Spray-applied cell therapy with human allogeneic fibroblasts and keratinocytes for the treatment of chronic venous leg ulcers: a phase 2, multicentre, double-blind, randomised, placebo-controlled trial

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Summary

Background Many patients with venous leg ulcers do not heal with standard care. HP802-247 is a novel spray-applied cell therapy containing growth-arrested allogeneic neonatal keratinocytes and fibroblasts. We compared different cell concentrations and dosing frequencies of HP802-247 for benefit and harm when applied to chronic venous leg ulcers.

Methods We enrolled adult outpatients from 28 centres in the USA and Canada with up to three ulcers, venous reflux confirmed by doppler ultrasonography, and adequate arterial flow in this phase 2, double-blind, randomised, placebo-controlled trial if at least one ulcer measured 2–12 cm² in area and had persisted for 6–104 weeks. Patients were randomly assigned by computer-generated block randomisation in a 1:1:1:1:1 ratio to $5·0\times10^6$ cells per mL every 7 days or every 14 days, or $0·5\times10^6$ cells per mL every 7 days or every 14 days, or to vehicle alone every 7 days. All five groups received four-layer compression bandages. The trial sponsor, trial monitors, statisticians, investigators, centre personnel, and patients were masked to treatment allocation. The primary endpoint was mean percentage change in wound area at the end of 12 weeks. Analyses were by intention to treat, excluding one patient who died of unrelated causes before first treatment. This trial is registered with ClinicalTrials.gov NCT00852995.

Findings 45 patients were assigned to $5·0\times10^6$ cells per mL every 7 days, 44 to $5·0\times10^6$ cells per mL every 14 days, 43 to $0·5\times10^6$ cells per mL every 7 days, 46 to $0·5\times10^6$ cells per mL every 14 days, and 50 to vehicle alone. All required visits were completed by 205 patients. The primary outcome analysis showed significantly greater mean reduction in wound area associated with active treatment compared with vehicle (p=0·0446), with the dose of $0·5\times10^6$ cells/mL every 14 days showing the largest improvement compared with vehicle (15·98%, 95% CI 5·56–26·41, p=0·0028). Adverse events were much the same across all groups, with only new skin ulcers and cellulitis occurring in more than 5% of patients.

Interpretation Venous leg ulcers can be healed with a spray formulation of allogeneic neonatal keratinocytes and fibroblasts without the need for tissue engineering, at an optimum dose of $0·5\times10^6$ cells per mL every 14 days.

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Introduction

Individuals with chronic venous insufficiency of the legs are prone to the development of venous leg ulcers on the ankles and lower leg.[1] The annual prevalence of venous leg ulcers is estimated to be between 1·65% and 1·74% in adults aged 65 years and older—a population expected to grow substantially during the next several decades.[2]

Chronic venous insufficiency occurs most often as a result of abnormal obstruction or valvular dysfunction affecting the superficial, perforator, or deep veins. Venous insufficiency subsequently leads to venous hypertension and small vessel damage, progressing to oedema, haemosiderin deposition, and low-grade tissue inflammation. Various interventional approaches can improve venous return and, in the case of superficial or perforator surgery, can reduce the recurrence of venous leg ulcers, yet no venous interventions have been shown to speed healing of chronic ulcers.[3]

Standard treatment with infection control, primary dressings, and the application of high-strength compression heals between 30% and 75% of venous leg ulcers.[4] The remainder become chronic, with unresolved inflammatory processes within the extracellular matrix of the wound bed and dysfunction of wound fibroblasts and keratinocytes.[5] Skin autografts have been used for refractory wounds, but grafting of autologous tissue necessitates creation of a donor site wound. Early success with sheets of cultured allogeneic adult keratinocytes suggested an alternative,[6] but the fragility of the construct meant that tissue engineering was needed to incorporate one or more dermis-like substrates.[7] The most complex variation so far contains allogeneic neonatal fibroblasts cultured within a bovine collagen matrix, on which neonatal keratinocytes are placed and subsequently differentiated. The result is a two-layer human skin equivalent with histological

similarity to skin.[8] In one large randomised controlled trial, repeated application of this construct healed 63% (92 of 146) of venous leg ulcers at 24 weeks compared with 49% (63 of 129) treated with standard compression therapy.[9]

Because allogeneic cells do not engraft, the need for an elaborate tissue construct is uncertain. Successes with fresh autologous keratinocytes in a self-assembling fibrin matrix,[10] and foreskin-derived allogeneic keratinocytes in a two-component fibrin glue,[11] support the hypothesis that these cells and their released products are most important for stimulation of healing.[12] HP802-247 consists of cryopreserved allogeneic, growth-arrested fibroblasts and keratinocytes derived from neonatal foreskin.[13] After rapid thawing, the cells are delivered to the wound surface in a modified fibrin spray. In-vitro studies have allowed optimisation of the cellular formulation to enhance release of essential growth factors including vascular endothelial growth factor, basic fibroblast growth factor, keratinocyte growth factor, transforming growth factor α, and, when the cells are combined, granulocyte-macrophage colony-stimulating factor. The aim of this multicentre trial was to compare different cell concentrations and dosing frequencies of HP802-247 for benefit and harm when applied to chronic venous leg ulcers.

Methods

Study design and participants

We did this phase 2, multicentre, randomised, placebo-controlled trial at outpatient facilities in the USA (34 centres) and Canada (one centre), with 28 centres each enrolling at least one patient between June 15, 2009, and May 5, 2011. A complete list of participating centres is in the appendix. Eligible patients were at least 18 years of age, with venous reflux confirmed by duplex doppler ultrasonography and up to three venous leg ulcers between the knee and ankle, at or above the malleolus, at least one of which measured between 2 cm² and 12 cm² in area without exposed tendon, muscle, or bone, serving as the target ulcer. Target wound duration was limited to 6–104 weeks. Perfusion was assessed and only patients with great toe pressure of 50 mm Hg or more, ankle to brachial systolic pressure index of 0·8 or more, or transcutaneous oxygen tension of at least 40 mm Hg measured at the foot were included.

See Online for appendix

Patients were excluded if they had glycated haemoglobin greater than 108 mmol/mol (ie, poorly controlled diabetes mellitus), serum prealbumin concentration of 150 mg/L or less, a history of Hashimoto's thyroiditis, non-toxic nodular goitre, or Grave's disease; had a previous diagnosis of systemic lupus erythematosus with raised anti-DNA titres; clinical impression of ulcer bed infection; documented history of osteomyelitis at the target wound location within the 6 months before screening; or refusal of or inability to tolerate compression therapy. Women who were pregnant or breastfeeding were excluded. No protocol changes were made after the trial started. The protocol was approved by institutional review boards at every participating centre. All patients provided written informed consent before screening.

Randomisation and masking

Before randomisation, patients underwent a 2 week run-in period during which a cadexomer-iodine dressing (Iodoflex, Smith & Nephew, Largo, FL, USA) was applied together with four-layer compression bandaging (Profore, Smith & Nephew). Patients with infection, unresolved severe oedema, wound deterioration, or a rapidly closing wound according to Gilman's method[14] did not progress past the run-in stage. At each centre, patients who successfully completed the run-in period were registered by the investigator to receive the next available treatment assignment for that centre, with remote concealed allocation made by an interactive voice response system, with computer-generated random sequences in blocks of five (ClinPhone, Perceptive Informatics, Waltham, MA, USA). The use of randomly numbered containers further required that the centre contact the interactive system each week to match a treatment to a given patient. The trial sponsor, trial monitors, statisticians, investigators, centre personnel, and patients were masked to treatment allocation until data verification was completed and the database locked.

Procedures

Treatment was with vehicle alone applied every 7 days, or with cells in vehicle (either $0·5\times10^6$ cells per mL or $5·0\times10^6$ cells per mL) applied either every 7 days or every 14 days, thus creating five parallel groups with a 1:1:1:1:1 allocation. Patients assigned cell treatment every 14 days at either concentration received vehicle spray on the intervening weeks to maintain masking of assignment. The vials, pump pressure, spray pattern, and appearance of self-assembling fibrin on the wound surface were indistinguishable between the different interventions.

The vehicle consisted of a human fibrinogen solution (fibrinogen, synthetic aprotinin, human albumin, sodium citrate, histidine, nicotinamide, polysorbate 80, and water), and a separate human thrombin solution (thrombin, calcium, human albumin, and glycerol), based on a 1:14 diluted commercial tissue sealant (Tisseel, Baxter Healthcare, Deerfield, IL, USA). Growth-arrested (80 Gy of γ irradiation) neonatal foreskin-derived keratinocytes and fibroblasts in a 1:9 ratio were suspended at double concentration in the thrombin component. Vials were stored at –80°C until shortly before application, when they were quickly moved (<2 min) to a 37°C water bath. After thawing for 5 min, the caps were replaced with pump heads and the cells resuspended by gentle inversion. Within 30 min, the pumps were primed with four actuations, then the wound surface was treated with sequential single 130 µL sprays (first fibrinogen, then thrombin to cause

polymerisation): we controlled the spray area by holding the spray nozzle about 10 cm from the wound surface. A foam dressing was added after the matrix had formed on the wound surface, and four-layer compression bandaging was applied according to the standardised hands-on training provided by Smith & Nephew to each investigative centre. Compression bandages were changed at each weekly visit.

Patients attended weekly assessment visits for 12 weeks, or until the wound was re-epithelialised without drainage or need for dressing. Complete wound closures were confirmed after 2 additional weeks of compression. Assessment of open wounds consisted of measurement of area (cm^2), perimeter (cm), and depth (cm) with the ARANZ Silhouette measuring device (ARANZ Medical, Christchurch, New Zealand). Wound debridement with sharp instruments was allowed at each investigator's discretion, but wound surface cleansing was not otherwise specified.

The primary efficacy endpoint was the mean of individual percentage changes from baseline in wound area during the 12-week treatment period. Secondary efficacy endpoints were the proportion of participants with complete wound closure at each of the 12 treatment weeks, time to wound closure, percentage change in wound area at each visit, proportion of participants with more than 50% reduction in wound area, and change in wound pain as measured on a visual analogue scale. Safety variables were all adverse events, measurement of vital signs before and 30 min after application, blood chemistry and haematological test results, titres of antinuclear antibody, and anti-HLA. Data were collected on electronic case report forms, and were verified by trial monitors. Details about the effect on outcome of wound location, baseline bodyweight, quantitative bacteriology, serum glycated haemoglobin, peripheral neuropathy, and goniometry will be presented in a separate report.

Statistical analysis

We used SAS (version 9.1.3) for all analyses. With an assumption of 17% difference (SD 26%) in change from baseline area between treated individuals and controls, and 80% power and a two-sided alpha level of 0·05, the planned number per group was 40 patients. Enrolment compensated for dropouts, allowing sensitivity analysis of complete cases only. Primary analyses were by intention to treat, with one individual who died of unrelated causes before first treatment excluded. Post-hoc sensitivity analysis showed no change in results on inclusion of the patient.

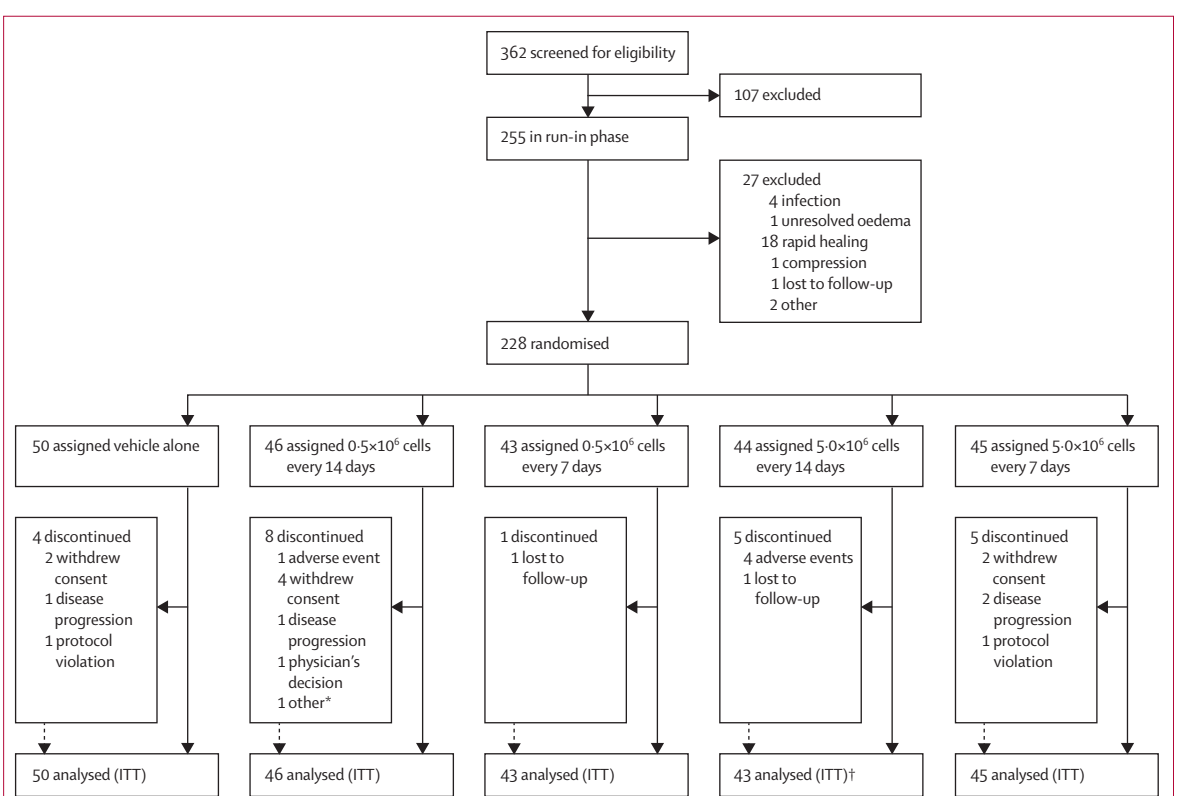

Figure 1: **Trial profile**
Initial screen failures were mostly attributable to non-qualifying wound size, failure to confirm the diagnosis with duplex doppler ultrasonography, and low serum pre-albumin concentration. ITT=intention-to-treat. *Voluntary discontinuation. †Excluding one patient who died of unrelated causes before starting study treatment.

We used a two-way ANCOVA model to analyse the primary efficacy endpoint of average percentage wound area reduction from baseline during the 12-week double-blind treatment period. The ANCOVA model included treatment (the effect of interest) and centre as the main effect, and baseline wound area as the covariate. By contrast with absolute reduction, percentage reduction from baseline is adjusted for the baseline wound area and provides a more meaningful measure that is consistent with the landmark analysis of proportion of wounds healed, in that 100% reduction in wound area is synonymous with complete wound closure. Wound duration was not included as a covariate in the primary analyses. To avoid the issue of only a few patients being enrolled at some centres, those that had fewer than ten patients in the intention-to-treat population were pooled to form a combined centre until the number of patients reached at least ten.

The null hypothesis of treatment efficacy stated that there were no differences in the average percentage wound area reduction during the treatment period between the five groups, with the alternative of non-zero differences among them. For the ANCOVA, the type I error rate for rejection of the null hypothesis was set at 0·05. In view of a significant treatment effect disclosed by the ANCOVA, pairwise comparisons were further done for each active group versus vehicle alone, by a model-based *t*-test procedure (two-sided) with a closed-test procedure for handling multiplicity. Under the assumption of a linear dose-response, and always versus vehicle alone, the closed-test procedure was planned to begin at the highest 7-day dose, then the highest 14-day dose, and continue to the lowest 7-day dose, and finally the lowest 14-day dose.

We used group mean values for the corresponding visit to substitute for missing data for target wound areas because of missed visits or voluntary discontinuations. Missing values subsequent to complete wound closure were imputed, by last observation carried forward. Post-hoc sensitivity analysis showed no change in results after adjustment of denominator degrees of freedom based on independent, recorded values only.

As a planned secondary analysis, the average percentage reduction in wound area from baseline during the 12-week treatment period was also analysed, based on a 2×2 factorial design with four active treatment groups, to assess the main effects of dose (two levels) and regimen (two levels) and their interaction.

We assessed time in days to target wound closure by Cox proportional hazards, and estimated median days to closure by Kaplan-Meier survival analysis. Reported pain scores (visual analogue scale) were analysed by ANCOVA. Analyses of safety variables included frequency of treatment-emergent adverse events, number of patients

	Vehicle every 7 days (n=50)	0·5×10⁶ every 14 days (n=46)	0·5×10⁶ every 7 days (n=43)	5·0×10⁶ every 14 days (n=44)	5·0×10⁶ every 7 days (n=45)
Clinical characteristics					
Sex					
Male	33 (66%)	26 (57%)	29 (67%)	22 (50%)	25 (56%)
Female	17 (34%)	20 (43%)	14 (33%)	22 (50%)	20 (44%)
Ethnic origin					
Non-Hispanic	39 (78%)	32 (70%)	33 (77%)	37 (84%)	32 (71%)
Hispanic	11 (22%)	14 (30%)	10 (23%)	7 (16%)	13 (29%)
Race					
Alaskan native	1 (2%)	0	0	0	0
Asian	0	0	0	0	1 (2·2)
African American	9 (18%)	10 (22%)	9 (21%)	12 (27%)	8 (17·8)
White	37 (74%)	34 (74%)	30 (70%)	30 (68%)	33 (73%)
Other	3 (6%)	2 (4%)	4 (9·3)	2 (4·5%)	3 (7%)
Age (years)	62·1 (14·1)	61·7 (15·7)	62·6 (15·4)	59·8 (15·0)	61·8 (16·1)
Weight (kg)	107·8 (39·7)	96·3 (26·0)	102·2 (34·5)	101·4 (28·8)	106·8 (38·2)
Clinical history					
Wound area (cm²)	5·4 (3·1)	5·1 (2·5)	5·4 (2·7)	6·0 (3·4)	5·9 (3·6)
Wound duration (weeks)	29·5 (24·6)	34·8 (29·1)	36·1 (31·3)	34·1 (29·2)	33·8 (27·0)
Target wound location					
Ankle	23 (46%)	20 (43%)	24 (56%)	18 (41%)	18 (40%)
Lower calf	26 (52%)	22 (48%)	17 (40%)	21 (48%)	24 (53%)
Upper calf	1 (2%)	4 (9%)	2 (5%)	5 (11%)	3 (7%)

Data are n (%) or mean (SD).

Table 1: **Baseline characteristics**

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and proportion reporting the event, severity of the event, relation to trial treatment, and event-related withdrawals, summarised for each body system by treatment group.

Changes in vital signs from baseline were analysed for differences between treatment groups, with ANCOVA, for each assessment before treatment and after the randomisation visit and for the exit visit, with the baseline measurement as the covariate. For each treatment visit, changes from vital signs before treatment were analysed for differences between treatment groups, by ANCOVA, with the assessment before treatment as the covariate. Clinical laboratory tests (haematology, serum chemistry), antinuclear antibodies, and antibody titres against HLA antigens expressed by the test article cells were summarised descriptively by treatment groups, with shift-tables used for qualitative analysis. Quantitative bacteriological analysis was done only at screening.

This trial is registered with ClinicalTrials.gov, number NCT00852995.

Role of the funding source

The study sponsor was primarily responsible for trial design, data collection, data analysis, and data interpretation, with crucial assistance from the trial lead investigators and the consulting biostatistician (Yuxin Zhang, Xtiers Consulting, Potomac, MD, USA). All authors had complete access to the data and mutually made the decision to submit for publication.

Results

We screened 362 patients, of whom 255 entered the run-in phase. 27 patients were excluded during this phase, leaving 228 who were enrolled. 205 (90%) patients completed the trial (figure 1). Demographic variables and wound characteristics were well balanced at baseline (table 1). 63 (28%) wounds had been present for between 1 and 2 years. Some deviations from protocol occurred: six (3%) enrolments violated inclusion criteria and three (1%) violated exclusion criteria; seven (3%) participants received fewer than 80% of scheduled treatments. 23 patients did not complete all required visits, yielding a 10% overall dropout rate, with data imputation accounting for 3·5–11·9% of wound area data for weeks 1–12 progressively.

For the primary analysis, cells produced a greater reduction in wound area than did vehicle alone during the 12-week treatment period (figure 2; p=0·0446). The dose of $0·5\times10^6$ cells per mL every 14 days yielded a 16% greater reduction on average than vehicle, with average decreases in the other dose groups ranging from 7·60 to 11·7% relative to vehicle (table 2). For the active treatment groups, we identified no significant effects for either high versus low dose (p=0·51), or for application every 7 days versus every 14 days (p=0·79), or for the dose-by-regimen interaction (p=0·12). Results were confirmed by ad-hoc analysis of complete cases only (n=205), and cases defined as having adhered sufficiently (eg, did not miss two consecutive or three total doses) to the protocol (n=194; data not shown). No differences were identified in the proportion of patients achieving at least 50% area reduction; 88–98% of patients reached this endpoint (data not shown).

Wound area began to decrease rapidly after initiation of treatment (figure 2A). After one application of the $0·5\times10^6$/mL every 14 days dose, mean reduction in wound area was 40% (SD 32%), compared with 23% (27%) for the vehicle group. This effect persisted with the maximum difference in mean reduction between the group assigned $0·5\times10^6$/mL every 14 days and the vehicle group occurring at week 7 of treatment (87% [SD 19%] *vs* 65% [43%]). Significant differences between the group assigned $0·5\times10^6$/mL every 14 days and the vehicle group occurred at ten of the 12 weekly timepoints (p<0·05, all but weeks 4 and 12). At week 12, mean wound area in the group assigned $0·5\times10^6$/mL every 14 days was reduced by 91% (SD 21%), compared with 80% (30%) with vehicle. The mean decrease in area for the three remaining treatment groups was 87% (SD 20%) for the $0·5\times10^6$/mL every 7 days group, 84% (26%) for the $5·0\times10^6$/mL every 14 days group, and 87% (23%) for the $5·0\times10^6$/mL every 7 days group.

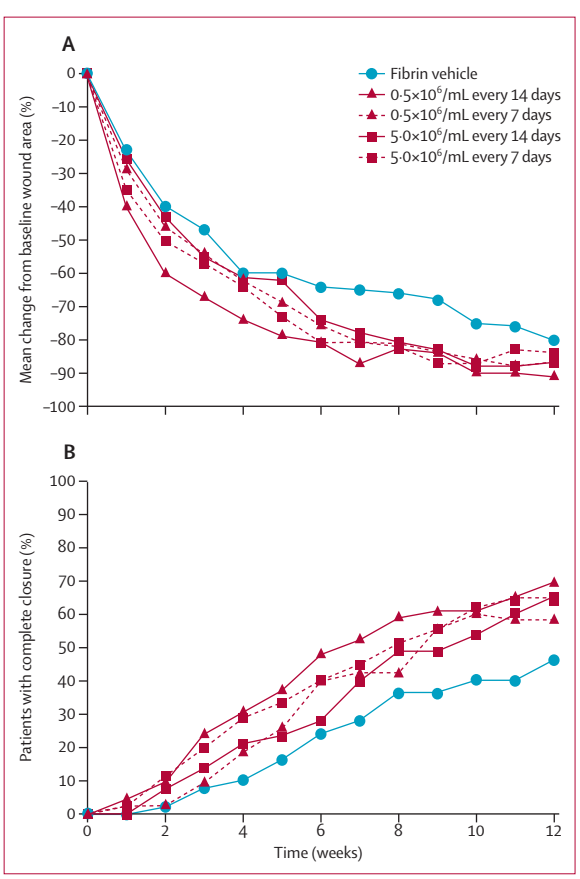

***Figure 2*: Wound area reduction and wound closure**
(A) Wound area reduction. (B) Complete closure.

	Vehicle every 7 days (n=50)	0·5×10⁶ every 14 days (n=46)	0·5×10⁶ every 7 days (n=43)	5·0×10⁶ every 14 days (n=44)*	5·0×10⁶ every 7 days (n=45)
Difference in percent area reduction over 12 weeks†					
Difference (95% CI)	..	−16·0% (−26·4 to −5·56)	−9·16% (−19·8 to 1·44)	−7·60% (−18·2 to 2·99)	−11·7% (22·2 to −1·17)
p value	..	0·0028	0·09	0·16	0·0295
Proportion healed by week 12					
Number (%)	23 (46%)	32 (70%)	25 (58%)	28† (65%)	29 (64%)
p value	..	0·0267	0·36	0·09	0·09
Kaplan-Meier days to closure					
Days (95% CI)	71 (57 to NA)	50 (37 to 66)	64 (43 to NA)	57 (50 to 85)	57 (43 to 71)
p value	..	0·0211	0·59	0·26	0·10
Cox HR*					
HR (95% CI)	..	1·83 (1·09 to 3·04)	1·19 (0·69 to 2·05)	1·43 (0·84 to 2·44)	1·64 (0·97 to 2·77)
p value	..	0·0212	0·53	0·19	0·06

The dimensions of wound closure are proportion closed over the treatment interval, and time to closure. NA=unable to assess upper limit. HR=hazard ratio. *Data for one patient (who died before starting treatment) are missing. † Versus fibrin vehicle.

***Table 2:* Effect of HP802-247 on wound area and wound closure**

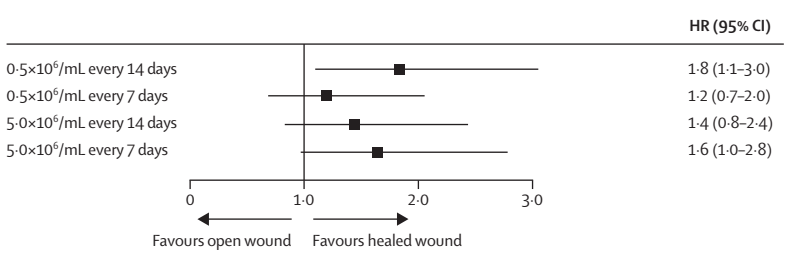

***Figure 3:* Cox proportional hazards analysis of wound healing**
The Cox proportional hazards model incorporates a comparison of healed versus open wounds across treatment groups at each of the weekly trial visits, with censoring of missing data. The HR is the likelihood of healing at any given point in time, by group, relative to the vehicle control group. CIs show the range of potential values. HR=hazard ratio.

	Vehicle every 7 days (n=50)	0·5×10⁶ every 14 days (n=46)	0·5×10⁶ every 7 days (n=43)	5·0×10⁶ every 14 days (n=44)	5·0×10⁶ every 7 days (n=45)
Infection, target wound	2 (4%)	0	0	2 (5%)	2 (4%)
Infection, non-target wound	0	0	1 (2%)	0	2 (4%)
Cellulitis, non-target wound	3 (6%)	1 (2%)	2 (5%)	0	0
Excoriation	2 (4%)	0	2 (5%)	0	0
Skin irritation	2 (4%)	1 (2%)	0	0	0
Skin ulceration	8 (16%)	3 (7%)	3 (7%)	4 (9%)	5 (11%)
Congestive heart failure	1 (2%)	0	0	0	2 (4%)
Pain in extremity	0	2 (4%)	1 (2%)	1 (2%)	0
Nervous system disorder	2 (4%)	2 (4%)	2 (5%)	2 (5%)	0
Psychiatric disorder	0	0	2 (5%)	0	0
Respiratory disorder	2 (4%)	1 (2%)	2 (5%)	1 (2%)	0
Vascular disorder	0	2 (4%)	1 (2%)	2 (5%)	0
Vomiting	0	0	0	0	2 (4%)

Data are n (%). 27 (54%) of vehicle recipients and 73 (41%) of cell recipients reported at least one adverse event.

***Table 3:* Adverse events reported by at least 3% of patients**

At each visit and during the 12-week treatment period, the proportion of patients with complete wound closure was always greater in the four active groups than in the vehicle alone group (figure 2B). The group assigned 0·5×10⁶/mL every 14 days had the highest proportion of closed wounds between weeks 3 and 9 and was significantly better than was vehicle alone at week 4 and weeks 4–12. Overall, patients treated with cells had higher proportions of healed wounds than did those assigned vehicle alone, but only the group assigned 0·5×10⁶/mL every 14 days differed significantly compared with control (table 2). Baseline wound duration showed a significant inverse correlation with wound closure overall ($p<0·001$, post-hoc analysis, multiple logistic regression), but was not significant as a covariate compared with treatment, with wound closure as the dependent variable (post-hoc analysis, two-way ANOVA; data not shown). Number of debridements did not correlate with healing (data not shown).

On the basis of Kaplan-Meier survival analysis, median time to complete wound closure was 21 days (IQR 14–NA) earlier for the group assigned 0·5×10⁶/mL every 14 days than for the vehicle group (table 2). Cox regression analysis identified baseline wound area as a significant covariate for wound closure ($p=0·0058$). After adjustment for baseline wound area, the likelihood of wound closure was 83% greater with 0·5×10⁶/mL every 14 days than with vehicle; differences between vehicle and other active groups were not significant (figure 3). We did not identify significant differences between active groups (data not shown).

Pain associated with the treated wound decreased steadily in all treatment groups during the 12-week treatment period. A week after initiation of treatment, a greater reduction in scores on visual analogue scale was recorded for groups treated with cells (ranging from −3·8 to −8·7) than for the group treated with vehicle

alone (+0·2, p=0·0464). Except for a significant difference favouring cell treatment at week 12 (p=0·0251), pain score reductions were much the same between groups at the weekly visits (data not shown).

A total of 194 adverse events were spontaneously reported by 100 patients; 73 of 178 (41%) of those assigned active treatment and 27 of 50 (54%) assigned vehicle alone. Most were non-serious, generally mild (126, 65%) to moderate (52, 27%), most resolved, and generally did not interrupt the patient's continuation in the trial. Only new skin ulcers and cellulitis had a frequency greater than 5% (table 3). Few adverse events were assessed as related to treatment (six [3%] for active groups combined and two [4%] for vehicle), or resulted in trial discontinuation (three [2%] for active groups combined and none for vehicle). 16 patients reported 18 serious adverse events, one of which (wound infection) was assessed by the investigator as potentially related to treatment. Adverse events were not reported more frequently for the active groups than for vehicle (table 3). On the basis of analysis of the reported adverse events, and haematological tests, blood chemistry, and vital signs before and after exposure, no safety issues were identified.

Immunotoxicity testing did not identify any meaningful treatment-induced alloantibody formation or induction of autoimmunity. Before cell exposure, 24% (48 of 200) of paired serum samples contained antibodies to one or more HLA-I antigens in the general test panel, with 23 samples containing antibody specific for one to six antigens expressed by the product cells (relevant antigens). Of these 48, 42 were positive at the end of the trial. 145 patients had no anti-HLA-I antibodies either before or after exposure. Eight patients became positive for alloantibodies to one or more relevant antigens after exposure. Six of these patients were in the high-dose treatment groups, with one each in the two low-dose groups. None were in the vehicle group. Six of these eight patients achieved complete wound closure. Six patients with positive titres for HLA-I antigens expressed by applied cells before exposure had negative results for the same antigens after exposure to either $5·0\times10^6$ cells (five patients) or $0·5\times10^6$ cells (one patient).

Discussion

Treatment with growth-arrested neonatal cells produced significantly greater reduction in wound area, time to healing, and in proportion of wounds closed, than did standard care alone. The rapidity with which healing began and the high proportion of wounds healed in the $0·5\times10^6$/mL group confirms and extends the benefit recorded previously with adult cells, which theoretically have a lesser healing potential than do neonatal cells (panel).[13] Vehicle response rate was consistent with results of clinical trials of four-layer compression bandage treatment in non-infected venous leg ulcers.[4,24] In acute wounds, vehicle alone does not speed healing compared with standard care, which is consistent with earlier reports that fibrin sealant alone does not promote healing.[10] Although product formulation, trial design, and trial conduct had been adjusted since the previous trial,[13] consistency of results suggests that cell treatment can be expected to close 60–70% of chronic venous leg ulcers of 2–12 cm² in area within 12 weeks.

We recorded the best results with $0·5\times10^6$/mL given every 14 days. We judged this dose interval to be viable on

Panel: Research in context

Systematic review

Although autologous skin grafts have not been rigorously studied and as yet have not been shown to be effective for venous leg ulcers, they are commonly used. Isolation and ex-vivo expansion of skin cells has allowed other approaches to the use of autologous skin for treatment of non-healing venous leg ulcers.[15,16] Cells derived from small biopsy specimens or plucked hair follicles can be constructed as suspensions or sheets of cells with various substrates, with results similar to those for split thickness grafts.[17] However, in most cases, use of autologous tissue as a cellular source requires creation of a donor site wound and some delay for production, whereas products that make use of allogeneic cells such as keratinocytes and fibroblasts can be made ready to use when needed. Unlike intact allografts, cultured cells do not provoke adaptive immune responses.[18] Systematic reviews of cell-based therapies for venous leg ulcers have identified little high quality evidence of benefit, attributable partly to the inherent challenge of masking engineered tissue application.[19,20] In a Cochrane review,[21] Jones and Nelson noted that 15 of 17 qualifying randomised controlled trials were underpowered, with all having poor quality of reporting. The best data are from one large open-label trial[9] reporting significant benefit in the proportion of wounds healed compared with compression alone, with a bilayered skin substitute containing neonatal foreskin-derived keratinocytes and fibroblasts. Not included in the systematic reviews are a randomised controlled trial reporting benefit with adult cells in fibrin spray,[13] and various engineered tissue formats that have shown no benefit, including allogeneic fibroblasts in fibrin-based matrix (ICXP007, Intercytex, Manchester, UK; NCT00232973, n=396), allogeneic fibroblasts in a polyglactin mesh scaffold (Shire, Dublin, Ireland; NCT01181453, n=314; NCT00909870, n=537), frozen sheets of allogeneic keratinocytes (Celaderm, Advanced BioHealing, San Diego, CA, USA; NCT00399308, n=40), and combined allogeneic fibroblasts and keratinocytes in a bovine collagen sponge (OrCel, Ortec International, Atlanta, GA, USA; NCT00270946, n=136). The sum of evidence suggests that although allogeneic cell therapy for venous leg ulcers is likely to be effective, more work is needed to define the optimum characteristics of the cells to be used, the format for cell delivery, and the application of properly designed randomised controlled trials for assessment of benefits.[22,23]

Interpretation

The use of growth-arrested neonatal keratinocytes and fibroblasts in a spray-applied, self-assembling fibrin matrix is a novel and rigorous extension of previous work. By contrast with many reports of small trials and case series suggesting benefit with allogeneic cells, our trial was large, properly randomised, and well masked, with complete reporting of prospectively determined outcomes and with intention-to-treat analyses. As such, the trial provides much needed high-quality evidence in support of the benefit of allogeneic cell therapy. By contrast with engineered tissue constructs, our use of a cell suspension has allowed exploration of cell ratios (1:1, 1:9) and concentrations ($0·5\times10^6$/mL to $10·0\times10^6$/mL), in addition to application frequencies (every 7 days, every 14 days). Within the range of wounds 2–12 cm² at baseline, we were able to establish the best dose, which was the lowest dose tested in each trial. The finding of alternate week dosing to be most effective in the present trial is consistent with the ability of the applied cells to persist for 2 weeks in an acute wound model (unpublished). Tolerability was very good, with no treatment-emergent adverse events.

the basis of in-vitro cell survival studies, and it has since been supported by evidence showing 2-week survival of HP802-247 cells in acute wounds (unpublished). Because three of the dose groups achieved wound closure proportions within 5% of each other, no further dose exploration seems warranted. The dose showing the least measured benefit did not differ with regard to baseline characteristics such as wound size, duration, location, presence of infection or comorbidities, age of the patient, mobility, or ability to wear and tolerate compression devices—factors reported to be associated with differential healing.[25]

Limitations of our trial include a wound area upper boundary of 12 cm², which represents about 80% of all venous leg ulcers, but a smaller proportion of chronic venous leg ulcers. The upper size limit was chosen to allow treatment with one spray (spray pattern 12–15 cm²), because the use of several sprays on larger wounds could have produced overlapping and thus a random difference in cell concentrations at the wound surface. Wound episode duration was limited to 2 years to define a potentially responsive population for dose testing. Information about the anatomy and pathophysiological mechanisms of each patient's underlying venous insufficiency was not collected prospectively. We attempted to improve generalisability and limit bias by having investigators from different disciplines (dermatologists, vascular surgeons, and podiatrists at private practices and universities) and providing uniform training of application of compression bandages.

The use of individual growth factors to promote closure of chronic venous leg ulcers has had little success, partly because of problems with delivery of these factors. Although granulocyte-macrophage colony-stimulating factor has shown promise,[26] combinations of cytokines seem to be more effective in promotion of wound healing, at least when applied in the less hostile wound environment of animal models.[27] Ideally, the combination would be tailored to the needs of the wound at any given timepoint. Allogeneic cells are potentially capable of delivering such combinations continuously.

Unlike tissue therapy with cellularised skin substitutes that bear some histological similarity to and thus mimic differentiated skin,[28] the described spray-delivered neonatal cells mixes less differentiated fibroblasts and keratinocytes into a fibrin gel. The use of γ irradiation to arrest growth of applied cells is thought to both provoke a stress response, and to impose self-limitation of that response through eventual apoptosis. Although not intuitive that arrested cells would be an appropriate therapy for wounds marked by the presence of senescent fibroblasts, several signalling pathways lead to induction of cell-cycle arrest in response to deterministic (terminal differentiation, telomere shortening) or stressful (oxidative, oncogene, ionising radiation) events. Several genes are differentially regulated dependent on the particular pathway.[29] The characteristics reported for oxidatively stressed fibroblasts in non-healing venous leg ulcers are not perfectly matched to those of other stressed fibroblasts.[30,31] Fibroblasts in chronic wounds might be rescued from senescence by cell-secreted factors,[32] including those released by other growth-arrested cells.[33]

The introduction of non-engrafting cells into a chronic wound presents a potential burden, in that physiological removal of the introduced materials must be achieved for the wound to heal. The normal removal of apoptotic bodies by scavenger macrophages might additionally aid healing through triggered release of growth factors; however, whether excessive numbers of dying cells inhibit healing is not entirely clear.[34] As with growth factors and agents affecting angiogenesis, fewer cells given less frequently might improve benefit as a result of a bell-shaped dose response involving a threshold and an upper limit beyond which responses are suppressed.[35] The use of a sprayed cell suspension has allowed us to explore dose response in a manner that would be more difficult for living tissue therapies that require a specific physical structure. In this trial, several dosing regimens seemed to provide benefit to patients compared with vehicle. The results are sufficiently promising that larger randomised trials comparing HP802-247 to standard treatment for venous leg ulcers are now warranted.

Contributors

All authors participated in design of the protocol and interpretation of the results of the trial. RSK, WAM, and RJS served as lead investigators for the trial. HBS, DIC, and TDL were responsible for site selection, with advice from RSK, WAM, and RJS. HBS, DIC, and TDL were responsible for overseeing the conduct of the trial and for data collection. HBS and DIC took the lead in drafting the report, after which every author participated in editing and finalisation.

Conflicts of interest

RSK, WAM, and RJS received consulting fees for their assistance with protocol design and data interpretation. HBS, DIC, and TDL are employed by Healthpoint, a privately owned US company. HBS and DIC hold adjunct academic appointments at the University of North Texas Health Sciences Center in Fort Worth, Texas, USA.

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