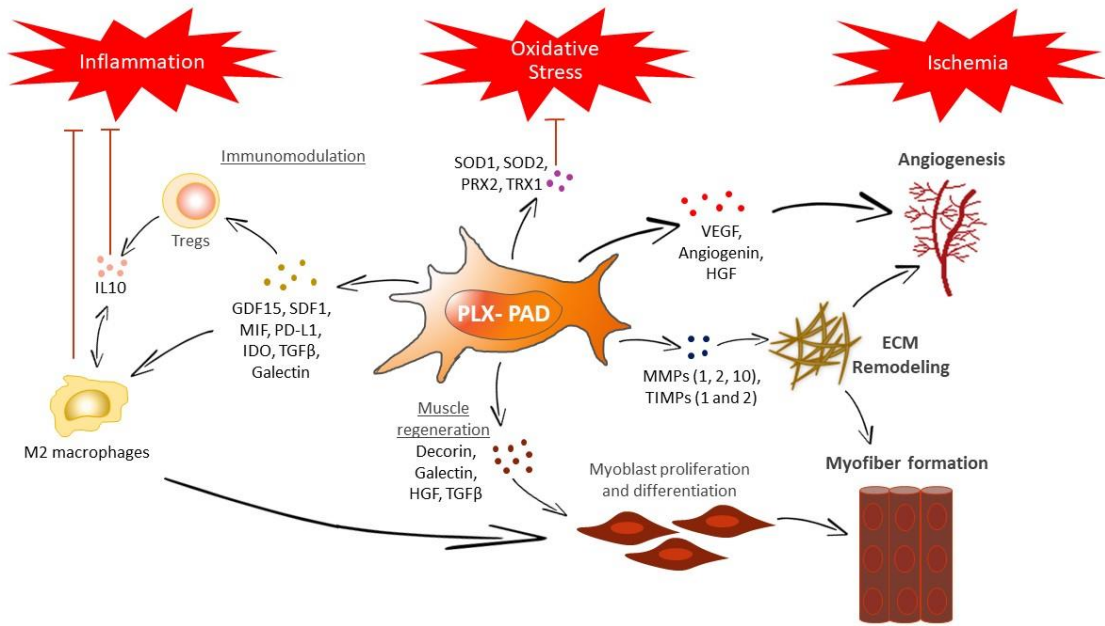
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509 Figure 2
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558   Figure 3
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TcPO$_2$ (mmHg) - Change from Baseline

Legend: single 280M, repeated 280 M, All
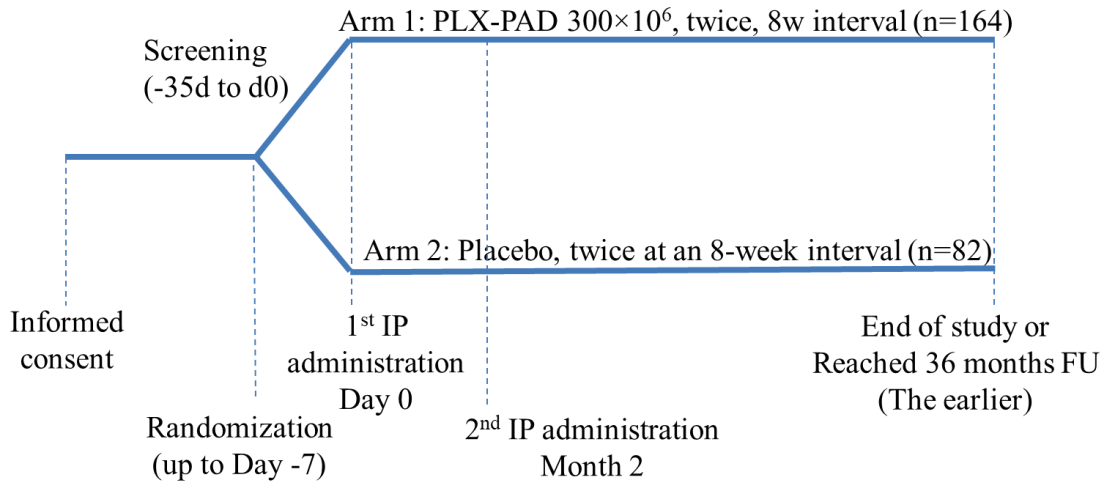
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591   Figure 4
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Screening
(-35d to d0)

Arm 1: PLX-PAD 300×10^6, twice, 8w interval (n=164)

Arm 2: Placebo, twice at an 8-week interval (n=82)

Informed
consent

1st IP
administration
Day 0

End of study or
Reached 36 months FU
(The earlier)

Randomization
(up to Day -7)

2nd IP administration
Month 2

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619	**Table 1**	
620	Cytokines secreted by PLX-PAD and their function	
621		
622	Angiogenesis	VEGF (Vascular Endothelial Growth Factor)
623		Angiogenin
624		Angiopoietin 1
625		HGF (Hepatocyte Growth Factor)
626		Osteopontin
627		MMP-1 (matrix metalloproteinase 1)
628		MMP-2
629	Immunomodulation	Osteopontin
630		CXCL12 /SDF 1 (Stromal Cell-derived Factor 1)
631		GDF 15 (Growth Differentiation Factor 15)
632		MIF (Macrophage Migration Inhibition Factor)
633		IDO (Indoleamine 2,3-dioxygenase)
634		TGF-β (Transforming growth factor beta)
635		PD-L1 (Programmed death ligand 1)
636		HGF
637		IL-11 (Interleukin 11)
638		CCL5 (RANTES- regulated on activation, normal T cell
639		expressed and secreted)
640		
641	Muscle regeneration	Decorin
642		MMP 1
643		HGF
644		TGF β
645		Galectin 1
646		IGFBP-3 (Insulin growth factor binding protein 3)
647		FLRG (FSTL3- Follistatin-related protein 3)
648		Osteopontin
649		CXCL12 /SDF 1
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Table 2

Main inclusion criteria:
- Age 45-99 years.
- CLI due to atherosclerosis with minor tissue loss (Rutherford 5) up to the ankle level.
- Ankle pressure ≤70 mmHg or toe pressure ≤50 mmHg.
- Subject unsuitable for revascularization (by any method) in the index leg, based on unfavorable risk-benefit assessment.
- Ischemic lesions neither healing, nor significantly worsening (within 2 weeks during screening)
- Ischemic 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 Category 6) in either leg.
- Evidence of active infection (e.g., cellulitis, osteomyelitis).
- Subject having undergone surgical revascularization <1 month prior to study, or endovascular revascularization/minor amputation <2 weeks prior.
- Planned or potential need for major/minor amputation or revascularization within 1 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 3 months prior to 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 5 years prior to study entry.

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Table 3

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:
    - o Major amputation of the index leg.
    - o Revascularization due to worsening of CLI in the index leg.
    - o Doubling of total ulcer area from baseline in the index leg.
    - o De novo necrosis in the index leg.
    - o All-cause mortality.
- Time to major amputation of the index leg.
- Complete healing of all ischemic lesions at 12 months.
- Change from baseline in ischemic pain (Numerical rating scale (NRS)) at 6 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 6 months.
- Complete healing of all ischemic 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 $tcPO_2$, ankle-brachial index (ABI), toe-brachial index (TBI).
- Revascularization procedure in the index leg within 12 months from treatment.
- Hospitalization days.
- Change from baseline in plasma cytokine levels after PLX-PAD administration.
- Change from baseline in mRNA expression profile after PLX-PAD administration.

Legends to figures

750

751   **Figure 1**

752

753   Suggested mechanism of PLX-PAD effect in CLI. PLX-PAD secretions can mitigate CLI
754   pathology by simultaneously affecting several disease associated pathways. PLX-PAD secrete
755   immunomodulatory cytokines which support the induction of M2 macrophages and elevate
756   the level of circulating regulatory T cells, leading to elevation in IL-10 and resolution of
757   inflammation. In addition, PLX-PAD secrete factors which directly support angiogenesis and
758   muscle regeneration. These processes are further supported by the PLX-PAD secretion of
759   enzymes with antioxidant activity, which can protect blood vessels from oxidative damage,
760   and the secretion of ECM (extracellular matrix) remodeling enzymes which enable
761   regeneration.

762

763   **Figure** 2

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765   PLX-PAD cells are effective in re-establishing blood flow in the HLI mouse model.
766   Intramuscular (IM) administration to the ischemic or contralateral limb, were effective in
767   rescuing blood flow to the ischemic limb compared to placebo control. PLX-PAD were
768   administered 1 and 21 days (depicted by arrows on graph) following induction of HLI. n=10
769   for each PLX-PAD treated group and n=5 for placebo group. $F_{(39,70)}=30.82$, $p<0.0001$. Blood
770   flow is measured as perfusion ratio relative to the contralateral limb. *$p<0.05$; ***
771     $p < 0.0001$, compared to placebo control.

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773   **Figure 3**

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775   Change of TcPO2 in Study 1202-2. M=million cells

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780   **Figure 4**

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782   Study design, timing of injections and follow up.

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