Check for updates

Received: 28 April 2021 | Revised: 27 August 2021 | Accepted: 5 October 2021

DOI: 10.1111/wrr.12977

ORIGINAL RESEARCH-CLINICAL SCIENCE

Wound Repair and Regeneration — WILEY

Evaluation of LL-37 in healing of hard-to-heal venous leg ulcers: A multicentric prospective randomized placebo-controlled clinical trial

Margit Mahlapuu PhD[1] | Adam Sidorowicz MD[2] | Jacek Mikosinski MD, PhD[3] | Mikołaj Krzyżanowski MD[4] | Jakub Orleanski MD[5] | Krystyna Twardowska-Saucha MD[6] | Andrzej Nykaza MD[7] | Michal Dyaczynski MD, PhD[8] | Beata Belz-Lagoda MD[9] | Grzegorz Dziwiszek MD[10] | Monika Kujawiak MD[11] | Marek Karczewski MD, PhD[12] | Folke Sjöberg MD, PhD[13] | Tomasz Grzela MD, PhD[14] | Adam Wegrzynowski MD, PhD[15] | Fredrik Thunarf MSc[16] | Jakob Björk PhD[1] | Jonas Ekblom PhD[1] | Arkadiusz Jawien MD, PhD[17] | Jan Apelqvist MD, PhD[18]

[1]Promore Pharma AB, Stockholm, Sweden

[2]ETG Warsaw, Warsaw, Poland

[3]NZOZ MIKOMED, Łódź, Poland

[4]Allmedica Badania Kliniczne, Nowy Targ, Poland

[5]EZ-MED Swidnica, Swidnica, Poland

[6]Specjalistyczna Pomoc Medyczna, Zabrze, Poland

[7]Centrum Medyczne Pratia Warszawa, Warsaw, Poland

[8]Centrum Medyczne Angelius Provita, Katowice, Poland

[9]ETG Zamość, Zamość, Poland

[10]CM Pratia Ostrołęka, Ostrołęka, Poland

[11]ETG Siedlce, Siedlce, Poland

[12]Solumed Centrum Medyczne, Poznań, Poland

[13]Burn Center, Linköping University Hospital, Linköping, Sweden

[14]Clinic of Phlebology and the Medical University of Warsaw, Warsaw, Poland

[15]Angiodiabetica Vascular and Diabetic Foot Clinic, Poznan, Poland

[16]LINK Medical Research AB, Uppsala, Sweden

[17]Department of Vascular Surgery and Angiology, Ludwik Rydygier Collegium

Abstract

Many patients with venous leg ulcers do not reach complete healing with compression treatment alone, which is current standard care. This clinical trial HEAL LL-37 was a phase IIb double-blind, randomized, placebo-controlled study, with the aim to evaluate the efficacy and safety of a new drug LL-37 for topical administration, in combination with compression therapy, in 148 patients suffering from hard-to-heal venous leg ulcers. The study had three arms, consisting of two groups treated with LL-37 at concentrations of 0.5 or 1.6 mg/mL, and a placebo cohort. Patients had a mean age of 67.6 years, a median ulcer duration of 20.3 months, and a mean wound size at the time of randomization of 11.6 cm^2. Efficacy analysis performed on the full study population did not identify any significant improvement in healing in patients treated with LL-37 as compared with the placebo. In contrast, a post hoc analysis revealed statistically significant improvement with LL-37 treatment in several interrelated healing parameters in the subgroup of patients with large target wounds (a wound area of at least 10 cm^2 at randomization), which is a known negative prognostic factor for healing. The study drug was well tolerated and safe in both dose strengths. In summary, this clinical trial did not detect any significant differences in healing of venous lower leg ulcers in the entire study cohort comparing patients treated

Krystyna Twardowska-Saucha is deceased.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2021 Promore Pharma AB. *Wound Repair and Regeneration* published by Wiley Periodicals LLC on behalf of The Wound Healing Society.

Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Torun, Poland
[18]Department of Endocrinology, University Hospital of Malmö, Malmö, Sweden

Correspondence
Margit Mahlapuu, Promore Pharma AB, Stockholm, Sweden.
Email: margit.mahlapuu@promorepharma.com

Funding information
Promore Pharma AB

with LL-37 versus placebo. A subgroup analysis provided an interesting observation that LL-37 could offer a treatment benefit in patients with large ulcers, exigently warranting a further study adequately powered to statistically assess the treatment outcome in this patient group.

KEYWORDS
LL-37, phase II clinical trial, venous leg ulcers, wound healing

1 | INTRODUCTION

Venous leg ulcers (VLUs) are the most prevalent type of chronic wounds, affecting approximately 1%–3% of the older population in Western countries.[1,2] These ulcers are painful and distressing, and are responsible for a considerable impairment in patients' quality of life (QoL).[3] Even with appropriate wound management using external compression therapy, about 50% of VLUs remain unhealed after 12 months,[4] with the recurrence rate within 3 months after wound closure as high as 70%.[2] In spite of intense nonclinical and clinical research in this area, there are no pharmaceutical prescription products approved today for the oral or topical treatment of hard-to-heal (HTH) VLUs. Thus, there is an urgent medical need for novel effective pharmacological treatments to advance chronic leg ulcer therapy.

LL-37 is one of the investigational medicinal products (IMP) currently under clinical development for the treatment of HTH VLUs. LL-37 is a 37-meric peptide derived by proteolytic cleavage from the 18-kDa human cathelicidin antimicrobial protein (hCAP18). Initial observation that endogenous LL-37 was abundantly present in acute wounds, but was absent in chronic wounds, suggested a role of this peptide in natural wound healing.[5,6] Furthermore, local administration of LL-37 has been shown to stimulate healing of chronic wounds in experimental animals and in an ex vivo model of human acute wounds,[6–11] and reciprocally, antibodies against LL-37 have been demonstrated to impair healing.[6] Consistent with these observations, a phase I/II clinical trial LL-37001B (EudraCT: 2012-002100-41) revealed that supplementation of synthetic LL-37 to nonhealing VLUs significantly enhanced the healing rate without causing any systemic safety or local tolerability concerns.[12] This first-in-man trial recruited 34 VLU patients in Sweden and comprised a 3-week run-in period on placebo, followed by a 4-week randomized double-blind treatment phase with twice weekly local applications of LL-37 (0.5, 1.6, or 3.2 mg/mL) or placebo, and a 4-week follow-up. The healing rate constants for the two lower doses of LL-37 were about sixfold and threefold higher than for placebo, respectively ($P = 0.003$ for 0.5 mg/mL and $P = 0.088$ for 1.6 mg/mL), whereas no improvement was observed in patients receiving the highest dose of 3.2 mg/mL of LL-37.[12] Notably, the mechanism by which LL-37 increases healing is not fully understood, but likely involves regulation of several physiological processes including wound re-epithelialization, angiogenesis, and inflammation.[13]

The aim of this study was to characterize the efficacy and safety of LL-37 in patients with HTH VLUs. This phase IIb trial comprised a 3-week, open-label, run-in phase on placebo, followed by a 13-week randomized double-blind treatment phase with twice weekly local applications of LL-37 (0.5 or 1.6 mg/mL) or placebo, and a 4-months follow-up, and was performed in 149 participants recruited in Poland and Sweden.

2 | MATERIALS AND METHODS

2.1 | Overall study design

The clinical study protocol, the amendments, and information provided to patients were reviewed and approved by the independent ethics committees (IECs) in Sweden and Poland, in accordance with regional requirements. The study was reviewed and approved by the Swedish Medical Products Agency and the Polish Office for Registration of Medicinal Products, Medical Devices, and Biocidal Products, before the start of patient recruitment. The trial was performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki that are consistent with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)/Good Clinical Practice (GCP) and applicable regulatory requirements. Informed consent was obtained from all participants prior to initiation of the study.

This was a phase IIb, double-blind, randomized, placebo-controlled, parallel-group efficacy, and safety study in patients of HTH VLUs conducted at 15 sites in Poland and Sweden (EudraCT: 2018-000536-10; Figure 1 displays the schematic presentation of the study design). The first patient's first visit in the study (first patient screened) was on 26 September 2018, the first patient was randomized and treated on 15 October 2018, the last patient's last visit in the treatment period was on 20 March 2020, and the last patient's last follow-up visit was on 13 July 2020. In an attempt to restrict investigation to participants with HTH ulcers, patients entered a 3-week, open-label, run-in period involving standard compression therapy and application of placebo. Participants with a mean weekly decrease in ulcer area during the run-in period of >7% (initial area > 10 cm^2) or >10% (initial area 2 to ≤10 cm^2) were excluded from the study and recorded as screening failures. At the end of the run-in period, the eligible participants underwent baseline assessments before randomization to receive either placebo or active treatment with LL-37 (0.5 or 1.6 mg/mL). A computer-generated randomization schedule (following a permuted block design) was

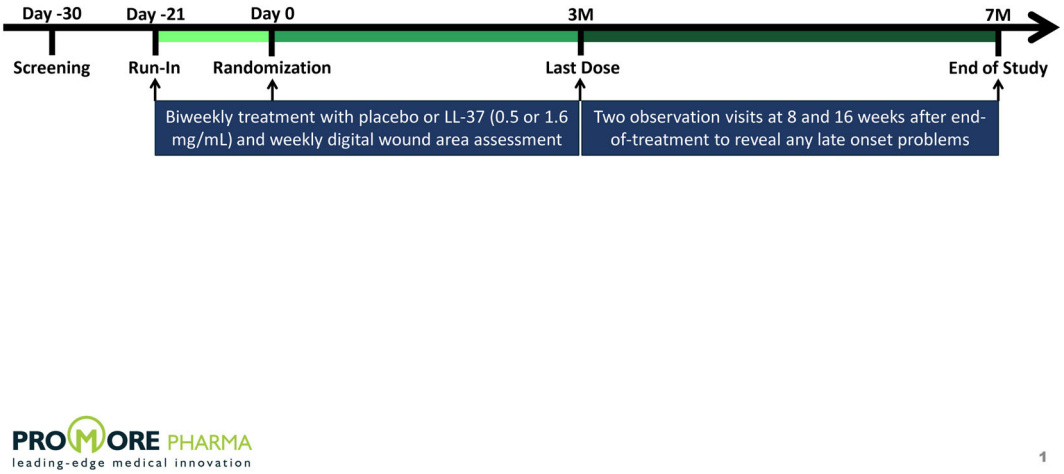

FIGURE 1 Schematic presentation of the study design. The trial comprised a 3-week, open-label, run-in phase on placebo, followed by a 13-week randomized double-blind treatment phase with twice weekly applications of LL-37 (0.5 or 1.6 mg/mL) or placebo, and a 4-months follow-up period

used to assign each eligible patient to one of the three treatment groups (allocation ratio 1:1:1). The randomization schedule was stratified by study site and wound size (<10 cm^2 or ≥10 cm^2) based on investigator's assessment. During the 13-week double-blind treatment period, the patients received twice weekly local applications of LL-37 (0.5 or 1.6 mg/mL) or placebo, which was followed by a 16-week observation (follow-up) period to reveal any late onset problems. In case the target wound was assessed as closed during the treatment period, four post-wound closure visits were scheduled every third day (±1 day), not more than two times per week, during the 2 weeks. The purpose of the post-wound closure visits was to ensure that the patient applied proper standard wound care to avoid that the wound opened due to lack of proper compression therapy.

2.2 | Study population

Eligible participants were males aged 18 years or over and postmenopausal women who were diagnosed with venous or mixed arteriovenous leg ulcers with predominant venous component (ankle brachial pressure index [ABPI] >0.70), with an area of 2–40 cm^2, which had not healed after 6 weeks of standard therapy. The list of inclusion and exclusion criteria is provided in Table 1.

2.3 | Sample size justification

The main aim of this study was to detect a difference in complete wound closure between the lower dose of LL-37 (0.5 mg/mL) and placebo, as the lower dose was likely to have a greater effect than the higher dose (1.6 mg/mL) based on the results from the previous study LL-37001B. The individual rates of wound area reduction over the 4 weeks of treatment observed in the previous study were used to predict the percentage of patients with complete wound closure. Assuming a response rate of 25% or 30% for placebo and a treatment effect expressed as an odds ratio (OR) (low dose of LL-37 vs. placebo) of at least 3.3, a sample size of between 30 and 39 patients per group would have 80% power to achieve the stated aim, using a one-sided test with a significance level of $\alpha = 0.05$. Based on this estimation, the aim was to have a total of 120 patients (40 per group) completing the treatment period. Assuming a drop-out rate of 15%, a total of 141 randomized patients were required. A total of 149 patients, instead of 141 patients, were randomized, as it was not considered ethical to withdraw patients who had already entered the run-in period.

2.4 | Study objectives

The primary objective of the study was to determine the efficacy of LL-37, at concentrations of 0.5 and 1.6 mg/mL, in increasing the incidence of complete wound closure compared with placebo in the treatment of HTH VLUs. The secondary objectives of the study were to determine the efficacy of LL-37, at concentrations of 0.5 and 1.6 mg/mL, in promoting wound healing in relation to secondary efficacy endpoints, as well as to evaluate local tolerability and safety of LL-37, compared with placebo in the treatment of HTH VLUs. The list of outcome measures is provided in Table 2.

2.5 | Drug substance, drug product, and diluent

The active substance used in this trial, LL-37, is a synthetic peptide with an amino acid sequence identical to the human endogenous wound healing peptide LL-37. The drug substance acetate salt of LL-37 was manufactured by AmbioPharm Inc., (North Augusta, North Carolina), using solid phase peptide synthesis applying the Fmoc (9-fluorenylmethyloxycarbonyl) strategy. A purification step was performed by preparative reverse phase high-pressure liquid chromatography (LC). The drug product, a sterile concentrate of LL-37, consisted

TABLE 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Capable and voluntarily giving signed informed consent, which included compliance with the requirements and restrictions listed in the ICF and in the protocol.	Other known predominant aetiology than VLU of the target ulcer, such as trauma. Malignant disease (excluding basal cell carcinoma) unless in remission for 5 years.
Male or female ≥18 years of age at the time of signing the ICF.	P-albumin <25 g/L or haemoglobin A1c (HbA1c) >10%.
Female patients had to be 1 year post-menopausal, surgically sterile, or using an acceptable method of contraception[a] for the duration of the study to prevent pregnancy.	Presence of active psoriasis skin lesions within 1.5 cm of the ulcer area. Ulcer which by location or extension was either difficult to assess or treat according to the protocol.
Negative pregnancy test (women of child-bearing potential only).	Presence of a non-study ulcer within 2 cm of the target VLU.
Lower leg ulcers presumed to be caused by venous insufficiency.	Exposure of bone, tendon, or fascia within the target ulcer.
Target leg ulcer that had failed to heal within a minimum of 6 weeks of compression therapy.	Clinical signs or symptoms of an infection of the target ulcer, erysipelas, or osteomyelitis requiring systemic antibiotic treatment.
ABPI >0.70 at screening. Ulcer localisation above the foot and below the knee (ankle and malleoli included). Surface area of target ulcer 2–40 cm^2 at screening. Ulcer essentially free of necrotic tissue.	Systemic immunosuppressive drugs with the exception of low-dose oral steroids and mineral corticoids: glucocorticoids corresponding to oral prednisolone ≤10 mg/day were allowed provided that drug treatment had been initiated not earlier than 4 weeks prior to the screening visit and was expected to be maintained at similar dose level throughout the study period.
Ability to tolerate compression bandaging.	Known hypersensitivity to any component of the study drug or standard ulcer dressing.
Appropriate state of health to participate in the study, as determined by the Investigator. This was determined by medical history, physical examination, and clinical laboratory evaluations.	Systemic treatment with antibiotics within 7 days prior to screening visit. Participation in another clinical study within 7 days prior to screening visit.
Willing to attend study visits and judged able to comply with the protocol requirements.	Treatment with topical antibiotics or potassium permanganate on the target ulcer on the day of screening.
	Heavy ulcer exudation that requires more frequent dressing changes than allowed in the study (i.e., twice weekly) as judged by the Investigator.
	For women only: currently pregnant (confirmed by positive pregnancy test) or breast-feeding.
	Any clinically significant disease judged by the Investigator to affect the patient's capability to participate in the study or to possibly influence the evaluation of study data.

Abbreviations: ABPI, ankle-brachial pressure index; ICF, informed consent form.
[a]An acceptable method of contraception was defined as a barrier method in conjunction with a spermicide. In addition, approved contraceptive contraception, intrauterine device, or tubal ligation were allowed.

of drug substance LL-37, sodium acetate (pH buffering agent), sodium chloride (tonicity agent), and water (solvent). The LL-37 concentrates were manufactured by an aseptic process: LL-37 acetate was dissolved in 50 mM sodium acetate buffer, and the pH was controlled before filtration through a sterile 0.22-μm polyvinylidene difluoride membrane. The content of LL-37 and the amount of impurities in the drug product were determined by LC with ultraviolet detection at 217 nm, and the identity was determined by LC–mass spectrometry. All batches of drug product used in this trial were within specification regarding content (90%–110%) and positive identity, and sum of impurities was 0.5% according to release testing. The diluent was a sterile aqueous solution of 13.1% polyvinyl alcohol (PVA). Manufacturing of the drug product as well as diluent, and all analytical testing, were performed by Apoteket Production & Laboratories AB (Kungens Kurva, Sweden).

Immediately before application onto the wound bed, the drug product with the concentration of LL-37 of 2.5 or 8 mg/mL was mixed with 13.1% PVA diluent to obtain viscous solutions of LL-37 in PVA with final concentrations of 0.5 or 1.6 mg/mL of LL-37 in 10.5% PVA. The doses were selected based on the results of the phase I/II study LL-37001B, which demonstrated the most pronounced effect on early wound healing response for the doses of 0.5 and 1.6 mg/mL.

2.6 | Treatment protocol

LL-37 in PVA solution or placebo (PVA) was administered onto the cleansed and dried ulcer. During the run-in phase, placebo was applied for 3 weeks. After randomization, participants received placebo or active treatment for a period of 13 weeks. The product was applied on the wound bed, using 25 μL solution per cm^2 ulcer area, at concentrations 0.5 or 1.6 mg/mL of LL-37 and active doses of 12.5 and 40 μg/cm^2, respectively (see section below for would area estimation).

TABLE 2 Outcome measures

Efficacy assessments
Primary efficacy endpoint
Confirmed complete wound closure of the target ulcer, defined as skin re-epithelialisation without drainage or dressing requirements at any time up to the end-of-treatment visit at 13 weeks, which was sustained at the post-wound closure visit, 2 weeks after the first reported closure. The wound closure was always to be documented by photography, both when first reported and when confirmed 2 weeks later.
Secondary efficacy end points
1. Wound healing rate of the target ulcer within the treatment period/or until complete closure, as applicable, estimated from the exponential decay model $Y = \alpha \times e^{-\beta t}$, where Y denotes the wound area, α denotes the estimated initial wound area, β denotes the estimated healing rate, and t denotes the time in days since baseline (randomisation).
2. Time to confirmed complete wound closure of the target ulcer as defined above.
3. Attainment of target ulcer area reduction of ≥50% compared with baseline (randomisation) at the end-of-treatment visit (Yes/No).
4. Attainment of target ulcer area reduction of ≥70% compared with baseline (randomisation) at the end-of-treatment visit (Yes/No).
Exploratory efficacy endpoints
1. Linear wound margin advance estimated from a segmented ("broken stick") regression analysis of wound area data.
2. Wound area reduction (%) at the end-of-treatment visit compared with baseline (randomisation).
Safety assessments
1. Incidence of local reactions as exemplified by clinical signs of inflammation of the target ulcer and the wound margin (oedema, redness, and raised temperature) and irritation of the adjacent skin (scaling, redness, papules, vesicles, and pustules). Any local reaction was recorded on a graded scale (0–3: none, mild, moderate, and severe).
2. Incidence of infection of the target ulcer.
3. Overall incidence of AEs, including SAEs.
4. Change in laboratory values from baseline (randomisation).
5. Change in vital signs from baseline (randomisation).
6. Incidence of >50% increase in target ulcer area compared with baseline (randomisation).
7. Physical examination assessments.
Other outcomes
1. Change in wound characteristics of the target ulcer (scores of slough, granulation tissue, necrosis, odour, and exudation level) compared with baseline (randomisation).
2. Change in local pain in the target ulcer compared with baseline (randomisation) using a graded visual analogue scale (VAS) score (0–10, where 0 = no pain and 10 = worst conceivable pain).

All treatments were applied twice weekly (every 3 ± 1 days) to coincide with routine dressing change intervals in clinical practice. The wound was covered with an appropriate dressing to control ulcer exudation before application of standard compression bandages. Patients with complete wound healing received standard post-wound closure care, including bedding (rather than dressing) and compression bandaging. At the follow-up visits, which consisted of two site visits at 8 weeks (±7 days) and 16 weeks (±7 days) after end-of-treatment, the patients received standard compression therapy.

2.7 | Outcome measures

Efficacy assessments, including incidence of complete wound closure and wound area measurements, were performed at each treatment/dressing change (wound closure) or once a week (wound area) during the treatment period. The wound area of the target ulcer in cm^2 was measured using Silhouette Lite+ (Aranz Medical, New Zealand), which is an iOS application allowing images and noncontact two-dimensional (2D) measurements to be obtained of wounds. Notably, being able to zoom in closely on a screen of a tablet or laptop also allows for careful and accurate consideration of the true boundary of the wound. Based on the photograph and tracing of the wound, the Silhouette Lite+ reader provides a digital calculation of the ulcer area. Local assessments of wound area were done at each site in order to assess the ulcer area for dosing purpose. To minimize inter-assessor variability, the statistical analysis of the outcome measures related to the wound area was based on the independent central reading of Silhouette photographs by a wound care specialist. The analysis of incidence of complete wound closure was based on the determination by the on-site assessor. Both the local study personnel assessing closure status and the wound care specialist performing the central reading were blinded to the study group.

Pain in the target ulcer and wound characteristics were checked weekly during the treatment period and a photography of the target ulcer was taken every week. Furthermore, the wound closure was always documented by photography, both when first reported and when confirmed 2 weeks later.

Local tolerability, infection of the target ulcer, and the occurrence of adverse events (AEs) were recorded at each treatment/dressing

change. Vital signs measurements were performed once a week during the treatment period and blood samplings for laboratory safety assessments were performed once a month.

Assessments at follow-up visits were identical to these performed during the treatment period.

2.8 | Statistical analysis

There were no interim analyses in this study, but the main analysis of data from the run-in and treatment periods (including post-wound closure visits) was performed on unblinded data when all patients had completed the treatment period and the potential 2 weeks of post-wound closure visits; that is, before the end of the study, since the follow-up period was still ongoing. All efficacy analyses were performed on both the full analysis set (FAS; main analysis) and the per protocol analysis set (PPAS; supportive analysis). Descriptive statistics on efficacy data were presented for both analysis sets. The primary efficacy variable, confirmed complete wound closure, was analysed using logistic regression models. A landmark analysis on the FAS including only subjects who completed the treatment period was performed as a sensitivity analysis. Wound healing rate (estimated individually for each patient by fitting exponential decay models to wound area data) was analysed using analysis of covariance (ANCOVA). Time to confirmed complete wound closure was analysed using regression analysis to estimate the restricted mean survival time (RMST). Attainment of target ulcer area reduction of at least 50% and 70% at end-of-treatment compared with baseline was analysed using logistic regression models. Linear wound margin advance was estimated from a segmented ("broken stick") regression analysis of square-root transformed wound area data. Wound area reduction at end-of-treatment compared with baseline was summarized descriptively only. Safety data and other outcomes (wound characteristics and local pain) were summarized descriptively only, for the safety analysis set. Post-hoc subgroup analyses of the primary and secondary efficacy variables were performed for the FAS and the PPAS on subgroups based on the baseline area of the target ulcer (<10 or ≥10 cm^2; stratification factor, assessed by the investigators). One-sided tests were used for the primary and secondary efficacy analyses, including subgroup analyses, and two-sided tests were used for the explorative analyses; all tests were performed at the 5% level of significance.

3 | RESULTS

3.1 | Disposition of patients and data sets analysed

A total of 190 patients were screened for the study and 149 patients were randomized while 148 patients were treated with IMP. The visit attendance was high in all groups during run-in and treatment periods: during the run-in, at least 97.9% of patients per group attended each visit; during the treatment, only three patients were withdrawn due to three or more missing treatment visits. The follow-up visits in this study were conducted during the period of restrictions pertaining to the COVID-19 pandemic, which resulted in relatively high numbers of patients lost to follow-up. Only 63.5% of patients visited at least one of the two follow-up visits, which is in contrast to the high adherence to the visit schedule during the treatment period.

A total of 148 patients (99.3% of the randomized patients) received treatment and were included in the safety analysis set, 144 patients (96.6%) were included in the FAS, and 129 patients (86.6%) were included in the PPAS (Figure 2 displays the study flow chart). Four patients were excluded from the FAS (and the PPAS) as they were randomized in violation of one or two eligibility criteria. Five patients were excluded from the PPAS due to major protocol deviations considered to affect the primary analysis (three or more missed visits during the treatment period and/or post-wound closure visit[s] not performed). Further, 10 patients were excluded from the PPAS since they did not complete the treatment period.

3.2 | Demographics and other baseline characteristics

The randomized and treated patients consisted of 83 females (56.1%) and 65 males (43.9%), aged between 33 and 94 years, in accordance with the eligibility criteria. All patients were of white ethnicity. Overall, the patients in the study had a mean (±SD) age of 67.8 (±11.5) years, a mean (±SD) height of 167.9 (±9.9) cm, a mean (±SD) body weight of 90.9 (±21.7) kg, and a mean (±SD) BMI of 32.2 (±7.1) kg/m^2.

The most common location of the target ulcer was the inner aspect of the left leg (43 patients, 29.1%), followed by the inner aspect of the right leg (25 patients, 16.9%), outer aspect of the left leg (23 patients, 15.5%), and outer aspect of the right leg (21 patients, 14.2%). The time that the patients had been affected by the target ulcer ranged between 60 and 9563 days (i.e., >26 years), the mean (±SD) duration of the target ulcer was 1574.4 (±2149.7) days and the median duration was 618.0 days. All patients had previous used compression therapy.

The majority of patients (81, 54.7%) were non-smokers, 41 (27.7%) were former smokers, and 26 (17.6%) of the patients were current smokers. Regarding the daily activity status, the majority of patients (102, 68.9%) were fully physically active, 44 (29.7%) were mainly sedentary, and 2 (1.4%) patients were incapable of any daily activity. The mean (±SD) ABPI was 0.991 (±0.153). All patients had an ABPI above 0.70 at screening, as per the inclusion criteria. There were no apparent differences between the groups with regards to demographics, history of the target ulcers, or other baseline characteristics (Table 3).

3.3 | Medical history and concurrent conditions

A total of 74 patients (50.0%) reported at least one past condition or procedure as medical or surgical history (events stopped prior to baseline). The most commonly reported medical history events were cholecystectomy and thrombophlebitis, both reported by 11 patients (7.4%) each. Based on the documented fulfilment of the inclusion

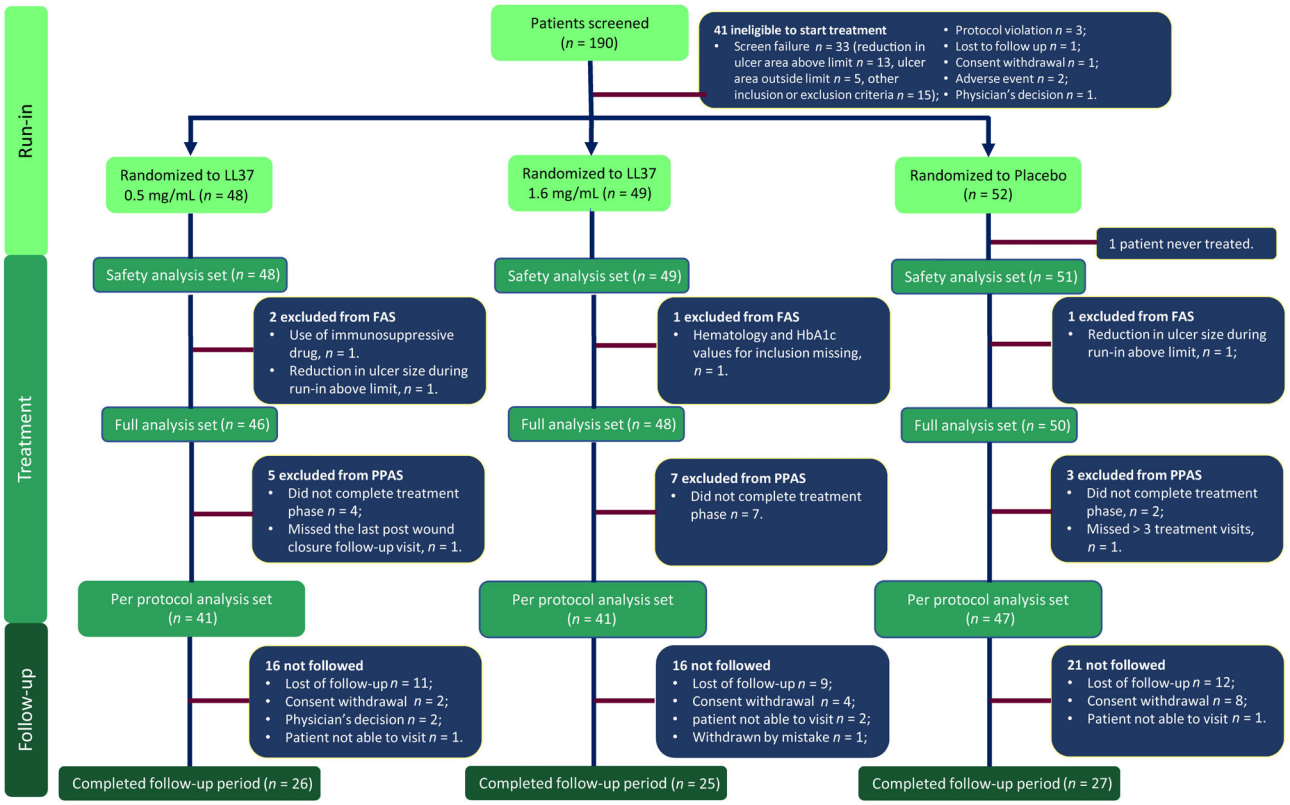

FIGURE 2 Flow of participants through the trial

criteria, all patients had lower leg ulcers presumed to be caused by venous insufficiency on inclusion in the study. This was also confirmed in the concurrent disease data, as peripheral venous disease was reported in 100.0% of the patients. Other common ongoing conditions at inclusion in the study were hypertension, reported by 62.2% of patients overall, followed by skin ulcer reported by 22.3%, varicose vein reported by 14.2%, and atrial fibrillation reported by 12.2% of patients. There were no apparent differences between the groups with regards to medical and surgical history or concurrent diseases.

3.4 | Prior and concomitant medications

A total of 18 patients (12.2%) reported at least one prior medication (stopped prior to baseline) and 136 patients (91.9%) reported at least one concomitant medication. The only prior medications used by more than one patient were clindamycin, other cicatrizants, and soft paraffin dressings, each used by two patients (1.4%) overall. The most common concomitant medications used during the study were sulodexide used by 33 patients (22.3%), ramipril used by 29 patients (19.6%), acetylsalicylic acid used by 24 patients (16.2%), bisoprolol used by 23 patients (15.5%), and metformin used by 22 patients (14.9%) overall. There were no apparent differences in use of medications prior to or during the study between the treatment groups.

3.5 | Efficacy evaluation

Efficacy analysis performed on full study population did not identify any significant improvement in healing parameters in patients treated with LL-37 as compared with the placebo (Table 4). In FAS, the estimated proportion of patients with confirmed complete wound closure was 26.5% in the LL-37 0.5 mg/mL group, 24.7% in the LL-37 1.6 mg/mL group, and 25.3% in the placebo group. The mean wound healing rate was 0.0261/day in the LL-37 0.5 mg/mL group, 0.0112/day in the LL-37 1.6 mg/mL group, and 0.0204/day in the placebo group. The time to confirmed complete wound closure (RMST) was 83.1 days in the LL-37 0.5 mg/mL group, 90.3 days in the LL-37 1.6 mg/mL group, and 87.9 days in the placebo group. A target ulcer area reduction of at least 50% was estimated in 56.4%, 35.0%, and 46.2% of patients in the LL-37 0.5 mg/mL group, the LL-37 1.6 mg/mL group, and in the placebo group, respectively, and a target ulcer area reduction of at least 70% was estimated in 43.1%, 34.6%, and 34.0% of patients, respectively. The linear wound margin advance (exploratory efficacy variable) was estimated to −0.0052 pre-randomization, and −0.0145 post-randomization in the LL-37 0.5 mg/mL group, −0.0115 in the LL-37 1.6 mg/mL group, and −0.0144 in the placebo group. The wound area (exploratory efficacy variable) decreased from baseline to end-of-treatment in all treatment groups. The mean decrease in the wound area from baseline to end-of-treatment was 35.63%, 4.02%, and 51.69% in the LL-37 0.5 mg/mL group, the LL-37 1.6 mg/mL group, and the placebo group, respectively. None of these

TABLE 3 Patient baseline characteristics

Patient characteristics	LL-37 0.5 mg/mL ($N = 46$)	LL-37 1.6 mg/mL ($N = 48$)	Placebo ($N = 50$)	Total ($N = 144$)
Demographics				
Age (years)				
Mean (SD)	67.6 (11.8)	66.8 (10.9)	68.4 (11.8)	67.6 (11.5)
Sex				
Female	24 (52.2%)	28 (58.3%)	28 (56.0%)	80 (55.6%)
Male	22 (47.8%)	20 (41.7%)	22 (44.0%)	64 (44.4%)
Race				
White	46 (100.0%)	48 (100.0%)	50 (100.0%)	144 (100.0%)
Height (cm)				
Mean (SD)	169.1 (9.6)	167.0 (9.9)	168.0 (10.6)	168.0 (10.0)
Body weight (kg)				
Mean (SD)	89.06 (23.78)	94.39 (21.25)	89.55 (20.65)	91.00 (21.87)
BMI (kg/m^2)				
Mean (SD)	31.10 (7.52)	33.80 (7.02)	31.68 (6.65)	32.20 (7.11)
History of the target ulcer				
Location of the target ulcer				
Left leg back	1 (2.2%)	3 (6.3%)	3 (6.0%)	7 (4.9%)
Left leg front	4 (8.7%)	3 (6.3%)	5 (10.0%)	12 (8.3%)
Left leg inner aspect	14 (30.4%)	17 (35.4%)	11 (22.0%)	42 (29.2%)
Left leg outer aspect	8 (17.4%)	7 (14.6%)	8 (16.0%)	23 (16.0%)
Right leg back	0	0	0	0
Right leg front	6 (13.0%)	3 (6.3%)	5 (10.0%)	14 (9.7%)
Right leg inner aspect	9 (19.6%)	5 (10.4%)	11 (22.0%)	25 (17.4%)
Right leg outer aspect	4 (8.7%)	10 (20.8%)	7 (14.0%)	21 (14.6%)
Duration of the target ulcer (days)				
Mean (SD)	1560.2 (2279.4)	1151.7 (1395.6)	2003.5 (2571.0)	1577.9 (2159.7)
Prior use of compression therapy				
Yes	46 (100.0%)	48 (100.0%)	50 (100.0%)	144 (100.0%)
No	0	0	0	0
Prior use of ulcer dressing				
Yes	42 (91.3%)	45 (93.8%)	47 (94.0%)	134 (93.1%)
No	4 (8.7%)	3 (6.3%)	3 (6.0%)	10 (6.9%)
Smoking status				
Current smoker	7 (15.2%)	11 (22.9%)	8 (16.0%)	26 (18.1%)
Former smoker	14 (30.4%)	10 (20.8%)	17 (34.0%)	41 (28.5%)
Non-smoker	25 (54.3%)	27 (56.3%)	25 (50.0%)	77 (53.5%)
Daily activity				
Fully physically active	29 (63.0%)	35 (72.9%)	34 (68.0%)	98 (68.1%)
Mainly sedentary	17 (37.0%)	12 (25.0%)	15 (30.0%)	44 (30.6%)
Incapable	0	1 (2.1%)	1 (2.0%)	2 (1.4%)
Ankle-brachial pressure index				
Mean (SD)	0.969 (0.141)	0.990 (0.172)	1.005 (0.148)	0.988 (0.154)

Note: Analysis is based on the number of subjects within each treatment group in FAS.

differences was statistically significant. Similar results were obtained in the subgroup of FAS including only the patients who completed the treatment period and PPAS.

Post-hoc efficacy analysis performed in the subgroup of patients with large target wounds (a wound area of at least 10 cm^2 at randomization) identified statistically significant improvement in several

TABLE 4 Efficacy results

Total population	**LL-37 0.5 mg/mL (*N* = 46)**		**LL-37 1.6 mg/mL (*N* = 48)**		**Placebo (*N* = 50)**
Closed ulcers (Estimated proportion [90% CI])	26.5 (17.1, 38.7)	$P = 0.4453$	24.7 (15.8, 36.4)	$P = 0.5274$	25.3 (16.4, 36.9)
Wound healing rate per day (Mean [90% CI])	0.0261 (0.0146, 0.0375)	$P = 0.2759$	0.0112 (−0.0003, 0.0227)	$P = 0.8326$	0.0204 (0.0097, 0.0312)
Time to wound closure (Days [90%CI])	83.1 (76.3, 89.8)	$P = 0.1644$	90.3 (86.2, 94.3)	$P = 0.7270$	87.9 (83.3, 92.6)
50% ulcer reduction (Estimated proportion [90% CI])	56.4 (44.2, 67.8)	$P = 0.1603$	35.0 (24.6, 47.0)	$P = 0.8666$	46.2 (34.9, 57.9)
70% ulcer reduction (Estimated proportion [90% CI])	43.1 (31.6, 55.3)	$P = 0.1839$	34.6 (24.2, 46.6)	$P = 0.4788$	34.0 (23.9, 45.9)
Subgroup with wounds ≥10 cm^2	***N* = 21**		***N* = 21**		***N* = 24**
Closed ulcers (Estimated proportion [90% CI])	28.1 (14.9, 46.6)	$P = 0.0458$	19.6 (8.8, 38.0)	$P = 0.1393$	8.1 (2.5, 23.1)
Wound healing rate per day (Mean [90% CI])	0.0367 (0.0174, 0.0559)	$P = 0.0439$	0.0159 (−0.0040, 0.0358)	$P = 0.3430$	0.0093 (−0.0087, 0.0274)
Time to wound closure (Days [90%CI])	87.4 (80.9, 93.9)	$P = 0.0066$	92.6 (88.3, 96.9)	$P = 0.0407$	97.5 (96.1, 98.8)
50% ulcer reduction (Estimated proportion [90% CI])	61.9 (43.6, 77.3)	$P = 0.0294$	38.2 (22.7, 56.6)	$P = 0.3669$	33.3 (19.6, 50.5)
70% ulcer reduction (Estimated proportion [90% CI])	47.2 (30.3, 64.8)	$P = 0.0149$	39.0 (23.3, 57.5)	$P = 0.0493$	16.2 (7.2, 32.5)
Subgroup with wounds <10 cm^2	***N* = 25**		***N* = 27**		***N* = 26**
Closed ulcers (Estimated proportion [90% CI])	26.0 (14.1, 42.8)	$P = 0.9062$	31.4 (18.7, 47.7)	$P = 0.8252$	44.2 (28.8, 60.8)
Wound healing rate per day (Mean [90% CI])	0.0138 (0.0020, 0.0256)	$P = 0.9693$	0.0076 (−0.0040, 0.0191)	$P = 0.9939$	0.0324 (0.0213, 0.0435)
Time to wound closure (Days [90%CI])	77.9 (67.4, 88.4)	$P = 0.5333$	86.1 (80.1, 92.2)	$P = 0.9181$	77.2 (68.6, 85.9)
50% ulcer reduction (Estimated proportion [90% CI])	51.6 (35.4, 67.4)	$P = 0.6920$	32.8 (19.9, 49.0)	$P = 0.9669$	58.7 (42.2, 73.4)
70% ulcer reduction (Estimated proportion [90% CI])	38.7 (24.2, 55.6)	$P = 0.8205$	32.1 (19.3, 48.3)	$P = 0.9223$	51.9 (35.8, 67.7)

Note: *Analysis* is based on the number of subjects within each treatment group in FAS.

interrelated healing parameters in a group of patients treated with LL-37 at the concentration of 0.5 mg/mL as compared with the placebo (Table 4). In this subgroup of FAS, the estimated proportions of patients with confirmed complete wound closure were 28.1%, 19.6%, and 8.1% in the LL-37 0.5 mg/mL group, the LL-37 1.6 mg/mL group, and the placebo group, respectively. The OR of 4.454 in the LL-37 0.5 mg/mL group was statistically significant in comparison with the placebo group ($P = 0.0458$). The mean wound healing rate was 0.0367/day, 0.0159/day, and 0.0093/day in the LL-37 0.5 mg/mL group, the LL-37 1.6 mg/mL group, and the placebo group, respectively. The difference in wound healing rate in the LL-37 0.5 mg/mL group was statistically significant compared with the placebo group ($P = 0.0439$). The RMST was 87.4, 92.6, and 97.5 days in the LL-37 0.5 mg/mL group, the LL-37 1.6 mg/mL group, and the placebo group, respectively, and both active treatment groups were statistically significant in comparison to the placebo group ($P = 0.0066$ and $P = 0.0407$, respectively). The target ulcer area reduction of at least 50% was estimated in 61.9%, 38.2%, and in 33.3% of patients in the LL-37 0.5 mg/mL, LL-37 1.6 mg/mL, and the placebo groups, respectively. The OR for reaching 50% wound closure was of 3.252 in the LL-37 0.5 mg/mL group versus placebo, which was statistically significant ($P = 0.0294$ vs. placebo). Also, the target ulcer area reduction of at least 70% was estimated in 47.2%, 39.0%, and 16.2% of patients in the respective groups. The ORs of 4.619 in the LL-37 0.5 mg/mL group and 3.307 in the LL-37 1.6 mg/mL group were both statistically significant compared with placebo group ($P = 0.0149$ and $P = 0.0493$, respectively). Similar results were obtained in the subgroup of FAS including only the patients who completed the treatment period and PPAS.

No improvement was detected in response to LL-37 administration when comparing the three treatment groups in patients with a wound area of less than 10 cm^2 at randomization (Table 4).

3.6 | Safety evaluation

Local reactions at the target ulcer included redness, oedema, and raised temperature and were reported in 63 (42.6%), 58 (39.2%), and

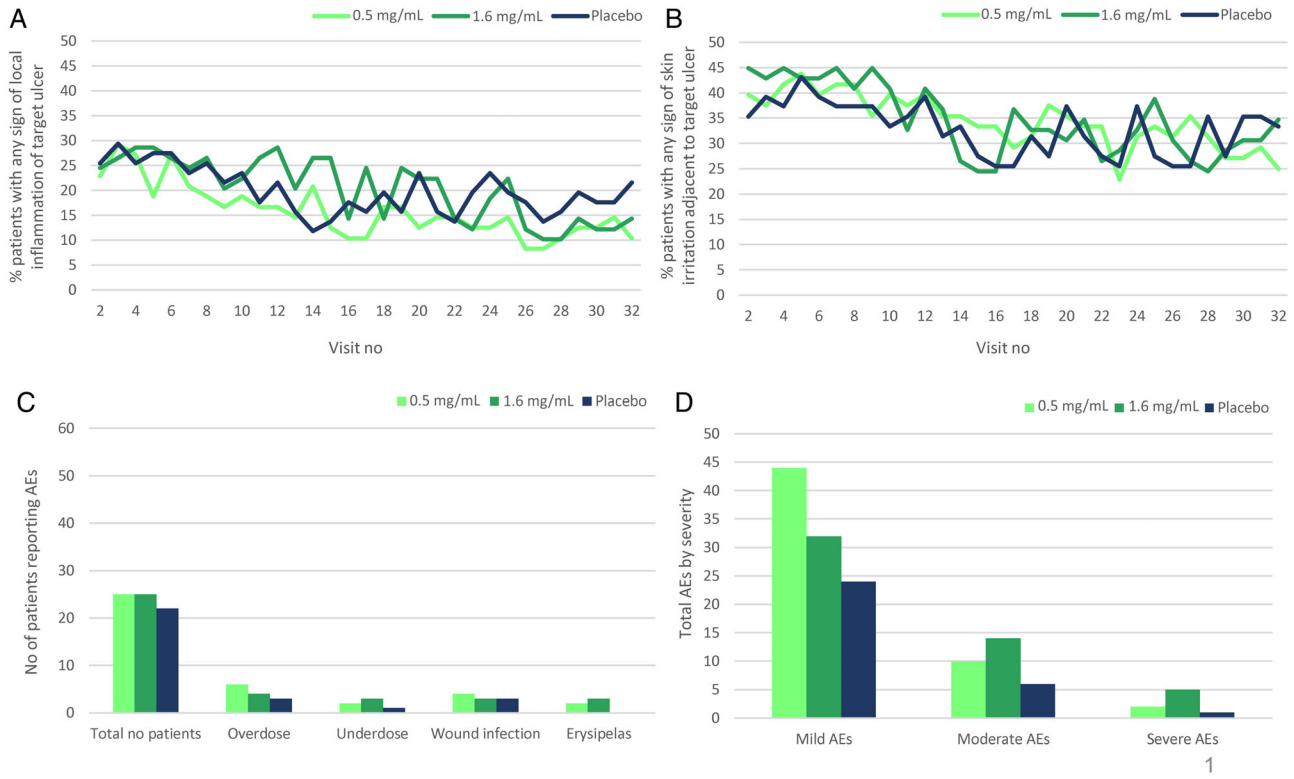

FIGURE 3 Safety analysis. (A,B) Percentage of patients in safety analysis set with any sign of inflammation on the target ulcer (A) or skin irritation adjacent to the target ulcer (B). Visits 1–6 were performed during run-in period and visits 7–32 were performed during the treatment period. (C) Number of study patients in safety analysis set reporting any AEs as well as selected categories of AEs. (C) Total number of AEs by severity

30 (20.3%) of patients in total, respectively. Local reactions of the adjacent skin included redness, scaling, papules, pustules, and vesicles and were reported in 80 (54.1%), 78 (52.7%), 29 (19.6%), 23 (15.5%), and 7 (4.7%) of patients in total, respectively. The majority of reactions at the target ulcer and the adjacent skin were mild or moderate at their peak intensity with no consistent trends of difference between the treatment groups (Figure 3A,B). Severe local reactions after the start of randomized treatment were rare and affected in total ≤5 patients (3.4%) at each visit.

Overall, few patients developed infections in the target ulcer during the study: one patient (2.1%) in the LL-37 0.5 mg/mL group, two patients (4.1%) in the LL-37 1.6 mg/mL group, and three patients (5.9%) in the placebo group. None of the infections were considered serious and all were assessed as unlikely related to the IMP.

In total, 122 AEs were reported by 64 patients (43.2%) in the study. The most commonly reported AEs by preferred term were *overdose* (29 AEs reported by 13 patients, 8.8%), *wound infection* (9 AEs reported by 8 patients, 5.4%), *hypertension* (7 AEs reported by 7 patients, 4.7%), *underdose* (12 AEs reported by 6 patients, 4.1%), and *erysipelas* (9 AEs reported by 5 patients, 3.4%) (Table 5). The total number of AEs was slightly higher in groups receiving active treatment compared with placebo group, which could at large be attributed to reports of overdosing and underdosing of IMP (generally minor with <30% difference from the planned dose) as well as erysipelas (reported by five patients) (Table 5; Figure 3C). It is, however, noteworthy, that none of the erysipelas cases occurred in the target wound site.

Most AEs were judged as unlikely related to IMP treatment. Six AEs in four patients (2.7%) were judged as possibly related to the IMP. These were two reports of *wound infections* in one patient and two reports of *dermatitis* in one patient, both in the LL-37 0.5 mg/mL group, one report of *peripheral swelling* in one patient in the LL-37 1.6 mg/mL group, and one report of *deep phlebitis* in the placebo group. No events were judged as probably related to IMP.

Most AEs reported were of mild (92 AEs in total) or moderate intensity (22 in total); eight severe AEs were reported in eight patients (Figure 3D). There were no deaths in the study and 12 non-fatal serious adverse events (SAEs) occurred in 11 patients (7.4%). None of these were assessed as related to the study drug treatment. The most common SAE was *erysipelas* reported on three occasions in two patients (none of these affected the target ulcer). Other SAEs were reported as single preferred terms (Table 5). Two patients in the LL-37 1.6 mg/mL group withdrew treatment due to the SAE *femur fracture* and the AE *deep vein thrombosis* and three patients in the LL-37 1.6 mg/mL group withdrew from the study due to SAEs that occurred after their last IMP dose (*cerebrovascular accident*, *lower limb fracture*, and *blood creatinine increased*). All the AEs leading to the withdrawals were assessed as unlikely related to the IMP.

There were small fluctuations in the levels of haematological and clinical chemistry parameters over time and no clinically important

TABLE 5 Adverse events

	LL-37 0.5 mg/mL ($N = 48$)	LL-37 1.6 mg/mL ($N = 49$)	Placebo ($N = 51$)
Summary of AEs			
Patients reporting at least one AE	20	24	20
Total no of AEs reported	49	45	28
Total no of AEs possibly or probably related to IMP treatment	4	1	1
Total no of AEs of severe nature	2	5	1
Total no of serious AEs	4	7	1
Most common AEs reported (no. of patients [no. of AEs])			
Overdose	6 (15)	4 (9)	3 (5)
Underdose	2 (5)	3 (5)	1 (2)
Wound infection	3 (4)	2 (2)	3 (3)
Erysipelas	2 (4)	3 (5)	
Hypertension	2 (2)	2 (2)	3 (3)
Nasopharyngitis		1 (1)	2 (2)
SAEs			
Erysipelas	1	2	
Cellulitis	1		
Cardiac failure	1		
Myocardial infarction		1	
Femure fracture		1	
Lower limb fracture		1	
Blood creatinine increase		1	
Cerebrovascular accident		1	
Acute kidney injury			1
Asthma	1		

Note: Adverse events in safety analysis set are coded according to MedDRA 22.0.
Abbreviation: No, number.

differences between the treatment groups, or any specific trend in change over time, could be seen. The vast majority of the laboratory values were within the normal reference range. Four patients in the actively treated groups reported clinically significant deviations in haematological and clinical chemistry parameters in association with AEs.

There were no consistent changes in the mean vital signs values (blood-pressure, heart rate, and body temperature) during the treatment period related to any particular treatment group and there were no obvious differences between treatment groups in the physical examinations performed.

Overall, 25 (16.9%) patients showed more than a 50% increase in their target ulcer area at any of the post-baseline visits, but no apparent differences between the treatment groups were seen.

No differences in wound characteristics (scores of slough, granulation tissue, necrosis, odour, and exudation level) could be seen between the treatment groups. A trend for improvement over the treatment period in patient-reported VAS scores for local pain was apparent in all treated groups both for pain experienced during the last 24 hours and at dressing change, without any differences between treatment groups.

3.7 | Analysis of follow-up data

Of the 39 patients with healed ulcers, who attended at least one follow-up visit, six patients (zero patient in the LL-37 0.5 mg/mL group, two patients in the LL-37 1.6 mg/mL group, and four patients in the placebo group) had reportedly re-opened wounds at one or both of the follow-up visits; however, due to the low visit attendance it was not possible to draw any conclusion on possible differences between groups. There were no safety concerns observed during the follow-up period and no differences between treatment groups in safety parameters could be seen.

4 | DISCUSSION

Here we describe the efficacy and safety of local supplementation of synthetic peptide LL-37 to nonhealing VLUs. Lower limb compression therapy is the most widely adopted treatment for VLUs and this concept has been applied in different forms for more than four centuries.[14] Unfortunately, the proportion of VLUs that remain refractory

to compression therapy is still significant and the management of HTH VLUs remains expensive and time consuming.[4] Thus, it is important to develop efficient and safe treatments for chronic wounds, which are also simple to use and compatible with the compression therapy and ulcer dressings.

Interestingly, in this clinical study, improved healing after LL-37 treatment was only observed in relatively large wounds (≥10 cm^2), while no improvement was observed in small ulcers (<10 cm^2). The reasons for the different response to LL-37 in large versus small ulcers remain elusive. However, it is important to emphasize that the wound area measurements by marking the edge of the ulcer as were used in this study are inherently less precise in case of small versus large ulcers. The unprecise area assessment, in turn, may result in wrongful determination of the efficacy endpoints which assess the reduction of wound size (i.e., healing rate and % reduction of wound area). Furthermore, errors in ulcer area measurement also result in wrongful estimations of the amount of medicinal product being administered to the wound and, thus, may contribute to lower efficacy due to too low or too high amount of LL-37 being applied.

In this study, the low dose of LL-37 (i.e., 0.5 mg/mL) was found to be more effective in enhancing the healing of large VLUs as compared with the high dose (i.e., 1.6 mg/mL). This observation is consistent with the inverted dose–response efficacy documented in the previous phase I/II trial, where 3-week treatment regimen with 0.5 mg/mL LL-37 demonstrated a more pronounced impact on improving the healing rate of HTH VLUs compared with the two higher doses of 1.6 and 3.2 mg/mL.[12] Importantly, the VLU patients treated with 3.2 mg/mL of LL-37 in this first-time-in-man trial also exhibited elevated frequency and severity of local reactions compared with the other groups,[12] suggesting that bell-shaped dose–response curve for LL-37 may relate to the increased inflammation caused by high doses of the peptide. Of note, bell-shaped concentration-response curves are not uncommon, in fact, there are more than 1000 literature citations to molecules with this behaviour.[15]

It is known that wounds of long duration and large size are particularly difficult to treat efficiently with current mainstay of VLU management.[16–20] As one example, the study by Margolis and coworkers conducted in a large cohort of over 20,000 VLUs patients demonstrated that a wound that is less than 10 cm^2 in size and less than 12 months in duration has a 29% risk of not healing by the 24th week of care, while a wound larger than 10 cm^2 with more than 12 months of duration has a 78% risk of not healing.[21] Consistently, the analysis including data from about 700 subjects with VLUs showed that ulcer area has a pronounced impact on healing, giving an approximately 10% reduction in the likelihood of closure with each 1 cm^2 increase in lesion size.[22] Notably, wound duration was modestly impactful in this study with an about 3% reduction in likelihood of closure of a wound for each 1-month increase in the ulcer duration at baseline.[22] Thus, wounds of large size and long duration represent an increased clinical challenge and should be referred to specialists to consider more aggressive management. With respect to the distribution of sizes of VLUs, it is well established that most VLUs are relatively small, while wound sizes vary over a large range. In a random cohort of VLU patients in an outpatient setting in Sweden, about 23% of patients had a wound size exceeding 10 cm^2,[23] which is well aligned with data from Margolis et al. showing about 22% of patients with large ulcers in a US cohort.[21] In the United Kingdom, it has been estimated that the average cost of wound care in clinical practice over 12 months was £7600 per VLU.[24] Remarkably, the monthly treatment cost of wounds larger than 10 cm^2 having a duration of more than 6 months is reportedly more than threefold higher compared with a smaller wound of shorter duration.[24] Correspondingly, it is postulated that this subpopulation of patients with large wounds of longer duration account for a disproportional fraction of the public wound care budget. To this end, it is interesting that administration of LL-37 significantly enhanced the healing in the VLU patients with large wound size and long duration (the median duration of the target ulcer in total subpopulation was 757.5 days).

The strengths of this study were the double-blind placebo-controlled design including the open-label run-in phase and the high patient adherence to the visit schedule during the treatment period, with very few major deviations from the protocol. Furthermore, the study arms were overall well-balanced in regards to pivotal patient characteristics. Conversely, the major limitation of the trial was a relatively high drop-off rate during the visits scheduled 8 and 16 weeks post-treatment, which lowered the value of the follow-up data. Moreover, while a subgroup analysis provided an interesting observation that LL-37 could offer a treatment benefit in patients with large venous lower leg ulcers, it should be acknowledged that the trial was not designed or powered to identify significant differences in the subgroups which were small with only 21–24 patients per group. Thus, an additional study adequately powered to statistically assess the efficacy of LL-37 in the subjects with large wounds is warranted. Further studies are also needed to understand why LL-37 has no benefit in the total population of leg ulcer patients, as well as to decipher the factors of the microenvironment which dictate its efficacy.

Together, the present clinical trial supports the concept that supplementation of LL-37 is safe and well tolerated when applied locally to nonhealing lower leg ulcers in combination with standard compression therapy and that a low dose of LL-37 may improve healing of HTH VLUs of large size, which in current medical practice is the patient segment with the most pronounced medical need.

ACKNOWLEDGMENTS

We thank the participants for taking part in the trial; the investigators and study nurses at each site; the independent safety monitor (Sabine Frank, PharmaLex GmbH, Germany); the trial project manager (Linn Skärberg, LINK Medical, Uppsala, Sweden), and medical writer (Rose-Marie Lindgren, LINK Medical). Statistical analysis was performed by FT, CStat. The study was financed by Promore Pharma AB, Solna, Sweden.

CONFLICTS OF INTEREST

At the time of the investigation, Margit Mahlapuu, Jakob Björk, and Jonas Ekblom were employees at Promore Pharma AB. Jonas

Ekblom is a minority shareholder at Promore Pharma AB. Promore Pharma AB is developing LL-37 under the non-proprietary name ropocamptide.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Graham ID, Harrison MB, Nelson EA, Lorimer K, Fisher A. Prevalence of lower-limb ulceration: a systematic review of prevalence studies. *Adv Skin Wound Care*. 2003;16(6):305-316.
2. Franks PJ, Barker J, Collier M, et al. Management of patients with venous leg ulcers: challenges and current best practice. *J Wound Care*. 2016;25:S1-S67.
3. Joaquim FL, Silva R, Garcia-Caro MP, Cruz-Quintana F, Pereira ER. Impact of venous ulcers on patients' quality of life: an integrative review. *Rev Bras Enferm*. 2018;71(4):2021-2029.
4. Guest JF, Fuller GW, Vowden P. Venous leg ulcer management in clinical practice in the UK: costs and outcomes. *Int Wound J*. 2018; 15(1):29-37.
5. Rivas-Santiago B, Rivas Santiago CE, Castaneda-Delgado JE, Leon-Contreras JC, Hancock RE, Hernandez-Pando R. Activity of LL-37, CRAMP and antimicrobial peptide-derived compounds E2, E6 and CP26 against Mycobacterium tuberculosis. *Int J Antimicrob Agents*. 2013;41(2):143-148.
6. Heilborn JD, Nilsson MF, Kratz G, et al. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. *J Invest Dermatol*. 2003; 120(3):379-389.
7. Carretero M, Escamez MJ, Garcia M, et al. In vitro and in vivo wound healing-promoting activities of human cathelicidin LL-37. *J Invest Dermatol*. 2008;128(1):223-236.
8. Ramos R, Silva JP, Rodrigues AC, et al. Wound healing activity of the human antimicrobial peptide LL37. *Peptides*. 2011;32(7):1469-1476.
9. Steinstraesser L, Hirsch T, Schulte M, et al. Innate defense regulator peptide 1018 in wound healing and wound infection. *PLoS One*. 2012; 7(8):e39373.
10. Kos S, Vanvarenberg K, Dolinsek T, et al. Gene electrotransfer into skin using noninvasive multi-electrode array for vaccination and wound healing. *Bioelectrochemistry*. 2017;114:33-41.
11. Steinstraesser L, Lam MC, Jacobsen F, et al. Skin electroporation of a plasmid encoding hCAP-18/LL-37 host defense peptide promotes wound healing. *Mol Ther*. 2014;22(4):734-742.
12. Gronberg A, Mahlapuu M, Stahle M, Whately-Smith C, Rollman O. Treatment with LL-37 is safe and effective in enhancing healing of hard-to-heal venous leg ulcers: a randomized, placebo-controlled clinical trial. *Wound Repair Regen*. 2014;22(5):613-621.
13. Hancock RE, Haney EF, Gill EE. The immunology of host defence peptides: beyond antimicrobial activity. *Nat Rev Immunol*. 2016;16(5): 321-334.
14. Kiev J, Kerstein MD. End-stage renal disease with venous occlusion in both upper extremities. *South med J*. 1993;86(11):1229-1232.
15. Owen SC, Doak AK, Ganesh AN, et al. Colloidal drug formulations can explain "bell-shaped" concentration-response curves. *ACS Chem Biol*. 2014;9(3):777-784.
16. Margolis DJ, Berlin JA, Strom BL. Which venous leg ulcers will heal with limb compression bandages? *Am J med*. 2000;109(1):15-19.
17. Gohel MS, Taylor M, Earnshaw JJ, Heather BP, Poskitt KR, Whyman MR. Risk factors for delayed healing and recurrence of chronic venous leg ulcers: an analysis of 1324 legs. *Eur J Vasc Endovasc Surg*. 2005;29(1):74-77.
18. Meaume S, Couilliet D, Vin F. Prognostic factors for venous ulcer healing in a non-selected population of ambulatory patients. *J Wound Care*. 2005;14(1):31-34.
19. Skene AI, Smith JM, Dore CJ, Charlett A, Lewis JD. Venous leg ulcers: a prognostic index to predict time to healing. *BMJ*. 1992;305(6862): 1119-1121.
20. Milic DJ, Zivic SS, Bogdanovic DC, Karanovic ND, Golubovic ZV. Risk factors related to the failure of venous leg ulcers to heal with compression treatment. *J Vasc Surg*. 2009;49(5):1242-1247.
21. Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. The accuracy of venous leg ulcer prognostic models in a wound care system. *Wound Repair Regen*. 2004;12(2):163-168.
22. Marston WA, Ennis WJ, Lantis JC 2nd, et al. Baseline factors affecting closure of venous leg ulcers. *J Vasc Surg Venous Lymphat Disord*. 2017;5(6):829-835. e1.
23. Ebbeskog B, Lindholm C, Ohman S. Leg and foot ulcer patients. Epidemiology and nursing care in an urban population in South Stockholm, Sweden. *Scand J Prim Health Care*. 1996;14(4):238-243.
24. Ragnarson Tennvall G, Hjelmgren J. Annual costs of treatment for venous leg ulcers in Sweden and the United Kingdom. *Wound Repair Regen*. 2005;13(1):13-18.

How to cite this article: Mahlapuu M, Sidorowicz A, Mikosinski J, et al. Evaluation of LL-37 in healing of hard-to-heal venous leg ulcers: A multicentric prospective randomized placebo-controlled clinical trial. *Wound Rep Reg*. 2021;29(6): 938-950. doi:10.1111/wrr.12977