Healing of Chronic Venous Ulcers Is Not Enhanced by the Addition of Topical Repifermin (KGF-2) to Standardized Care

Martin C. Robson, MD[a,b]
Jason Hanfnt, DPM[c]
Warren Garner, MD[d]
Jeffrey Jenson, DPM[e]
Thomas Serena, MD[f]
Wyatt G. Payne, MD[a,b]
Anthony Sussman, MD[g]
Adrian Barbul, MD[h]
Markete Limova, MD[i]
Robert Snyder, DPM[j]
Daniel J. Odenheimer, PhD[k]
Diane M. Cooper, PhD[k,l]
KGF-2 Venous Ulcer Study Group[m]

[a]*The Institute for Tissue Regeneration, Repair, and Rehabilitation, Department of Veterans Affairs Medical Center, Bay Pines, Florida*
[b]*Division of Plastic Surgery, Department of Surgery, University of South Florida, Tampa, Florida*
[c]*Foot and Ankle Institute of South Florida, Miami, Florida*
[d]*LAC-USC Medical Center, Los Angeles, California*
[e]*Diabetic Foot and Wound Center, Denver, Colorado*
[f]*Penn North Center for Advanced Wound Care, Warren, Pennsylvania*
[g]*Memorial Health University Medical Center, Savannah, Georgia*
[h]*Siani Hospital of Baltimore, Baltimore, Maryland*
[i]*Sierra Medical Research, Fresno, California*
[j]*Taramac, Florida*
[k]*Human Genome Sciences, Rockville, Maryland*
[l]*Johnson and Johnson Wound Management, Somerville, New Jersey*
[m]*The remaining investigators are listed in the acknowledgement at the end of the article.*

KEY WORDS: Growth factor, venous ulcer, KGF-2, compression therapy

ABSTRACT

Background: It is estimated that 2.5 million people in the United States have chronic venous ulcers. Standard care for these ulcers includes compression, debridement of necrotic tissue, treatment of edema, and control of infection. There has been a search for additional therapeutic compounds and/or devices to enhance ulcer healing beyond that provided by standard care. A previous phase 2a clinical trial suggested repifermin (KGF-2) might enhance ulcer healing.

Materials and Methods: A randomized, double-blinded, parallel group, multi-center clinical trial was conducted to evaluate the safety and efficacy of topical repifermin treatment, for up to 26 weeks, in the healing of chronic venous ulcers in 352 patients.

Results: The percent of ulcers achieving 100% closure by 20 weeks was not significantly different in ulcers treated with 60 μg/cm^2 or 120 μg/cm^2 of repifermin compared with placebo treatment. Similarly, there was no difference in total healing among the 3 treatment groups at 26 weeks or at 16 weeks. Time to closure was also statistically identical for the 3 treatment groups. Safety analyses showed that topical repifermin is well-tolerated.

Conclusion: Healing of chronic venous ulcers is not enhanced by the addition of topical repifermin (KGF-2) to standardized care.

INTRODUCTION

It is estimated that two and a half million people in the United States have chronic venous ulcers.[1,2] These ulcers result in two million lost work days at a cost of two to four billion dollars for treatment.[1,2] Only 50 to 60% of venous ulcers will heal within 6 months of treatment.[3,4] Even if complete healing of the venous ulcer occurs, recurrence is a major clinical problem. It has been reported that up to 70% of venous ulcers can recur after complete healing.[5,6]

The cornerstone of standard care of chronic venous ulcers involves application of compression therapy.[1,7] Other treatment includes debridement, treatment of edema, and control of infection. A more specific therapeutic approach would be to promote the proliferation and migration of epithelial cells to restore the epithelium.[7]

Repifermin (a truncated form of Keratinocyte growth factor-2 [KGF-2]) selectively promotes proliferation and migration of keratinocytes in culture.[7] Repifermin has shown, in both in vitro and in vivo experiments, a highly selective action on epithelial cells.[7] In addition to promoting re-epithelialization by a direct effect on keratinocytes causing them to proliferate and migrate, repifermin may also stimulate granulation tissue formation by a direct effect on fibroblasts.[8]

After extensive in vitro and in vivo preclinical studies suggested that repifermin would be effective in accelerating closure of wounds healing largely by the process of epithelialization, a phase 2a multi-center, randomized, double-blinded, parallel group, placebo-controlled, venous ulcer clinical trial was performed to assess the safety and efficacy of two different dose levels of repifermin (20 μg/cm^2 and 60 μg/cm^2) compared to placebo, following twice weekly topical applications for a period of 12 weeks.[7,9,10]

Repifermin was shown to be safe and well-tolerated in the clinical trial. Venous ulcer healing was evaluated by a number of parameters including complete wound closure, partial wound closure, and percent area healed. There was no difference in total ulcer healing in 12 weeks in the two repifermin-treated groups compared to the placebo. However, trends in favor of repifermin were observed for many of the parameters, including the percentage of subjects achieving partial wound closure and total area of the ulcer healed.[7] The difference between repifermin and placebo treatment was statistically significant for the 75% healed endpoint when the results from the 2 repifermin treatment groups were combined.[7] The treatment effect of repifermin appeared more marked for a subgroup of patients with initial ulcer areas ≤15cm^2 and wound ages of ≤18 months. In this subgroup,

statistically more ulcers treated with repifermin reached 75% closure and 90% closure than did placebo-treated ulcers.

The phase 2a clinical trial results suggested that there was a dose response effect with more patients with 90% or 100% closure being treated with the 60 μg/cm^2 dose of repifermin than with the 20 μg/cm^2 dose. Because ulcers in that trial tended to achieve 75% or 90% closure more frequently than 100% closure, it was concluded that 12 weeks may not have been a sufficient treatment period. Therefore, it was decided to perform a phase 2b clinical trial using a larger dose of repifermin and applying it for a longer period in an attempt to enhance healing of chronic venous ulcers.

MATERIALS AND METHODS

The study was designed to assess the efficacy and safety of 2 different dose-levels of repifermin (60 μg/cm^2 and 120 μg/cm^2) compared with placebo following twice-weekly topical application for up to 26 weeks. Topical therapy was in addition to conventional compression therapy and a standardized protocol of good ulcer care. It was conducted using a randomized, double-blinded, parallel group, multi-center design. The treatment period was preceded by up to a 48 day screening phase for evaluation of subject eligibility and standardized wound care. Follow-up visits at 1 and 4 weeks after the last dose of study agent were used to evaluate wound healing and drug safety. In addition, a follow-up visit 13 weeks following the last dose of study agent was used to assess the durability of response for subjects who were healed during the trial or at the early follow-up visit. All participants provided informed consent prior to the start of the protocol. Each institution's Human Investigation Committee gave approval to the protocol before any patients were enrolled.

Application of Growth Factor

A truncated form of KGF-2 was used in this study. It was expressed in E. coli and was purified by a series of chromatography and filtration procedures. The repifermin was prepared as a sterile lyophilized product. Upon reconstitution with 5.0 mL of bacteriostatic water for injection, each vial contained 1.0 or 2.0 mg/mL of repifermin (depending on the applied dose), 2% glycine, 0.5% sucrose, 10 mM sodium citrate, 20mM sodium chloride, 1 mM ethylenediamine tetra acetic acid disodium salt, 0.12% methyl paraben, and 0.12% propyl paraben, pH 6.2. The placebo was also prepared as a sterile lyophilized product. Upon reconstitution with 5.0 mL of bacteriostatic water for injection, each vial contained identical additives as the repifermin vials and a pH of 6.2. Once the repifermin or placebo was reconstituted, it was used within 5 days.

Subjects received 1 of 2 dose levels of repifermin (60 μg/cm^2 or 120 μg/cm^2) or placebo-control administered by topical spray twice weekly (separated by 2 to 3 days) for 26 weeks or until the ulcer healed, whichever was shorter. The ulcer edge was traced to determine its area on the first visit of each week. The area of the ulcer was used to calculate the amount of the study agent sprays for the subsequent week. The agent was sprayed 2 to 3 inches from the ulcer surface. Five minutes after application of the study agent, the wound was covered with a petrolatum-impregnated gauze (ADAPTIC, Johnson and Johnson Wound Management, Somerville, NJ) and then the leg encased in a multi-layer sustained, graduated compression bandage system (DYNAFLEX, Johnson and Johnson Wound Management, Somerville, NJ) which was only removed by study personnel during a study visit.

Protocol Design

The primary objective of the study was

Table 1. Inclusion Criteria

To be eligible for entry into this study, a subject must have met the following inclusion criteria:

- Have evidence of venous insufficiency documented by venous duplex scanning or impedance plethysmography.
- Have a venous ulcer with a wound size of 3 to 25 cm^2.
- Have been in prescribed compression for at least 7 consecutive days immediately prior to randomization, but not more than 28 consecutive or non-consecutive days.
- Have a wound that has been unhealed continuously for 3 to 36 months prior to treatment.
- Be available for twice-weekly visits.
- Be willing and able to comply with the protocol.

to evaluate the efficacy of topically administered repifermin in achieving complete wound closure within 20 weeks of initiating treatment. Secondary efficacy analyses included: the percentage of subjects achieving complete wound closure within 26 weeks of initiating treatment, the percentage of subjects achieving complete ulcer healing within 16 weeks, the time required to achieve complete ulcer healing, and the percent change in ulcer size from baseline to the final on-therapy wound measurement. In addition to the efficacy objectives, safety of topically administered repifermin was evaluated.

Based on the assumption that approximately 50% of subjects in the placebo group would show complete healing during the 20 week treatment period, a sample size of at least 113 subjects per group would provide a greater than 80% power to detect an absolute 20% difference in complete ulcer closure rates between placebo and either of the repifermin treatment groups at the 2.5% level of significance using a 2–sided test.

There was a screening period of up to 48 days prior to study enrollment. At the first visit of the screening period, the ulcer was debrided if necrotic tissue was present, to reveal a clean ulcer bed. The ulcer was photographed, traced for planimetry, and a quantitative tissue biopsy performed for bacterial analysis.[11] Following the biopsy, the ulcer was dressed with ADAPTIC gauze and placed in DYNAFLEX compression dressing. If the biopsy was not supportive of an active wound infection (background colony count of $< 10^6$ CFU/gram of tissue and no beta-hemolytic streptococci[12]), compression was continued until the period of compression totaled at least 7 days at which time the application of the study treatment was initiated. If the biopsy was supportive of an active wound infection (bacterial count $\geq 10^6$ CFU/gram of tissue or any beta-hemolytic streptococci), the infection was treated with systemic or topical antimicrobials for at least 10 days with the P.I. allowed to select a compression device of his/her choice which would facilitate self-application of a topical treatment by the subject. Up to 3 tissue biopsies were allowed to achieve an ulcer in bacterial balance.[12] Once the ulcer was in bacterial balance and protocol compression had been used for at least 7 days, the ulcer was re-photographed and traced for the randomization planimetry.

Prior to randomization, eligibility criteria had to be met. Inclusion criteria for the study are enumerated in Table 1. Exclusion criteria are listed in Table 2.

Measures of Treatment Efficacy and Statistics

Efficacy evaluations were made using wound areas measured by computer planimetry from wound tracings. Analyses of all end points were performed on the intention-to-treat popula-

Table 2. Exclusion Criteria

To be eligible for entry into the study, the subject must *not*:

- Have participated in a clinical trial of an investigational agent within the last 30 days.
- Have been treated with repifermin (KGF-2).
- Have the designated ulcer below the malleolus, on the foot, or above the base of the knee.
- Have had the study ulcer treated with Regranex (PDGF-BB) within the last 30 days.
- Have had the study ulcer treated at any time with a skin substitute or an autologous growth factor.
- Have had a surgical procedure to treat venous or arterial disease within the last 90 days.
- Have evidence of significant arterial insufficiency (an ankle brachial index of < 0.8). Subjects with an ABI > 1.2 must have a toe brachial index of > 0.6 or a supine transcutaneous oxygen measurement ($TcPO_2$) > 30 mmHg.
- Have clinical evidence of active infection at the ulcer site.
- Have a granulation tissue colony count ≥ 10^6/gram of tissue or beta-hemolytic *streptococci* at any level.
- Have evidence of active vasculitis, cellulitis, or collagen vascular disease.
- Have a history of malignant neoplasm within the last 5 years, except for adequately treated cancers of the skin or uterine cervix.
- Have significant acute or chronic diseases (ie, cardiovascular, pulmonary, gastrointestinal, hepatic, renal, neurological, or infectious diseases), which are not adequately controlled by medical treatment as determined by the investigator's judgment.
- Have diabetes mellitus with a hemoglobin A1c ≥8%.
- Have an active skin disease, such as psoriasis, which could impair the ability to assess the wound.
- Have an allergy to the dressings used in the study.
- Require treatment to the study ulcer with any topical agent other than normal saline within 7 days of the first repifermin / placebo treatment.
- Require treatment of the study ulcer with topical lidocaine for anesthesia prior to study ulcer debridement after the first repifermin / placebo treatment.
- Require concomitant use of pentoxifylline or clopidrogrel bisulfate during the study.
- Have undergone enzymatic debridement at any time during the screening period.
- Have a known history of allergies to *Escherichia coli*-derived or paraben-containing products.
- Have any requirement for the use of systemic steroids or immunosuppressive or cytotoxic compounds during the period of the study.
- Expected to undergo hyperbaric oxygen therapy at any time during treatment or through the 4 week follow-up visit.
- Be a pregnant female or nursing mother. All females with an intact uterus, regardless of age must have a negative serum pregnancy test result at screening and use contraception during the study.
- During the first 20 weeks of the study be expected to miss 3 or more consecutive visits or be expected to miss 2 consecutive visits more than once.

tion, defined as anyone randomized. The study was designed to test the null hypothesis that the percentage of subjects with complete ulcer closure at 20 weeks would be no different for the placebo group compared with the 60 µg/cm^2 repifermin-treated group or for the placebo group compared with the 120 µg/cm^2 repifermin-treated group. All tests were performed at the two-sided 2.5% level. All analyses were performed using the SAS software version 8.0 or later and R statistical software version 1.51 or later.

All baseline covariants were assessed to determine differences between the treatment groups. For categorical variables like gender and race, the likelihood ratio chi-squared test was used. For continuous variables like age,

Table 3. Study Completion Status

Treatment Groups	Placebo (n=117)	60µg/cm² (n=123)	120µg/cm² (n=112)	*P* Value
Subjects completed	102 (87.2%)	105 (85.4%)	92 (82.1%)	0.5628
Subjects discontinued due to:				
Subject request	2 (1.7%)	6 (4.9%)	4 (3.6%)	
Adverse Event	6 (5.1%)	6 (4.9%)	4 (3.6%)	
Protocol Violation	1 (0.09%)	2 (1.6%)	6 (5.4%)	
Lost to Follow-up	2 (1.7%)	3 (2.4%)	2 (1.8%)	
Death	2 (1.7%)		2 (1.8%)	
Other	2 (1.7%)	1 (0.8%)	2 (1.8%)	

height, weight, duration, and size of ulcer, ANOVA methods were used to assess differences between treatments.

The time to complete wound closure was compared across treatment groups using Kaplan-Meier methods. The percent change from baseline in the area healed was compared across the treatment groups using the ANOVA methods.

Safety Assessment

Safety evaluation criteria included the frequency, severity, and duration of adverse events (AE's), including changes from baseline of laboratory test parameters. The likelihood ratio chi-squared test was utilized to test if the occurrence of AE's was similar across treatment groups. Changes from baseline to endpoint in laboratory parameters were assessed using ANOVA methods.

RESULTS

A total of 352 patients were enrolled into the trial by 55 different investigators. Nine investigators enrolled at least 11 patients each and accounted for 174 of the subjects. The treatment assortment for enrolled patients was 117 for placebo treatment, 123 for repifermin 60 µg/cm², and 112 for repifermin 120 µg/cm². The number of subjects that completed the study was 102 placebo, 105 repifermin 60 µg/cm², and 92 repifermin 120 µg/cm² (Table 3).

As shown in Table 4, treatment groups were comparable for race, mean age, duration of the ulcer and size of the ulcer at time of randomization / enrollment into the study ($P > 0.05$). The percentage of males enrolled into the 120 µg/cm² repifermin treatment group was significantly less than the percentage of males randomized into the other 2 treatment groups ($P = 0.0361$).

Efficacy Outcomes

The percent of ulcers achieving complete closure by 20 weeks in the 60 µg/cm² repifermin treatment group (58.5%) or the 120 µg/cm² repifermin treatment group (51.8%) was not statistically different from the placebo-treated ulcers (61.5%) (Figure 1). The *P* values were 0.6351 and 0.1362 respectively. By 26 weeks of therapy more ulcers reached 100% healing in each treatment group but the differences between groups were not significant ($P = 0.7906$ for 60 µg/cm²repifermin vs placebo; $P = 0.1774$ for 120 µg/cm² repifermin vs placebo) (Figure 2). As expected, at 16 weeks, the closure rates were less, but still no statistical differences among treatment groups were demonstrated (Figure 3). Kaplan-Meier analysis of time to closure also showed no significant differences between placebo-treated ulcers and 60 µg/cm² repifermin ($P = 0.9849$) or 120 µg/cm²

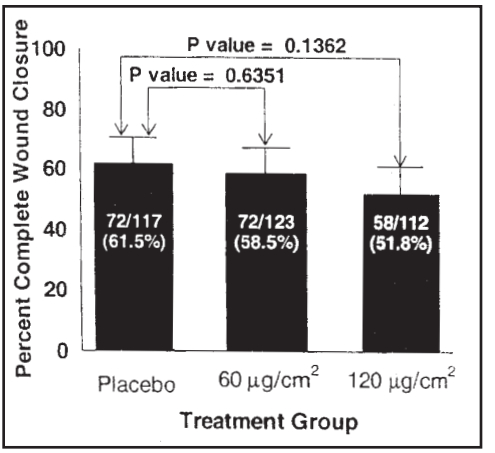

Figure 1. The primary efficacy analysis of percent of subjects attaining 100% ulcer healing by 20 weeks of treatment demonstrated no significant differences among the 3 treatment groups.

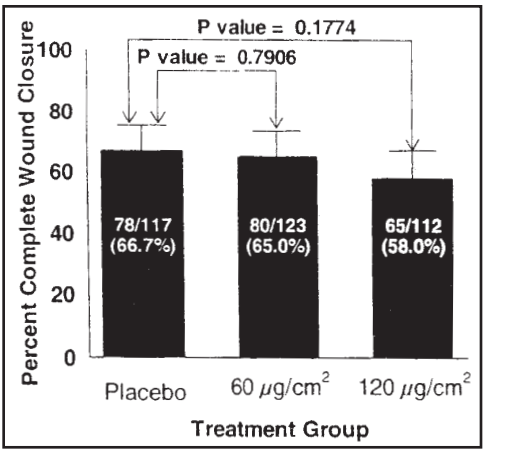

Figure 2. Extending treatment to 26 weeks increased the number of ulcers in each treatment group that attained complete ulcer closure, but showed no statistical differences among the 3 treatment groups.

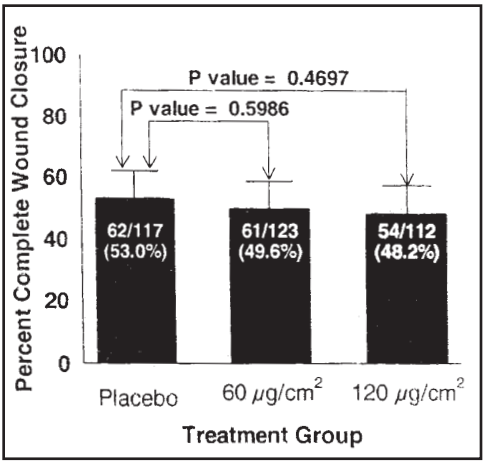

Figure 3. Earlier evaluation of complete ulcer healing at 16 weeks did not show acceleration of healing by any specific treatment group and there were no significant differences among the 3 treatment groups.

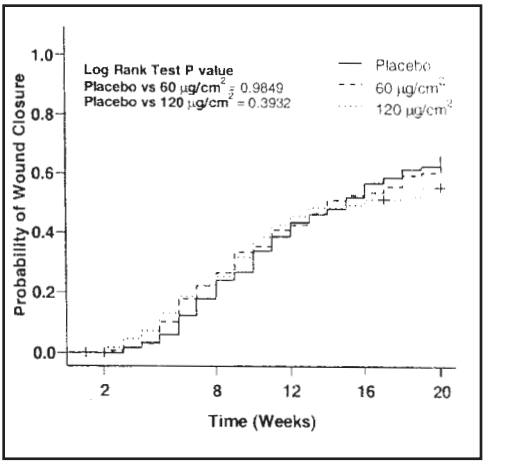

Figure 4. Time to ulcer closure was statistically identical between the repifermin-treated ulcers and the placebo-treated ulcers.

repifermin ($P = 0.3932$) (Figure 4). The mean percent change in ulcer size from time of enrollment to week 20 was not statistically different for the 3 treatment groups ($P > 0.05$).

Repifermin was well tolerated in this study. There were no significant differences in the number or severity of adverse events (AE's) reported among the 3 treatment groups (Table 5).

DISCUSSION

Venous ulcers have a prevalence of approximately one percent in the United States, with approximately 50% of the patients having an ulcer history of 10 years.[13,14] Despite standard therapy with compression dressings, only 50 to 60% of venous ulcers heal completely within 6 months and the recurrence rate is as high as 70%.[3-6,15,16] Direct and indirect costs associated with the treatment

Table 4. Demographics

	Placebo (n=117)	60µg/cm² (n=123)	120µg/cm² (n=112)	*P* Value
Sex				
				0.0361
Male	81 (69.2%)	76 (61.8%)	59 (52.7%)	
Race				0.08861
White	78 (66.7%)	74 (60.2%)	69 (61.6%)	
Black	25 (21.4%)	30 (24.4%)	25 (22.3%)	
Hispanic	12 (1.7%)	1 (0.8%)	2 (1.8%)	
Other	2 (1.7%)	1 (0.8%)	2 (1.8%)	
Age (Years)				0.08785
Mean ± SD	61.0 ± 15.4	60.9 ± 13.7	61.8 ± 15.6	
Duration of Ulcer (Months)				0.4540
Mean ± SD	11.8 ± 9.3	12.3 ± 9.2	8.7 ± 5.3	
Median	7.0	9.0	6.0	
Range	(3.0, 35.0)	(3.0, 36.0)	(3.0, 36.0	
Ulcer size at randomization (cm²)				0.2779
Mean + SD	9.9 ± 6.2	9.4 ± 5.4	8.7 ± 5.3	
Median	7.4	8.4	7.1	
Range	(3.0, 24.3)	(3.0, 25.0)	(2.4, 22.9)	

of these ulcers are considerable.[17] Patients affected with venous ulcers experience a decrease in their overall health-related quality of life, including severe pain, impaired mobility, limited work capacity, and negative emotions.[15,18]

Treatment of venous stasis ulcers varies from multiple surgical procedures to the use of various topical agents combined with external pressure support to the legs.[19,20] Standard care for these ulcers includes compression therapy, debridement of necrotic tissue, treatment of edema, and control of infection.[1,2,12] In the United States, the standard compression dressing is either the Unna boot, a zinc-oxide impregnated inelastic dressing, the Duke boot with an added elastic bandage, or the multiple-ply compression dressing.[5,21] The method chosen for this study was the DYNAFLEX multi-layer, sustained and graduated compression device. This is considered a Class 3 (most supportive) dressing.[1] A recent meta-analysis of the compression therapy literature reveals that high compression such as used in this study was more effective than low compression, but failed to identify any particular procedure or product as more effective than the other.[1,16]

High bacterial burdens have been shown to interfere with healing.[12] Each of the various processes of the wound healing scheme has been reported to be inhibited by high levels of bacteria.[22] Controlling bacterial levels is particularly important when proteins such as growth factors are used for wound treatment. Growth factors have been demonstrated to be degraded by the common bacteria that populate chronic wounds.[23] It has been recommended that the bacterial burden be controlled during conduct of clinical trials involving growth factors.[23,25]

Based on the results of the Phase 2a clinical trial previously performed evaluating repifermin (KGF-2) at 20 µg/cm² and 60 µg/cm² for 12 weeks, it was hypothesized that larger doses given for a longer time period would enhance healing of venous ulcers beyond that

Table 5. Summary of Adverse Events

	Placebo (n=117)	60 µg/cm² (n=123)	120 µg/cm² (n=112)	*P* Value
At Least One AE	89 (76.1%)	83 (67.5%)	79 (70.5%)	0.3270
At Least One Related AE	9 (7.7%)	13 (10.6%)	10 (8.9%)	0.7390
At Least One Serious AE	19 (16.2%)	13 (10.6%)	10 (8.9%)	0.2070
At Least One Severe AE	12 (10.3%)	9 (7.3%)	12 (10.7%)	0.6097

TABLE 6. Subgroup Analysis

120µg/cm2	Placebo (n=117)	60 µg/cm2 (n=123)	120 µg/cm2 (n=112)	*P* Value vs 60 µg/cm2	*P* Value vs
≤ 15 cm2	64 / 96 (66.7%)	66 / 102 (64.7%)	55 / 96 (57.3%)	0.7715	0.1805
3 – 18 months	63 / 91 (69.2%)	56 / 94 (59.6%)	50 / 92 (54.3%)	0.1698	0.0378
≤ 15 cm2 and 3 – 18 months	55 / 76 (72.4%)	52 / 79 (65.8%)	48 / 80 (60.0%)	0.3777	0.1020

provided by standardized care.[7] The data from this study show that was not the case. The 61.5% and 66.7% incidence of complete healing for the placebo-treated patients at 20 to 26 weeks respectively was comparable to the better outcomes for compression therapy in the literature.[1,3,4,16] These healing percentages were not enhanced by the addition of topical repifermin therapy (Figures 1 and 2). Similarly, time to closure was not speeded by the addition of repifermin (Figure 4), nor was the decrease in ulcer size.

In the previously reported clinical trial using repifermin for the treatment of chronic venous ulcers, a subgroup of patients with ulcers ≤15 cm² in area and a wound age ≤ 18 months appeared to have a better response to repifermin.[7] Therefore, a subgroup analysis was performed on the data from the present study. Table 6 demonstrates that there were no significant differences demonstrated on the smaller ulcers. The placebo treatment actually did better than repifermin in the ulcers of shorter duration and this difference reached significance when the placebo-treated ulcers were compared with the 120 µg/cm² repifermin-treated ulcers ($P = 0.0378$). When one combined ulcers of smaller size (≤ 15 cm²) and shorter duration (3 to 18 months), no significant differences were seen among treatments (Table 6).

The safety profile was expected. In the previous study of 94 patients treated with topical repifermin, it proved to be well-tolerated and non-immunogenic.[7] In conclusion, healing of chronic venous ulcers is not enhanced by the addition of topical repifermin (KGF-2) to standardized care.

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