

Baseline factors affecting closure of venous leg ulcers



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ABSTRACT

Objective: The objective of this study was to characterize factors associated with closure of venous leg ulcers (VLUs) in a pooled analysis of subjects from three randomized clinical trials.

Methods: Closure of VLUs after treatment with HP802-247, an allogeneic living cell therapy consisting of growth-arrested human keratinocytes and fibroblasts, vs standard therapy with compression bandaging was evaluated in three phase 3 clinical trials of similar design. Two trials enrolled subjects with VLUs ranging from 2 cm² to 12 cm² in area with 12-week treatment periods; the third trial enrolled subjects with VLUs between >12 cm² and ≤36 cm² with a 16-week treatment period. The first trial went to completion but failed to demonstrate a benefit to therapy with HP802-247 compared with placebo, and because of this, the remaining trials were terminated before completion. On the basis of no differences in outcomes between groups, subjects from both HP802-247 and control groups were pooled across all three studies. Cox proportional hazards regression analysis was employed to evaluate factors associated with VLU closure.

Results: This analysis included data from 716 subjects with VLU. Factors evaluated for association with healing included age, gender, race, diabetes, glycated hemoglobin level, body mass index, treatment (HP802-247 vs compression alone), and ulcer characteristics including location and area and duration at baseline. In an initial model including all of these putative factors, the following were significant at the $P < .10$ level: diagnosis of diabetes mellitus, gender, wound location (ankle or leg), baseline wound area, and wound duration at baseline. In a final model including only these factors, all but diabetes mellitus were significant at the $P < .05$ level. Effect sizes were as follows (hazard ratio [95% confidence interval]): female gender (1.384 [1.134-1.690]), wound location on the leg (1.490 [1.187-1.871]), smaller wound area at baseline (0.907 [0.887-0.927]), and shorter wound duration at baseline (0.971 [0.955-0.987]).

Conclusions: Factors associated with VLU lesions including location, area, and duration were important predictors of healing. Women were more likely than men to achieve wound closure. Factors including body mass index, the presence of diabetes mellitus, and higher concentrations of glycated hemoglobin were not significant independent predictors of wound closure in this analysis. (J Vasc Surg: Venous and Lym Dis 2017;5:829-36.)

Venous leg ulcers (VLUs) represent a large and growing burden to the U.S. health care system. The prevalence of VLUs in the United States is estimated at 1% to 2%^{1,2} and is expected to increase as we live longer. The cost to the U.S. health care system associated with VLU care is estimated to be in excess of \$2.5 billion annually.^{3,4} Treatment of VLU remains challenging. Even with optimal

conservative treatment, only 50% to 75% of VLUs will close within 6 months,⁵ and of these, 6% to 27% will recur annually.^{6,7} Thus, a substantial proportion of VLUs will become chronic. Currently, it is often difficult to predict which ulcers will fail to heal with current recommended treatment protocols for VLUs. Most previous reports have been based on limited data sets of fewer than

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300 cases, leading to a significant lack of predictive power. If we are better able to identify patients who will respond poorly to compression-based care, we may move immediately to other more aggressive therapies, such as intervention to correct venous hypertension or living cellular therapies to stimulate the wound. Improved prediction of the likelihood of healing would also allow better stratification of patients in clinical studies to improve our ability to compare treatments for specific types of VLU patients.

A large clinical development project was recently completed in VLUs for HP802-247, an allogeneic living cell therapy consisting of growth-arrested human keratinocytes and fibroblasts delivered in a fibrin matrix by a spray device. This product was developed to actively facilitate closure of VLU and was studied in multiple randomized clinical trials compared with standard VLU treatment. Although the phase 2 clinical studies of this product produced promising results,^{8,9} the phase 3 clinical program was terminated when the first of three studies failed to meet its primary end point.¹⁰

The HP802-247 phase 3 registry provides a more robust data set of >700 patients with which to evaluate factors associated with VLU closure. In this paper, we report the effect of both baseline patient and wound characteristics on VLU closure observed in the three randomized clinical trials composing the HP802-247 phase 3 development program. A better understanding of the risk factors for nonhealing VLUs can provide insight into the identification and management of patients with VLU and may also help streamline enrollment for future VLU intervention studies by optimizing eligibility criteria.

METHODS

Patient-level data for the current analysis were drawn from three phase 3 clinical trials of HP802-247: protocol 029 enrolled and randomized 447 subjects at 49 centers in the United States and Canada; protocol 031 enrolled and randomized 155 subjects at 39 centers in the United States and Canada; and protocol 032 enrolled and randomized 252 subjects at 47 centers in 5 European countries. Both 031 and 032 were terminated before completion after failure to meet the primary end point in protocol 029. All three studies were conducted in accordance with Good Clinical Practice guidelines and in accordance with the tenets of the Declaration of Helsinki, all protocols were reviewed by the relevant ethics agencies, and all subjects provided written informed consent to participate.

Each of the three studies followed a nearly identical protocol, which has recently been described.¹⁰ Briefly, all three were prospective, randomized, double-masked, vehicle-controlled trials. Key eligibility criteria were as follows: adults with venous reflux confirmed by duplex Doppler ultrasound and a VLU located between the knee and ankle, at or above the malleolus, between

ARTICLE HIGHLIGHTS

- **Type of Research:** Retrospective analysis of data of three prospective randomized trials
- **Take Home Message:** Location, area, and duration were important predictors of healing of 716 venous leg ulcers. Women were more likely than men to achieve wound closure.
- **Recommendation:** This study suggests that factors associated with venous leg ulcer healing include ulcer location, area, and duration in addition to female gender.

2 and 12 cm² in area (029 and 032) or between 12 and 36 cm² in area (031). Ulcers were required to be present for at least 6 weeks but not more than 104 weeks; adequate perfusion to the target ulcer limb was required and defined as an ankle-brachial systolic pressure index of at least 0.8, a transcutaneous oxygen tension of at least 40 mm Hg measured at the foot, or a great toe pressure of at least 50 mm Hg; and patients with diabetes mellitus were eligible provided their glycated hemoglobin (HbA_{1c}) level did not exceed 12%. Patients with occlusive disease (eg, a deep venous thrombosis diagnosed within 10 days of study entry, or one for which the investigator thought compression was contraindicated) were excluded from the study.

A 2-week run-in phase was included, and wounds healing rapidly during this time frame were eliminated from randomization. Baseline wound characteristics were determined after the run-in phase at randomization. All subjects underwent weekly application of four-layer compression bandages (Profore; Smith & Nephew, Hull, UK), at which time subjects in the active arm were treated with HP802-247 spray (fibrinogen solution followed by thrombin solution containing 0.5×10^6 cells/mL [human neonatal foreskin-derived fibroblasts and keratinocytes]) every 14 days (and vehicle on alternate weeks); subjects in the vehicle arm received weekly treatment with fibrinogen and acellular thrombin solution. Patients in both arms of the study received venous ulcer care as outlined in relevant guidelines documents including débridement to remove nonviable or necrotic tissue, exudate control, and absorptive primary wound dressings.^{11,12} The duration of active treatment was 12 weeks for protocols 029 and 032 and 16 weeks for protocol 031, given the larger lesion sizes, with all subjects then observed through 52 weeks. The primary end point of all three trials was wound closure (defined as re-epithelialization without drainage) achieved during the active treatment period and maintained through two subsequent visits.

Protocol 029 was the first of the three studies to be completed and failed to meet the primary objective. Consequently, protocols 031 and 032 were terminated

Table I. Sources of data for the current analysis and primary outcome of each study

Protocol	Randomized, No.	Intention to treat population ^a	Achieved wound closure		
			HP802-247, n/N (%)	Vehicle, n/N (%)	P value
O29	447	421	129/211 (61.1)	126/210 (60.0)	.5896
O31	155	139	17/69 (24.6)	23/70 (32.9)	.4280
O32	252	252	61/131 (46.6)	61/121 (50.4)	.5348
Total	854	812	207/411 (50.4)	210/401 (52.4)	—

^aExcludes subjects from sites that violated Good Clinical Practice.

early, and many subjects—including some with wound closure at the end-of-treatment time point—exited before completion of post-treatment follow-up. Given that cellular therapy was found to have no effect on wound healing, positive or negative, we elected to include all patients in these three studies in the analysis for this manuscript. To retain data from as many subjects as possible in this analysis, the end point for this analysis was wound closure by the end-of-treatment time point without the requirement for confirmation of closure at two subsequent time points. Subjects in protocols O31 and O32 who were unhealed but were not afforded the full time (12 or 16 weeks) to achieve healing because of study closure were excluded from this analysis. Subjects exiting all three protocols for other reasons (eg, adverse events, voluntary withdrawal, loss to follow-up) were included in the current analysis with their last observation carried forward. Thus, we included those who exited for nonadministrative reasons while excluding those who exited for administrative reasons on the basis that the final study sample best represents real-world experience.

The three studies were virtually identical in terms of design, visit structure, inclusion and exclusion criteria, investigators and investigative sites, protocol training, and study conduct. Based on this information and the lack of clinically significant differences between treatment groups in the proportion of subjects reaching the primary end point in any of the three studies (Table I), active treatment and control groups were pooled across all three studies.¹³ With the exception of body mass index (BMI), no significant differences were observed between the HP802-247 and vehicle groups in either demographics or wound characteristics after pooling across studies (data not shown), supporting the pooling of active and control groups for this analysis. Mean BMI was slightly higher in the pooled treatment group than in the pooled vehicle group (34.0 vs 32.6; $P = .034$). End-of-treatment visits were used exclusively for the analysis, which was at 12 weeks for protocols O29 and O32 and 16 weeks for protocol O31. Arbitrarily truncating the treatment period for protocol O31 to 12 weeks would have incorrectly classified ulcers healing between weeks 12 and 16 as “nonhealers” and also ignored the generally longer time needed for larger ulcers to heal.

Data analysis consisted of descriptive statistics for subject and wound baseline characteristics. Between-group comparisons of subject and wound characteristics were performed using analysis of variance (for continuous variables) or χ^2 tests (for categorical variables). To evaluate the significance of various putative predictive factors on VLU wound healing by end of treatment (12 weeks for O29 and O32 and 16 weeks for O31), Cox proportional hazards regression analysis was used to model the time to wound closure. Putative factors evaluated included age (per 1-year increase), HbA_{1c} level (dichotomized to $<6.4\%$ vs $\geq 6.4\%$), BMI (per unit increase), country of residence (United States vs other), diabetes mellitus (present vs absent), gender (female vs male), race (white vs other), treatment (HP802-247 vs vehicle), ulcer location (lower leg vs other), baseline wound area (per 1-cm² increase), and wound duration at baseline (per 1-month increase). An initial model was constructed that contained all the putative factors. Those attaining significance at $P \leq .10$ in the univariate analysis were included in a final multivariate model. Factors found in this final model to have a $P \leq .05$ were considered to have a statistically significant relationship to wound healing.¹⁴

RESULTS

A total of 854 subjects were randomized in the three studies included in this analysis, of whom 42 were excluded from their respective intention to treat analyses in protocols O29 and O31 because of violations of Good Clinical Practice identified through routine study monitoring (Table I).

An additional 96 subjects in protocols O31 and O32 were excluded because of failure to complete the treatment phase of their respective studies on the basis of early study termination. The current analysis includes data from the remaining 716 subjects (Table II).

Baseline demographic and wound characteristics of subjects in the analysis population who did and did not achieve VLU closure during the treatment period are given in Table III.

The Cox proportional hazards model with all putative risk factors associated with VLU wound closure is given in Table III. Putative factors included age, gender, race, country of residence, diabetes, HbA_{1c} level, BMI, ulcer

Table II. Derivation of analysis population

Study	Randomized (safety population)	Analysis population
O29	447	421
O31	155	124 ^a
O32	252	171 ^a
Total	854	716

^aExcludes subjects who exited before end-of-treatment time point because of early study closure.

location, wound area, wound duration, and treatment assignment (HP802-247 vs vehicle). Factors that were insignificant in the full model included age, HbA_{1c} level, BMI, nationality, race, and treatment.

Factors significant at the $P \leq .10$ level in the full model (diabetes mellitus, gender, wound location, area, and duration) were included in the final model (also given in Table III). In this final model, diabetes mellitus (of only marginal significance in the full model) did not attain significance. All other factors in the final model retained their significance at the $P < .05$ level with unchanged effect sizes. Closure rates for these predictive factors are listed in Table IV; wound size and duration are presented dichotomously according to the stratification used for randomization (larger or smaller than 5 cm², older or younger than 6 months).

Subjects were also assigned Margolis scores: 1 point if the ulcer was >5 cm², and 1 point if duration was >6 months, otherwise 0 for area and 0 for duration.¹⁵ This score was a strong predictor of closure as noted in Table IV.

DISCUSSION

Cellular therapy to promote wound closure in VLU with HP802-247 showed promise in phase 2 evaluation but failed to demonstrate efficacy of the product over current standard of care compression in phase 3 testing, leading to the termination of its development.^{8,10} Studies with negative results have intrinsic value, and much can be learned from the data about the disease state.

This study used a large data set (N = 716) to evaluate factors associated with healing of VLU. As expected, wound size, location, and duration were significant predictors of wound closure. Wound location had the greatest effect size, with lesions on the leg healing more frequently than those on the ankle. This may be attributable to higher venous pressures that would be experienced at the ankle in patients with incompetent axial reflux. It is also possible that compression bandaging is mechanically more difficult to achieve on the ankle compared with the leg. In addition, ankle inclusive disease may include a proportion of patients who could have a greater component of mixed disease in the form of vasculitis. Differential involvement of deep,

superficial, or perforator veins might also influence ability to heal. Doppler ultrasound is acknowledged to be the best method for determining the involvement of these systems; however, it was noted that the robustness of this test varied from center to center, and thus it was not possible to evaluate such differences. Perfusion was evaluated at baseline to ensure safe use of compression by various methods (eg, transcutaneous oximetry, ankle-brachial index). Periwound oxygen measurements were not taken but in future work could provide useful data relevant to the healing prognosis.

Wound size did have an impact on healing, giving a significant 10% reduction in the likelihood of closure with each 1-cm² increase in wound size. Wound duration was modestly impactful with an approximately 3% reduction in likelihood of closure for each 1-month increase in the duration of the lesion at baseline. Being a woman was highly significant and with a large effect size. Women in these three studies were nearly 40% more likely than men to achieve closure of VLUs. This is a novel finding, and the explanation is unclear. In a review of the epidemiology of venous ulcers, Gloviczki et al found that the majority of published studies reported a higher prevalence of VLUs in women.¹⁶ In a previous study of risk factors related to the healing of diabetic foot ulcers, female gender was similarly associated with a higher incidence of wound closure.¹⁷ The influence of androgen levels on wound healing has been studied in animal models. Ashcroft and Mills, using a mouse wound model, reported that castration of male mice resulted in acceleration of cutaneous wound healing.¹⁸ The authors hypothesized that testosterone may upregulate proinflammatory cytokine expression by macrophages, inhibiting wound healing, and that alternatively, estrogen or progesterone in their models contributed to macrophage activation, driving wound repair, angiogenesis, and tissue remodeling.¹⁹

These findings agree with those of a similar data analysis from the HP802 phase 2 program.²⁰ Although the phase 2 data set was smaller (N = 228), wound size, location, and duration were similarly significant and diabetes and HbA_{1c} level were not, as in the present phase 3 analysis. In the phase 2 analysis, bacterial bioburden—specifically the quantity of bacteria associated with inhibition of wound healing—was inversely associated with healing. This intriguing association was identified after finalization of the designs of the phase 3 studies, so it was unable to be included as a component of these studies. Likewise, BMI was significant in phase 2 but not in phase 3 analysis. This is somewhat counterintuitive in that higher BMI was associated with higher likelihood of healing; this might be a random type I error in the smaller phase 2 data set. Female gender, which was significant and highly predictive of wound closure in the phase 3 analysis, was not significant in phase 2 analysis.

Table III. Demographic and baseline wound characteristics based on wound status at end of treatment^a

Characteristic	All subjects (N = 716)	Wound closed (n = 408)	Wound open (n = 308)	Hazard ratio (95% CI) [P value]	
				Full model	Final model
Age, years	62.7 ± 13.7	62.5 ± 13.8	63.0 ± 13.7	Per year: 0.997 (0.989-1.005) [.4651]	—
Gender				Female vs male: 1.391 (1.129-1.713) [.0019]	Female vs male: 1.384 (1.134-1.690) [.0014]
Male	406 (56.7)	213 (52.2)	193 (62.7)		
Female	310 (43.3)	195 (47.8)	115 (37.3)		
Race				White vs other: 0.999 (0.762-1.309) [.9932]	—
White	593 (82.8)	334 (81.9)	259 (84.1)		
Black	100 (14.0)	59 (14.5)	41 (13.3)		
Other	23 (3.2)	15 (3.7)	8 (2.6)		
Country of residence				United States vs other: 0.945 (0.728-1.225) [.6674]	—
United States	545 (76.1)	310 (76.0)	235 (76.3)		
Other	171 (23.9)	98 (24.0)	73 (23.7)		
BMI, kg/m ²	33.3 ± 9.3	33.1 ± 8.9	33.5 ± 9.8	Per unit increase: 0.998 (0.986-1.011) [.7570]	—
Diabetes mellitus present	194 (27.1)	104 (25.5)	90 (29.2)	Present vs absent: 0.776 (0.581-1.036) [.0853]	Present vs absent: 0.835 (0.658-1.059) [.1364]
Baseline hemoglobin A _{1c} , %	6.1 ± 1.1	6.1 ± 1.1	6.2 ± 1.1	Per percent increase: 1.208 (0.900-1.620) [.2080]	—
Wound duration, months	8.7 ± 6.5	8.3 ± 6.1	10.5 ± 6.8	Per month increase: 0.971 (0.955-0.987) [.0005]	Per month increase: 0.971 (0.955-0.987) [.0005]
Wound area, cm ²	8.6 ± 7.8	6.1 ± 5.7	10.9 ± 9.1	Per cm ² increase: 0.907 (0.886-0.927) [<.0001]	Per cm ² increase: 0.907 (0.887-0.927) [<.0001]
Wound location				Lower leg vs ankle: 1.481 (1.175-1.867) [.0009]	—
Lower leg	504 (100)	297 (58.9)	207 (41.1)		
Ankle	212 (100)	111 (52.4)	101 (47.6)		

CI, Confidence interval; BMI, body mass index.
Categorical variables are presented as number (%). Continuous variables are presented as mean ± standard deviation.
^aEffect size (as hazard ratio) of each putative predictive factor on time to wound closure in univariate and multivariate Cox proportional hazards regression models is also given.

These findings are consistent with other studies of factors predicting closure of VLU lesions. A 12-week study in 165 subjects with VLU identified wound size and duration as predictors of healing, whereas age, gender, race, skin condition, and clinically evident infection were not predictive.²¹ Also, a large cohort study (n >20,000) identified wound size and duration to be strong predictors of healing, whereas age, gender, number of wounds, and wound depth were only weak predictors.²²

Wound duration is consistently associated with poor healing across the HP802 phase 2 and 3 studies as well as in the other studies described before. It is unclear whether this association represents a causal risk or rather reflects the possibility that long-standing wounds are a subset of all wounds that demonstrate recalcitrance to therapy and may represent distinct underlying pathophysiologic processes (for instance, specific bacterial bio-burden). Certainly, wounds of greater duration represent an increased clinical challenge.

Table IV. Target wound closure rate by gender, baseline wound area, duration, and location

Comparison	Parameters	Margolis score	Closure, %	P value
Gender	Female (n = 310)	N/A	62.9	.005
	Male (n = 406)	N/A	52.5	
Baseline duration	≤6 months (n = 337)	0	62.6	.004
	>6 months (n = 379)	1	52.0	
Baseline wound area ^a	≤5 cm ² (n = 344)	0	71.8	<.001
	>5 cm ² (n = 371)	1	43.4	
Wound position	Leg (n = 504)	N/A	58.9	.105
	Ankle (n = 212)	N/A	52.4	
Closure based on Margolis score of 0 and 2 ^b	Duration ≤6 months Area ≤5 cm ²	0	71.8	<.001
	Duration >6 months Area >5 cm ²	2	34.8	

N/A, Not applicable.
^aBy laser-assisted digital imaging.
^bMargolis score⁵ points: wounds ≤5 cm², 0; wounds ≤6 months, 0; wounds >5 cm², 1; wounds >6 months, 1.

The current analysis is strengthened by a large sample size representing a large range of lesion sizes (2-36 cm²). This was, however, a post hoc analysis; the studies included in this pooled evaluation were not specifically designed to evaluate factors predictive of VLU wound closure. We chose to pool the treatment and control groups for this analysis, which assumes no treatment effect and similar natural histories of the two groups. Protocol O29 was carried through to completion, providing a robust, full-powered evaluation of treatment effect (none was found). Protocols O31 and O32 terminated early and may have lacked adequate power to be certain of no treatment effect. However, in all three studies individually and in the pooled intention to treat data set, the wound closure rate in the active treatment groups was mathematically lower than in the control groups. Finally, the impact of the evaluated putative risk factors on healing is limited in this analysis to the ranges of these factors permitted by the eligibility criteria. At extreme values, factors that were deemed insignificant in this analysis may be relevant.

Additional limitations of this study include the variable length of treatment, whereby the larger ulcers in protocol O31 were assessed at 16 weeks rather than 12 weeks, and the fact that protocol O32 was performed in Europe whereas the others were carried out in the United States and Canada. However, the protocol designs were otherwise identical, as were the methods of monitoring the data collected.

In an effort to develop a prognostic scoring system for VLU healing, Margolis et al¹⁵ performed a retrospective cohort study on a group of 260 VLU patients. They assigned 1 point for VLU >5 cm² or 0 if smaller and 1 point for VLU >6 months in duration or 0 if of shorter duration. Ulcers with a combined score of 0 were found to heal 93% of the time by 24 weeks and only 13% of the time if the combined score was 2. This was validated in

another group of 140 patients with 95% closure for those scored 0 and 37% for those scored 2. The present study further confirms this model. Large-long duration ulcers (Margolis score of 2) achieved 34.8% closure (70 of 201) by 12 weeks, whereas small-shorter duration ulcers had 71.8% closure (120 of 167). The lower percentage of closures (71.8%) in this report for the subjects with a score of 0 compared with that of Margolis (95%) may in part be attributable to the 2-week run-in period in the current three studies. At the end of this period, subjects who were healing rapidly and were predicted to close within the treatment period were excluded from the study. Furthermore, the subjects in the Margolis study were evaluated during a 24-week treatment period compared with the 12 to 16 weeks of treatment in the current studies. Continued studies into factors that affect VLU closure may eventually produce a more accurate assessment for predicting VLU closure.

CONCLUSIONS

In this analysis of >700 patients with venous ulcers, wound location, area, and duration were identified as important predictors of healing. Also, gender was found to be important, as women were more likely than men to achieve wound closure. Factors including BMI, the presence of diabetes mellitus, and higher concentrations of HbA_{1c} were not significant independent predictors of wound closure.

The fact that wounds of larger size and longer duration experience significantly lower healing rates would indicate that those who do not heal rapidly with initial treatment should be referred to specialists to consider more aggressive management. The findings from this analysis have implications for future trials of interventions to promote VLU wound closure. Eligibility criteria pertinent to factors found to be insignificant predictors of VLU healing can perhaps be relaxed in future studies, which

may increase the pool of eligible study subjects and therefore shorten enrollment time and reduce the length and cost of future studies. In contrast, factors found to be significant should be considered stratification variables in future studies to ensure even distribution among treatment groups to minimize the risk of confounding.

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APPENDIX (online only).

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