ORIGINAL ARTICLE

# Safety and efficacy of intramuscular human placenta-derived mesenchymal stromal-like cells (cenplacel [PDA-002]) in patients who have a diabetic foot ulcer with peripheral arterial disease[†]

Stephanie C Wu[1], Richard Pollak[2], Robert G Frykberg[3], Wei Zhou[4], Maha Karnoub[5], Vladimir Jankovic[6], Steven A Fischkoff[6] & Denesh Chitkara[6]

1 Rosalind Franklin University, North Chicago, IL USA
2 Endeavor Clinical Trials PA, San Antonio, TX USA
3 Phoenix VA Health Care System, Phoenix, AZ USA
4 VA Palo Alto Health Care System, Palo Alto, CA USA
5 Celgene Corporation, Summit, NJ USA
6 Celgene Cellular Therapeutics, Warren, NJ USA

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## Abstract

The objective of this study was to examine the safety of cenplacel (PDA-002) in patients with peripheral arterial disease (PAD) and a diabetic foot ulcer (DFU). Cenplacel is a mesenchymal-like cell population derived from full-term human placenta. This phase 1, dose-escalation study investigated cenplacel in diabetic patients with chronic DFUs (Wagner grade 1 or grade 2) and PAD [ankle-brachial index (ABI) $>0.5$ and $\leq 0.9$], enrolled sequentially into each of four dose cohorts ($3 \times 10^6$, $10 \times 10^6$, $30 \times 10^6$ and $100 \times 10^6$ cells; administered intramuscularly on study days 1 and 8 in combination with standard of care). Overall, cenplacel was well tolerated in all 15 patients in the study. Before enrollment, nine patients had an ulcer for $\geq 6$ months and 11 had an ABI of $0.7–0.85$. No patient met dose-limiting toxicity criteria and no treatment-related serious adverse events were reported. There was preliminary evidence of ulcer healing in seven patients (five complete; two partial) within 3 months of cenplacel treatment, and circulating endothelial cell levels (a biomarker of vascular injury in PAD) were decreased within 1 month. Cenplacel was generally safe and well tolerated in patients with chronic DFUs and PAD. Outcomes from this study informed the doses, endpoints, biomarkers and patient population for an ongoing phase 2 trial.

## Introduction

In 2014, the estimated global prevalence of adult patients with diabetes was 8·3%, or around 387 million people worldwide, with approximately 26 million and 52 million patients in the USA and Europe, respectively (1). Many serious complications accompany diabetes, including peripheral arterial disease (PAD) as well as diabetic foot ulcers (DFUs), which have a lifetime incidence as high as 25% in this patient population (2). Approximately 50% of diabetic patients with DFUs have PAD (3).

**Key Messages**

- mesenchymal-like cell therapies can modulate inflammation, secrete factors that promote angiogenesis and have been shown to accelerate wound closure in pre-clinical studies, thereby making them attractive candidates for the treatment of chronic diabetic foot ulcers (DFUs)
- cenplacel, a mesenchymal-like cell population derived from full-term human placenta, is under investigation as a treatment for chronic DFUs in patients with peripheral arterial disease (PAD)
- this phase 1 study established the safety of cenplacel in patients with chronic DFUs and PAD and provided preliminary evidence for ulcer healing with improvement in vascular parameters
- outcomes from this study informed the patient population, cenplacel doses, endpoints and biomarker analyses for an ongoing, randomised, placebo-controlled, phase 2 trial of cenplacel for the treatment of chronic DFUs with PAD

Foot ulcers in patients with diabetes can lead to lower limb amputation and increased mortality. The mortality rate of patients who have DFUs with PAD has been reported as 44% at 5 years (4). The pathophysiology of DFUs is complex, and disease management in diabetic patients who have DFUs can be further complicated by the inadequate blood flow from PAD. Arterial insufficiency that results from PAD compromises the wound healing process, and diabetic patients with PAD have more severe outcomes, including decreased and delayed ulcer healing, increased risk of ulcer recurrence and increased risk of amputation compared with patients without PAD (5). The probability of ulcer healing is decreased and the risk of amputation increased in patients with reduced limb perfusion, as measured by ankle-brachial index (ABI), and is further complicated by the presence of infection, sites of necrosis and poor glycaemic control (6–8). Currently, no product is approved for treating patients with DFUs and PAD, and there are limited treatment options for patients who do not respond to conservative treatment methods or for patients who are not candidates for bypass surgery or percutaneous revascularisation procedures.

Mesenchymal stromal cell-based therapies can modulate inflammation, secrete factors that promote angiogenesis (9) and in pre-clinical and clinical studies they have been shown to accelerate wound closure and reduce size (10). Cenplacel (PDA-002) is an intramuscular formulation of placenta-derived adherent cells – a culture-expanded, mesenchymal stromal cell-like population derived from normal human placental tissue with immunomodulatory, anti-inflammatory and pro-regenerative properties (11,12). The cells in cenplacel display the nominal phenotype CD34−, CD10+, CD105+ and CD200+ (11) and constitutively express moderate levels of human leukocyte antigen class I and undetectable levels of human leukocyte antigen class II (12).

In pre-clinical studies using rodent models of hind-limb ischaemia, cenplacel increased angiogenesis and regeneration through the induction of blood vessel formation and muscle repair, enhanced endothelial cell survival and increased blood flow (13). Animals treated with cenplacel demonstrated decreased limb necrosis with enhanced vascular collateralisation effects as evidenced by enhanced perfusion when measured by Doppler-enhanced ultrasound, and by angiographic imaging of the affected limb. Histological analysis revealed enhanced maturation of blood vessels in animals treated with cenplacel (13).

Based on the pre-clinical data, cenplacel was determined to have the potential to improve peripheral perfusion and to lead to improved ulcer healing and therefore was advanced to clinical investigation. The current phase 1 study investigated the safety of cenplacel in patients who had DFUs with PAD; exploratory analyses into evidence of wound healing and biomarkers of vascular injury were also performed.

## Materials and methods

### Patient population

Adults (age 18–80 years) with type 1 or type 2 diabetes mellitus with a full-thickness DFU (Wagner grade 1 or grade 2 severity) of more than 1-month duration in combination with PAD [ABI >0·5 and ≤0·9 or toe-brachial index (TBI) >0·35 and ≤0·7), inadequate response to conventional ulcer therapy, and no planned revascularisation or amputation 3 months after the screening visit were eligible. Patients with a body mass index >40 kg/m$^2$ during screening, aspartate transaminase or alanine transaminase >2·5 times the upper limit of normal, alkaline phosphatase >2·5 times the upper limit of normal, human immunodeficiency virus positive status, known osteomyelitis, ≥50% increase or decrease in ulcer size during screening, uncontrolled hypertension (diastolic blood pressure >100 mmHg or systolic blood pressure >180 mmHg), poorly controlled diabetes mellitus [haemoglobin A1c >10% (>86 mmol/mol)] or history of malignancy within 5 years (with the exception of basal cell or squamous cell carcinoma of the skin) were excluded.

### Study design

This phase 1, multicentre, open-label, dose-escalation study (Clinical Trials Registration: NCT01859117; https://clinicaltrials.gov/ct2/show/NCT01859117) was divided into three periods: a 28-day screening period prior to treatment initiation to determine patient eligibility; a treatment period that consisted of cenplacel administration on study days 1 and 8; and a follow-up period that included evaluations on study days 15 and 29 and at study months 3, 6, 9, 12 and 24 (Figure 1). Cenplacel (PDA-002; Celgene Corporation, Summit, NJ, USA) was administered by deep intramuscular injection into the calf of the leg with the index ulcer. The dosage was divided into 15 portions that were injected in three rows of five sites along the calf. All patients received standard-of-care treatment (typically included foot hygiene, debridement, antibiotic therapy for infection and offloading) in addition to cenplacel administration. The primary objective was to evaluate safety and determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of cenplacel. The secondary objective was to explore the clinical efficacy of cenplacel, and
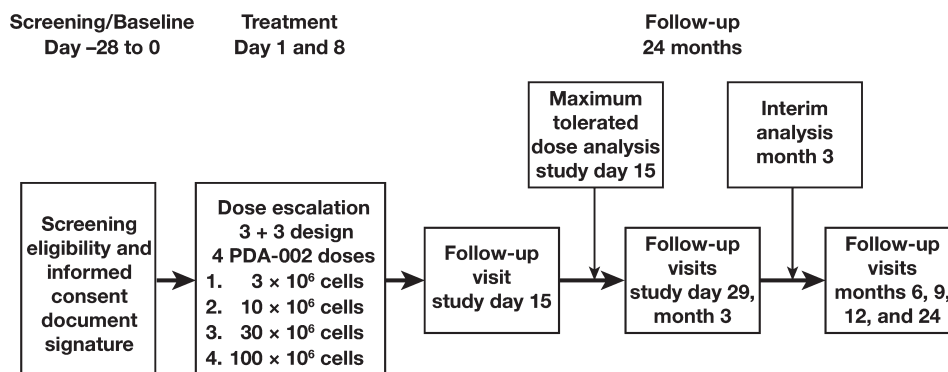
**Figure 1** Study design.

included changes in ABI and TBI and the number and extent of ulcers. Exploratory objectives included the development of novel biomarkers of tissue repair and immune modulation.

During the treatment period, patients who had a DFU with PAD were assigned to one of four cenplacel dosing cohorts based on study entry order. A 3 + 3 dose-escalation design was used and included three to six patients in each of the cenplacel dose level cohorts: $3 \times 10^6$ cells, $10 \times 10^6$ cells, $30 \times 10^6$ cells or $100 \times 10^6$ cells. Dose levels were selected based on safety and efficacy considerations using weight scaling from doses used in the animal models of hind-limb ischaemia. Prior to administration, cell viability and function were confirmed per our standard cell-handling protocols. A minimum of three patients were sequentially enrolled in each dosing cohort until the MTD was determined or the highest planned dose level was tested. The number of patients in the cohort would be increased to six if no more than one patient experienced a DLT during the first 14 days of follow-up. For this study, a DLT was defined as a grade 2 toxicity suspected to be related to cenplacel not resolving within 14 days or any toxicity grade $\geq 3$ suspected to be related to cenplacel. The MTD was defined as the highest cenplacel dose level for which the incidence of DLTs was less than or equal to one of six patients. The MTD was considered exceeded if two or more patients experienced a DLT within 14 days of dosing.

## Assessments and procedures

### Ulcer and PAD assessment

Ulcers were graded based on the Wagner Ulcer Classification (14) and were measured using the E-Z Graph® Wound Assessment System (E.Z. Graph of Victoria, Inc., Victoria, TX, USA). Specific criteria to enter patients were as follows: grade 1, superficial diabetic ulcer (full thickness) or grade 2, ulcer extension to ligament, tendon, joint capsule or deep fascia without abscess or osteomyelitis. Complete ulcer healing was defined as skin closure without drainage or need for dressing. The assessment of the wound via the Wagner Ulcer Classification was performed by the evaluating physician at each participating site and was not centrally confirmed by the study sponsor. Patients were assessed for severity of symptoms of PAD at screening; on study days 1, 8, 15 and 29; at months 3, 6, 9, 12 and 24; and at early termination using the Rutherford Classification of Chronic Limb Ischemia Criteria (15).

### Ankle-brachial index

The ABI was obtained bilaterally by measuring the posterior tibial and dorsalis pedis arteries and dividing the systolic blood pressure at the ankle by the systolic blood pressure in the arm using the Doppler technique. Patient ABI was assessed during screening; on study days 1 and 8 prior to cenplacel administration; on study days 15 and 29; at months 3, 6, 9, 12 and 24; and at early termination.

### Circulating endothelial cells

Circulating endothelial cells (CECs) were measured prior to cenplacel administration on study days 1 and 8, and approximately 2 hours post-dose on study days 1, 8, 15 and 29. The CEC analysis was performed using the CellSearch® endothelial cell kit (Veridex LLC, now Janssen Diagnostics, LLC, a subsidiary of Johnson & Johnson, Raritan, NJ, USA), as previously described (16). Briefly, CECs in whole blood were enriched with anti-CD146 antibody–conjugated magnetic nanoparticle selection, stained with $4',6$-diamidino-2-phenylindole (DAPI) for nuclei and with fluorescent antibodies against CD105 and CD45. The immune-magnetically enriched and fluorescent-labelled cells were further processed for imaging using the fluorescent microscope and quantitative analysis software, as described (17).

## Ethical considerations

The study protocol was approved by the Institutional Review Board/Independent Ethics Committee at each participating centre prior to commencement and performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines as described in International Conference on Harmonization Guideline E6. All the patients provided written informed consent. This study was registered at www.clinicaltrials.gov (NCT01859117).

## Statistics

Data were analysed after all patients in each cohort completed the initial 3-month follow-up and at later timepoints as data were available. Efficacy analyses were performed on a modified

**Table 1** Baseline patient demographics and disease status

| | Cohort 1, $3 \times 10^6$ cells ($n = 3$) | Cohort 2, $10 \times 10^6$ cells ($n = 3$) | Cohort 3, $30 \times 10^6$ cells ($n = 3$) | Cohort 4, $100 \times 10^6$ cells ($n = 6$) |
|---|---|---|---|---|
| Age, mean (years $\pm$ SD) | 72 $\pm$ 6·4 | 67 $\pm$ 6·7 | 65 $\pm$ 9·9 | 71 $\pm$ 9·7 |
| Sex, male (%) | 67 | 100 | 33 | 83 |
| Weight, mean (kg $\pm$ SD) | 86 $\pm$ 27·6 | 111 $\pm$ 6·5 | 88 $\pm$ 11·7 | 93 $\pm$ 12·8 |
| BMI, mean (kg/m$^2$ $\pm$ SD) | 27 $\pm$ 5·7 | 32 $\pm$ 1·5 | 33 $\pm$ 6·0 | 30 $\pm$ 4·5 |
| Hypertension [$n$ (%)] | 3 (100) | 3 (100) | 3 (100) | 5 (83) |
| Hyperlipidemia [$n$ (%)] | 2 (67) | 2 (67) | 1 (33) | 3 (50) |
| Coronary artery disease [$n$ (%)] | 1 (33) | 1 (33) | 0 | 2 (33) |
| Ulcer size, cm$^2$ | 0·7 $\pm$ 0·29 | 1·0 $\pm$ 0·87 | 1·0 $\pm$ 0·87 | 0·9 $\pm$ 0·58 |
| Ulcer duration, weeks | 52 $\pm$ 22 | 62 $\pm$ 60 | 82 $\pm$ 123 | 27 $\pm$ 15 |
| ABI, mean | 1·02 $\pm$ 0·25 | 0·85 $\pm$ 0·01 | 0·95 $\pm$ 0·14 | 0·75 $\pm$ 0·18 |
| Ulcer grade ($n$) | Grade 1 (3) | Grade 1 (2) | Grade 1 (2) | Grade 1 (4) |
| | | Grade 2 (1) | Grade 2 (1) | Grade 2 (2) |
| Rutherford score | 0,1,2 | 0,2,5 | 0,0,2 | 0,0,2,5,5,5 |

ABI, ankle-brachial index; BMI, body mass index; SD, standard deviation.

intent-to-treat population, defined as all treated patients who had a baseline and at least one post-baseline efficacy assessment. Descriptive statistics were provided for summaries of efficacy endpoints. Safety analyses were tabulated and summarised by cohort as appropriate.

## Results

Fifteen patients with type 1 ($n = 1$) or type 2 ($n = 14$) diabetes mellitus were sequentially enrolled in cohorts 1 to 4 (Table 1). At baseline, the majority of patients had hypertension (93%; $n = 14$), more than half the population had hyperlipidemia (53%; $n = 8$), and about 27% of total patients had coronary artery disease. Overall, 13% and 27% of patients had prior foot and toe amputations, respectively. Prior to enrollment, ulcer duration was ≥6 months in nine patients and >1 year in four patients. In addition, 11 patients had a pretreatment ABI between 0·7 and 0·85. Two patients in cohort 1 had ABI > 0·9 and were enrolled on the basis of a qualifying TBI.

The MTD was not reached after cenplacel treatment, as no cases of dose-limiting toxicity, adverse events (AEs) leading to the discontinuation of cenplacel 2 treatment, or treatment-related death were reported in this trial. No patients experienced *de novo* gangrene or reopening of closed ulcers within the 3-month follow-up period. All doses of cenplacel, including the maximum tested dose of $100 \times 10^6$ cells, were well tolerated. Overall, 11 patients (73%) experienced an AE. One patient (7%) had a non-serious treatment-related AE of pruritus that occurred 1 day after receiving cenplacel therapy. The AE spontaneously resolved the same day. The most common AEs (occurring in ≥10% of the total population) included pruritus (13%), nausea (13%), pyrexia (13%), hypoglycemia (13%) and dyspnea (13%) (Table S1, Supporting Information). No treatment-related serious AEs were reported. Cellulitis, osteomyelitis and hypoglycemia were the most common grade 3 AEs and were reported in 7% of the overall population. In the long-term follow-up period, one non–treatment-related death due to acute myocardial infarction was recorded in a patient with prior history of cardiovascular, peripheral vascular and cerebrovascular disease. This death was judged to be

non–treatment–related as no indications of cardiovascular effects were observed with cenplacel in animal models and as the patient had long-standing cardiovascular disease. No treatment-related grade 4 or 5 AEs were reported.

At 3 months, seven of 15 patients had evidence of ulcer healing (Figure S1). A total of five patients had complete ulcer healing and two patients had partial ulcer healing. No association between cenplacel dose and ulcer healing was observed. Follow-up data from 6 to 12 months were available for 14 patients. Among the five patients who had wound healing within 3 months, four patients continued to have ulcer closure between 6 and 12 months (6 months, $n = 1$; 9 months, $n = 2$; 12 months, $n = 1$). One patient had a closed DFU at 3 months that reopened at 6 months and remained open at 12 months.

In the overall population, change in median ABI from baseline was observed by study day 8 (0·11; $n = 14$) and after 3 months of cenplacel treatment (0·160; $n = 14$). Further analysis demonstrated that the increase in ABI was limited to patients whose DFUs healed compared with those whose DFUs did not heal (Figure 2). At 3 months, median ABI change from baseline in patients with ulcers that healed was 0·2 ($n = 5$) compared with −0·01 in patients with ulcers that did not heal ($n = 9$). Follow-up data at 6 and 9 months were available for 11 and six patients, respectively (Figure 2). For patients whose ulcer healed, the median ABI remained elevated above baseline at 6 months (0·1) and 9 months (0·22). For non-healers, the median ABI remained similar to baseline at 6 months (0·05) and 9 months (0·06). The increase in median ABI was observed by study day 8 in patients whose ulcers healed (0·30; $n = 5$) and was maintained to the 12-month follow-up (0·44; $n = 2$).

CECs, a biomarker of vascular endothelial injury in patients with PAD (18), were measured (Figure 3). At baseline, on study day 1, varying levels of CECs (2–69 CECs/ml) were detectable in peripheral blood of all patients. In the overall population, no significant change in average CEC levels was observed throughout the study (data not shown). However, similar to the observed trend of change in ABI, a decrease in CEC was observed in patients with healing DFUs ($n = 5$) throughout the study days 8–29, but not in patients with non-healing
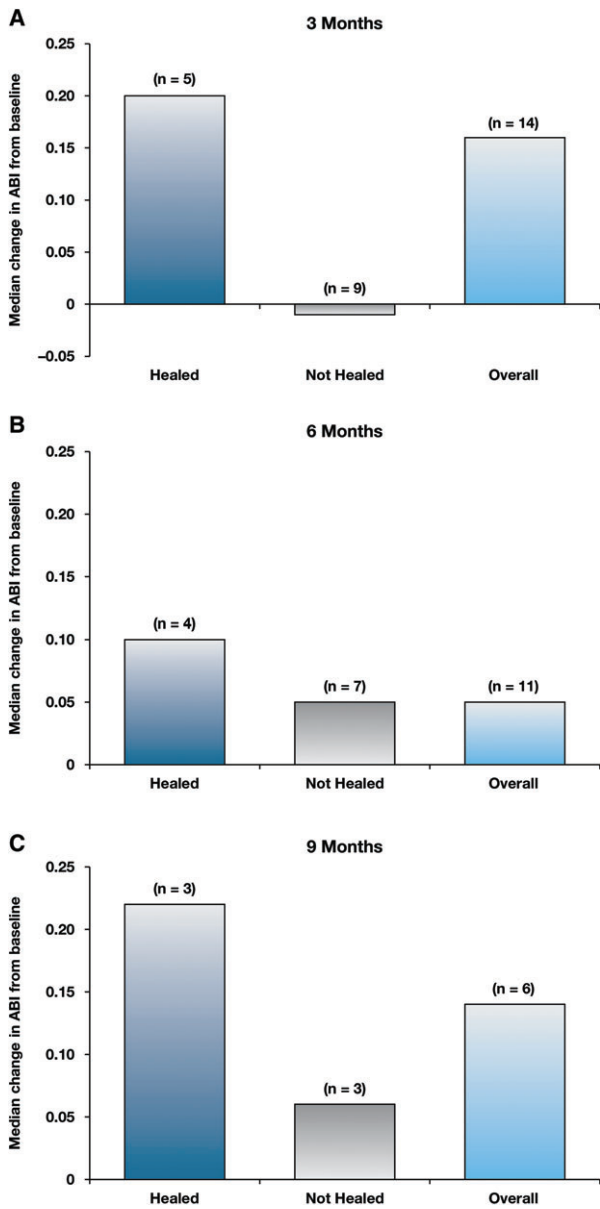
**Figure 2** Change in ankle-brachial index in patients treated with cenplacel at (A) 3, (B) 6, and (C) 9 months, respectively.



**Figure 3** Exploratory biomarker analysis in circulating endothelial cells: (A) healers versus (B) non-healers. CI, confidence interval.

DFUs (*n* = 10), resulting in a statistically significant difference (*P* = 0·02) in the median change from baseline in CEC between the two study subsets (Figure 3).

## Discussion

In this phase 1 trial, cenplacel was safe and well tolerated in patients who had DFUs with PAD and were refractory to previous conventional therapy. The MTD was not reached and no DLTs were reported, regardless of cenplacel 2 dose level. Observed serious adverse events, including hypoglycemia, cellulitis and osteomyelitis, were consistent with what would be expected in patients who had DFUs and with previously published investigations of other biologic agents (19,20). The one reported death in the long-term follow-up period was in a
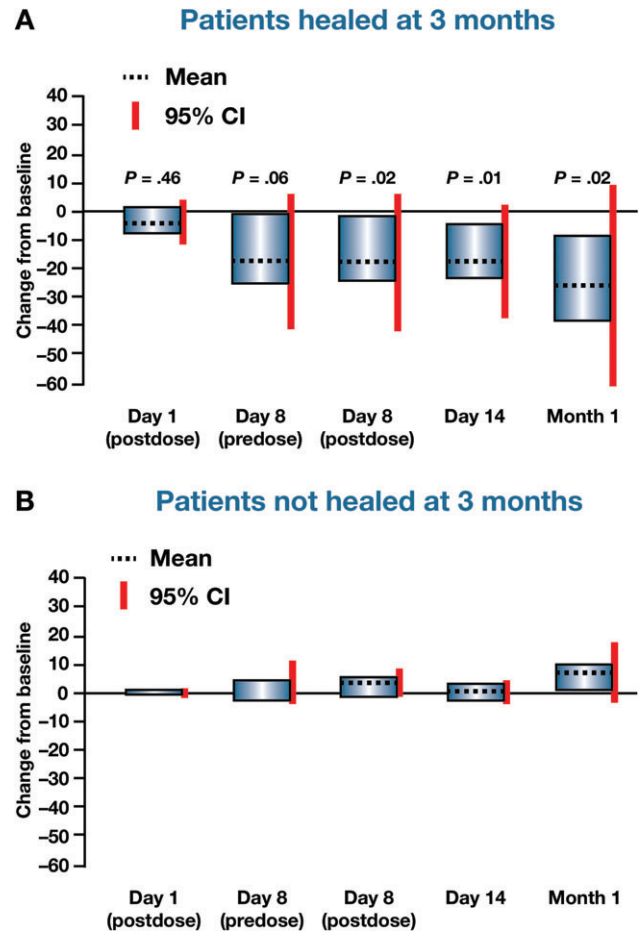
patient with severe pre-existing coronary artery disease, and although the cause of death was unknown, it was determined to be unrelated to the study intervention.

Seven out of 15 patients (most of whom had chronic ulcers that had been present for 6 months or longer) had evidence of ulcer healing. Five patients had complete ulcer healing and two patients had partial ulcer healing within 3 months. Among the patients who had complete ulcer healing within 3 months, most (four of five) continued to have durable healing of their ulcer from 6 months up to 1 year.

Based on pre-clinical evidence (13), it was hypothesised that the effects of cenplacel might be systemic rather than localised, leading to analysis of vascular parameters including biomarkers of endothelial injury. The exploratory outcomes from this study are novel in linking improvements in vascular parameters with DFU healing and suggest modification of the underlying disease by cenplacel. There were notable differences in the changes in limb perfusion (ABI) and vascular inflammation (CEC levels) between patients with ulcers that healed and those with ulcers that did not heal. Between screening and prior to dosing, patients had no change in ABI. At 3 months following dosing with cenplacel, a median increase of 0·16 in ABI was observed in patients with healed ulcers, which is considered a clinically significant change in ABI

(6). This increase in ABI was not observed in patients with ulcers that did not heal. In patients whose ulcer healed, an increase from baseline in ABI persisted at 6 months and 9 months. Consistent with the change in ABI, patients with healed ulcers had a decrease in CEC levels, a biomarker of endothelial injury, which was not observed in patients with unhealed ulcers. Although preliminary, the results support pre-clinical observations that cenplacel may improve compromised peripheral limb circulation and provide insights for future placebo-controlled studies.

Currently, there are no Food and Drug Administration-approved products indicated for the treatment of patients who have a DFU with PAD. Nearly all products used in patients with a DFU are topical, and the mechanisms by which they work (i.e. maintaining an optimal wound environment, suppressing infection, delivering growth factors to support wound healing) would not be expected to improve limb perfusion and would only be appropriate for patients who have preserved tissue oxygenation.

The use of such cell-based therapies in DFUs is promising to promote wound healing through revascularisation and modulation of the immune system. Single-agent therapies (e.g. fibroblast growth factor) have shown limited efficacy and may not be sufficient for the prevention of amputation or death (21). A pre-clinical study showed that placenta-derived adherent cells can secrete a versatile assortment of proangiogenic factors and demonstrated that administration of PDA-002 stimulated blood vessel formation, increased blood flow and vascular density as well as improved muscle tissue regeneration, in rodent models of hind-limb ischaemia (13).

There is evidence that biological therapies can increase perfusion to the lower limbs in patients with critical limb ischaemia, characterised by ischaemic rest pain or tissue loss (22). A meta-analysis of randomised controlled trials ($n = 510$ patients) that evaluated autologous bone marrow-derived cell therapy for critical limb ischaemia reported significant benefits in ABI and transcutaneous oxygen measurements ($P < 0.00001$) as well as reduced amputation rates compared with placebo (22). Autologous cell therapy has limitations, as patients undergoing therapy require bone marrow aspiration, which makes the assessment of dose response and the administration of multiple doses a challenge. Cenplacel has the advantages of being derived from a safe and plentiful source of non-embryonic cells from full-term placenta (11,12), and production scalability is comparable with traditional pharmaceuticals.

This phase 1 trial had an open-label design with a small sample size and was designed to evaluate the safety of cenplacel. As a result, data exploring the efficacy of cenplacel should be interpreted with caution and are best considered to provide early suggestions of what might be observed in a larger placebo-controlled trial. In this study, all patients understood they were receiving active therapy. Despite most patients having chronic ulcers, enrollment in a clinical trial might have resulted in increased wound care from their health care providers. It is also possible that the improvements in ABI and CEC may reflect improvements that occur in patients with ulcers that healed with standard of care and may not be related to cenplacel treatment. However, the patients who had

an increase in ABI had a notable change following the administration of cenplacel that continued through the long-term follow-up. This durable effect that occurred shortly after dosing suggests that cenplacel could have a therapeutic effect on the peripheral vasculature similar to the observations in the pre-clinical animal model (13), and awaits confirmation in a phase 2 study.

The current study has demonstrated that cenplacel is safe and well tolerated in patients who had a chronic DFU with PAD. There were preliminary indications of ulcer healing after treatment with cenplacel, together with increased peripheral circulation (as indicated by measurements in ABI) and decreases in a biomarker of vascular injury. These observations form the basis for an ongoing, placebo-controlled, phase 2 study (NCT02264288) to evaluate the efficacy and safety of cenplacel in patients who have DFUs with PAD.

## Supporting Information

The following supporting information is available for this article:

**Figure S1**. Representative images of ulcer healing in patients treated with cenplacel.

**Table S1**. Adverse events observed in patients treated with cenplacel

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