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Once daily Bromelain-based enzymatic debridement of venous leg ulcers versus gel vehicle (placebo) and non-surgical standard of care: A three-arm multicenter, double blinded, randomized controlled study

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Abstract:	Background: Debridement is considered the first step in treatment of chronic wounds, however, current enzymatic and autolytic debridement agents are slow or ineffective. Previous studies have shown positive initial results with EscharEx® (EX-02 formulation), a Bromelain- based enzymatic debridement agent in development for chronic wounds. The main objective of this study was to assess its efficacy in debriding venous leg ulcers (VLU), compared to gel-vehicle (GV) as a placebo control and to non-surgical standard of care (NSSOC). Methods: A prospective, randomized, multicenter, placebo-controlled trial in VLU patients from 20 medical centers and clinics in the United States, Switzerland and Israel was undertaken. Patients were treated with daily topical applications of either EX-02, GV, or NSSOC (in a 3:3:2 ratio), until reaching complete debridement or up to 8 daily treatments (within 2 weeks), and then followed-up for up to 14 weeks. Findings: A total of 196 patients were enrolled, and 119 randomized (between November 12th, 2019, and February 15th, 2022); 46 to the EX-02 arm, 43 to the GV arm, and 30 to the NSSOC arm. Eight patients dropped out of the study (2 in EX-02, 2 in GV, 4 in NSSOC). The incidence of complete debridement within 8 daily treatments was 63% (29/46 patients) in the EX-02 arm as compared to 30.2% (13/43 patients) in the GV arm ($p=0.004$) and 13.3% (4/30 patients) in the NSSOC arm ($p<0.001$). Sixty-five patients reported wound related adverse events throughout the study; 24 (52.2%), 27 (62.8%) and 14 (46.7%) patients in the EX-02, GV and NSSOC arms (p=NS). No deaths occurred during the study. Interpretation: EX-02 lead to a significantly higher incidence of complete debridement as compared to GV and NSSOC, without significant safety issues. Additional studies are needed to explore the benefits of EX-02 in VLU and other chronic wound etiologies. Funding: MediWound Ltd.

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July 8th , 2024

Dear Editor,

Please find the 5th revision of our manuscript “Once daily Bromelain- based enzymatic debridement of venous leg ulcers versus gel vehicle (placebo) and non-surgical standard of care: a three-arm multicenter, double blinded, randomized controlled study” attached (in tracked changes and in a clean version).

Additionally, please find our response (in red font) to the comments below.

Thank you very much for your feedback and comments.

Yaron Shoham MD

EDITORIAL POINTS:

The Editor in Chief has now done the final check of your paper. They have just asked that you please remove this sentence in the “Evidence before this study” section of the Research in context: “However, this was done with 4-hour applications whose applicability for home use, especially in an aging population, seems questionable.”.

After this, we should be able to accept the paper for publication.

Please make this change and upload the revised manuscript.

Done.

Thank you very much.

Revised manuscript (clean)

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Once daily Bromelain- based enzymatic debridement of venous leg ulcers versus gel vehicle (placebo) and non-surgical standard of care: a three-arm multicenter, double blinded, randomized controlled study

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SUMMARY

Background: Debridement is considered the first step in treatment of chronic wounds, however, current enzymatic and autolytic debridement agents are slow or ineffective. Previous studies have shown positive initial results with EscharEx® (EX-02 formulation), a Bromelain- based enzymatic debridement agent in development for chronic wounds. The main objective of this study was to assess its efficacy in debriding venous leg ulcers (VLU), compared to gel-vehicle (GV) as a placebo control and to non-surgical standard of care (NSSOC).

Methods: A prospective, randomized, multicenter, placebo-controlled trial in patients with VLU from 20 medical centers and clinics in the United States, Switzerland and Israel was undertaken. Patients were treated with daily topical applications of either EX-02, GV, or NSSOC (in a 3:3:2 ratio), until reaching complete debridement or up to 8 daily treatments (within 2 weeks), and then followed-up for up to 14 weeks. The primary efficacy endpoint was the incidence of complete debridement. This study is registered with ClinicalTrials.gov (NCT03588130) and EudraCT (number 2020-004861-38).

Findings: A total of 196 patients were enrolled, and 119 randomized (between November 12th, 2019, and February 15th, 2022); 46 to the EX-02 arm, 43 to the GV arm, and 30 to the NSSOC arm. Eight patients dropped out of the study (2 in EX-02, 2 in GV, 4 in NSSOC). The incidence of complete debridement within 8 daily treatments was 63% (29/46 patients) in the EX-02 arm as compared to 30.2% (13/43 patients) in the GV arm (p=0.004) and 13.3% (4/30 patients) in the NSSOC arm (p<0.001). Sixty-five patients reported wound related adverse events throughout the study; 24 (52.2%), 27 (62.8%) and 14 (46.7%) patients in the EX-02, GV and NSSOC arms (p=NS). No deaths occurred during the study.

Interpretation: EX-02 lead to a significantly higher incidence of complete debridement as compared to GV and NSSOC, without significant safety issues. Additional studies are needed to explore the benefits of EX-02 in VLU and other chronic wound etiologies.

Funding: MediWound Ltd.

Keywords: VLU, venous leg ulcer, enzymatic debridement, EscharEx, RCT, chronic wounds

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed from database inception to July 1st, 2023, for papers published in English including the following terms: “venous leg ulcer” OR “VLU” AND “debridement”. We identified an article reviewing 10 randomized controlled trials comparing different non-surgical debridement methods of venous leg ulcers, which failed to demonstrate an optimal debridement method or duration of treatment. A recent multi-center, assessor blinded RCT, demonstrated that a Bromelain based enzymatic debridement agent achieved a significantly higher incidence of complete debridement of venous, diabetic and post-surgical/traumatic ulcers as compared to its gel vehicle.

Added value of this study

Once daily Bromelain-based enzymatic debridement led to a significantly higher incidence of complete debridement of venous leg ulcers within two weeks as compared to gel vehicle, and to a significantly shorter time to complete debridement as compared to both gel vehicle and various non-surgical standard of care debridement agents. Additionally, it led to a significantly higher incidence of >75% granulation tissue within two weeks as compared to gel vehicle. The efficacy of these 24-hour applications, combined with an acceptable safety profile demonstrated in this study, may provide a solution to the unmet need for a safe and efficient non-surgical debridement agent for venous leg ulcers.

Implications of all the available evidence

Non-surgical debridement of venous leg ulcers has thus far not been demonstrated to lead to earlier wound closure. A faster debridement as seen in this study may lend itself to earlier application of cellular, acellular, matrix-like products or spilt thickness skin graft to close the wound, hopefully leading to a shorter time to complete wound closure. This, along with the

efficacy, safety and ease of use demonstrated in this trial potentially allowing for home use by the patient or a caregiver, may prove to be substantial benefits for patients and health care professionals. Additional studies are needed to further demonstrate the efficacy and potential benefits of Bromelain based enzymatic debridement in venous leg ulcers and other chronic wounds.

1. INTRODUCTION

Venous leg ulcers (VLU) are the most common ulceration on the lower extremity and account for 70% of all leg ulcers.[1] The prevalence of VLU has been estimated to range up to 2% of the population based on observational studies, with an overall cost of treatment approaching 1% of the health care budget of some western European countries.[2] The constant increase in the aging population is expected to increase these numbers as age negatively affects healing and recurrence rates.[3-5]

Eschar or fibrinous debris, consisting of devitalized tissue or slough that may harden by desiccation, commonly exists on the surface of chronic wound-beds.[6] Therefore, wound bed preparation (WBP) is the first step in treatment of these wounds, which is initiated by debridement, that removes the local impediments to healing by eliminating devascularized tissue, necrotic material, and excessive bacterial burden.[7] The aims of debridement are to create a healthy wound bed, edges and peri-wound skin, with the objective of promoting and accelerating healing.[8] Previous studies have shown the importance of adequate debridement in VLU. Cardinal et al demonstrated a 47% closure rate in VLUs that were surgically debrided serially versus a 30% closure rate in those that were not.[9] Several debridement methods exist, including surgical (or sharp in case of a minor bedside procedure), enzymatic, autolytic, osmotic (i.e. honey), mechanical (i.e. wet to dry), biologic (larvae), and technical (i.e. jet lavage, ultrasound).[8] However, according to the clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum, surgical debridement is recommended over other methods for VLUs that contain slough, nonviable tissue, or eschar.[2] This is in line with the fact that there is little evidence to suggest that the use of non-surgical debridement agents is beneficial for VLU healing.[10] Nevertheless, as surgical debridement has its drawbacks such as associated pain and bleeding, and requires the availability of a clinician trained in surgical debridement, the use

of currently available slow acting non-surgical debridement agents remains common. EscharEx® (MediWound Ltd, Yavne, Israel), is a Bromelain- based enzymatic topical agent currently in development for debridement of chronic wounds. Bromelain-based enzymatic debridement has been shown to provide an efficient, rapid, selective and safe non-surgical debridement in burns (NexoBrid®, MediWound Ltd, Yavne, Israel).[11-13] The positive experience in burns lead to an initial proof-of-concept study, demonstrating efficacy in debridement of chronic wounds of various etiologies.[14] This study was followed by a multi-center, assessor-blinded RCT, that demonstrated a significantly higher incidence of complete debridement of lower extremity ulcers treated with EscharEx as compared to those treated with its gel vehicle.[15] However, these studies utilized 4 hour topical applications, whose applicability for home use, and in an aging population, seemed questionable. Therefore, a new EscharEx formulation (EX-02) was developed to allow for longer safe treatment times (24 hours).

The main objective of this study was to assess the efficacy of EX-02 compared to its gel vehicle and to current non-surgical standard of care (NSSOC) debridement agents in debridement of VLU.

2. METHODS

2.1 Study design

The ChronEx study was an international, multicenter, prospective, randomized, placebo-controlled trial conducted across 20 medical centers and clinics in the United States, Switzerland and Israel, under the ethical rules for human experimentation that are stated in the 1975 Declaration of Helsinki, including approval by the institutional review boards of all participating centers (clinicaltrials.gov identifier NCT03588130, EudraCT number 2020-004861-38). After giving written informed consent (to a study investigator) and completing a screening period of 1-week, patients suffering from VLUs were randomized (using a computer-generated randomization stratified by region, pain level and wound size) to daily treatments with either EX-02, its gel vehicle (GV) as a placebo control, or NSSOC (in a 3:3:2 ratio), until reaching complete debridement or up to 8 daily treatments (within 2 weeks). Following the debridement period, patients were followed-up for up to 14 weeks. The overall duration of the study for patients in all arms was up to 17 weeks (See supplementary material figure 1). EX-02 and GV arm randomizations were double-blinded. The NSSOC arm was not blinded for both patients and assessors due to the different appearance of topical agents in this arm. The use of standardized compression therapy was indicated throughout the study for all patients. The trial protocol can be found in the supplemental material.

2.2 Inclusion and exclusion criteria

Inclusion criteria: Adult patients between 18 and 90 years of age; Suffering from a VLU present 4 weeks to 2 years (determined by medical history, physical examination, and an ultrasound scan demonstrating venous insufficiency); nonviable tissue (necrotic/slough/fibrin) covering at least 50% of the wound area (assessed by clinical evaluation); wound surface area between 2-100 cm2 (assessed by inSight™, eKare Inc.,

Fairfax, VA, USA); patient understood the nature of the procedure, was able to adhere to the protocol regimen, and provided a written informed consent prior to any study procedure.

Exclusion criteria: Patients with more than one leg ulcer (area ≥ $2cm^2$) on the leg of the target wound; wound area decreased by >20% after one week of standard-of-care-only period (screening period); signs of clinically significant infection including purulent discharge, deep-tissue abscess, erysipelas, cellulitis, etc; severely damaged skin (e.g., abrasion, exfoliation) extending >2 cm around the wound edge; presence of gangrene, signs of systemic infection, sepsis, or osteomyelitis during screening phase; clinical suspicion of skin cancer not ruled out by biopsy; skin disorders unrelated to the wound that are present adjacent to the wound; chronic skin disorders (e.g., idiopathic pruritus, psoriasis, panniculitis, pyoderma gangrenosum) that may deteriorate as a result of local trauma or debridement; wound with sinus tracts or tunnels extending under healthy tissue or penetrating into joint capsule; vascular operations in proximity to the wound in the last month; patients with primary lymphatic edema; significant decrease in the arterial blood flow of the extremity, as demonstrated by either Toe-Brachial Index (TBI) ≤ 0.50, Ankle-Brachial Index (ABI) ≤ 0.70, Skin Perfusion Pressure (SPP) ≤ 40 mmhg, or Transcutaneous oximetry (TCOM) ≤ 40 mmhg; pre-enrollment wounds which were covered by eschar heavily saturated with iodine or silver sulfadiazine; history of allergy or atopic disease or a known sensitivity to pineapples, papaya, bromelain, or papain, as well as known sensitivity to latex proteins, bee venom or olive tree pollen; poorly controlled diabetes mellitus (Hba1c >12%); Hemoglobin<8 g/dl) or WBC<3,800/µl or >15000/µl or Platelets<100,000/µl; abnormal liver function (aspartate transaminase, alanine transaminase >2 × upper limit of normal range); BMI>48 or albumin <2.5 g/dl; patients undergoing renal/peritoneal dialysis or creatinine >2.5 mg/dl; any condition that would preclude safe participation in the study (e.g., evidence of significant or unstable cardiovascular, pulmonary, liver, hematological, immunological, positive for COVID-19 or neoplastic disease, or any immediate life-threatening condition);

recent history (<6 months) of myocardial infarction; concurrent acute injury or disease that might compromise patient welfare; patient receiving (or received at any time within 3 months prior to enrollment) any medications or treatments known to affect the wound healing processes, including chronic systemic steroid intake with topical skin changes (i.e., thin, fragile skin with multiple hematomas, or previous laceration history), immunosuppressive drugs, radiation therapy, immunomodulating medications and chemotherapy; mentally incapacitated adults who were incapable of giving informed consent; concurrent use of nonapproved drugs or alcohol abuse; pregnant women or nursing mothers; exposure to investigational intervention within 3 months prior to enrollment, or anticipated participation in any other interventional study.

2.3 Screening period

Study procedures performed during this period (6-9 days) included recording of demographics and medical history, concomitant medications, vital signs, physical examination, clinical laboratory tests, pain assessment, wound assessments and cultures, and wound photograph assessment. Additionally, arterial insufficiency was ruled out by either; measurement of ankle-brachial index (ABI), toe-brachial index (TBI), skin perfusion pressure (SPP), or transcutaneous oximetry (TCOM). During this week, the wounds were treated by non-surgical standard treatment according to the investigators' choice, and standardized compression therapy with the Coban™ 2 Two-Layer Compression System (3M, St Paul, MN, USA). Patients whose wound surface area decreased by more than 20% as assessed by the eKare inSight™ digital wound management system (eKare Inc., Fairfax, VA, USA) during this period were excluded.

2.4 Debridement period

This was a period of up to 2 weeks, where patients arrived for up to 8 daily visits, and were treated according to their randomization.

2.4.1 EscharEx and Gel Vehicle Arms

EX-02 is a biological product that consists of a concentrate of proteolytic enzymes enriched in Bromelain, extracted from the stem of pineapples (MediWound Ltd, Yavne, Israel). The mechanism of action is mediated by the proteolytic activity of the enzymes. The powder of EX-02 or GV (8.1 grams in a 50 milliliter type II glass vial) was reconstituted with 20 grams sterile water for injection to obtain 5% EX-02 or the gel vehicle. In order to maintain blinding, the appearance of the vials and powders of EX-02 and GV was identical, and so was their appearance and consistency after reconstituting with water for injection. Patients, study investigators, and the study sponsor were all blinded. The content of the mixed product was sufficient for a wound area of up to 80 cm^2. For wounds larger than 80 cm^2, 2 vials of IMP (investigational medicinal product) were used. EX-02 or GV were topically applied on the wound surface for 24±3 hours, up to 8 consecutive applications (4 treatments a week), with 3 days of IMP holiday during the weekends, or until a complete debridement was achieved, whichever occurred first. A single additional IMP holiday of up to 24±3 hours was allowed in case of medical needs (e.g., in case of tolerability issues). During IMP holiday, a non-active dressing such as a hydrocolloid, hydrogel, or foam, was applied. At all times, the peri-wound area was protected by Calmoseptine® ointment (Calmoseptine Inc, Huntington Beach, CA, USA) and analgesics were provided as needed. Between dressings the wounds were cleaned with either sterile saline solution or water and mild soap. A flow chart describing the method for EX-02 / GV application during the study can be seen in Supplementary material figure 2.

2.4.2 NSSOC Arm

The NSSOC arm consisted of a standardized selection of dressings (including enzymatic agents, alginates, foams, highly absorbents, honey, hydrocolloids, hydrofibers, hydrogels, and silver products) that were applied on the wound surface according to their approved label, instructions for use, and investigator discretion in accordance with ulcer status, until complete debridement was achieved.

2.4.3 Additional study treatments

The Coban™ 2 Two-Layer Compression System (3M, St Paul, MN, USA) was used continuously throughout the study in all patients, reapplied after each dressing change, and limb elevation was instructed 3 times a day. All active methods of debridement including surgical, mechanical (wet-to-dry), biological (maggots), and the use of negative pressure wound therapy were prohibited during the debridement (daily visits) and follow-up periods. Hyperbaric oxygen therapy was not allowed throughout the entire study period.

2.5 Follow-up period

This period consisted of 4 twice-weekly visits for 2 weeks, followed by once-weekly visits until achieving complete wound closure or reaching a total of 10 weeks. During this period a similar variety of NSSOC treatments could be used in all arms. Complete wound closure was defined as complete re-epithelialization of the wound surface without drainage or dressing, confirmed at two visits 2 weeks apart, in accordance with FDA definitions.[16] Therefore, a wound closure confirmation visit was performed 2 weeks after wound closure if applicable. Thus, the maximal duration of the follow-up period was up to 14 weeks.

2.6 Endpoints

The primary efficacy endpoint of the study was the incidence of complete debridement in the EX-02 and GV arms, scored dichotomously (yes/no), assessed clinically after each application during the daily visits period (up to 8 applications within 14 days).

Secondary endpoints included the time to complete debridement in the EX-02 and NSSOC arms (from the initiation of study treatments, throughout the entire study); change in pain from baseline to the end of the twice weekly period in the EX-02 and GV arms, as assessed by Numeric Pain Rating Scale (NPRS);[17] change in wound area from baseline to the end of the twice weekly period in the EX-02 and GV arms, as assessed by eKare inSight™; incidence of >75% granulation tissue at the end of the daily visits period in the EX-02 and GV arms

(clinical assessment); change in quality of life (QoL) from baseline to the end of the twice weekly period in the EX-02 and GV arms, as assessed by the Wound-QoL questionnaire (ranging from 0 to 4, higher values describe larger restriction).[18] *Safety outcome measures* included the incidence and to time to complete wound closure in the EX-02 and GV arms, and the incidence and severity of adverse events and tolerability issues, including the proportion of patients discontinuing treatment due to adverse events, assessed over the entire course of the study.

2.7 Sample size calculation

Sample size calculation was based on demonstrating superiority of EX-02 over GV on the primary endpoint. Based on the assumption of a complete debridement rate of 60% for EX-02 and 30% for GV, a sample size of 45 patients in the EX-02 arm and 45 patients in the GV arm were to provide a minimum of 80% power using a two-sided test, based on the asymptotic normal approximation for a difference in proportions with α=0.05. With 30 additional patients in the NSSOC arm (based on the 3:3:2 randomization scheme), the total minimal planned sample size was 120 patients. The final sample size for this study was determined at an interim sample size re-assessment planned after 80 subjects (67% of the subjects) had completed the daily visits period (thus, producing the results of primary endpoint). The final sample size was determined based on Mehta and Pocock's promising zone approach. The design criterion for this approach was predicated on achieving a final power of at least 80% for the primary efficacy endpoint.

2.8 Statistical analysis

Data was collected by investigators at the participating medical centers and wound care clinics in the United States, Switzerland and Israel, and were transferred to a central database. Numerical variables were tabulated using number of observations, mean, standard deviation,

minimum, median, and maximum. Categorical variables were tabulated using number of observations and percentages. Statistical tests were conducted against a two-sided alternative hypothesis, employing a significance level of 0.05. Fisher's exact test was used to compare rates and incidences between the study groups. The incidence of complete debridement, which was the primary endpoint of the study, was conducted on the full analysis set and compared the incidence of complete debridement between EX-02 and GV. The hypothesis was evaluated as follows: $H0$: $\pi EX\text{–}02 = \pi Gel\ Vehicle$; $H1$: $\pi EX\text{–}02 \neq \pi Gel\ Vehicle$ (where π was the proportion of subjects who achieved complete debridement during the daily visits period). This hypothesis was tested using the normal approximation with 2-sided α=0.05. The normal approximation (rather than Fisher's exact test) was used to match the calculation of conditional power done for sample size re-assessment. Treatment effect is represented by absolute risk reduction with its 95% confidence intervals. Kaplan–Meier's survival function curve was used to compare the time to complete debridement and time to wound closure. To avoid inflation of type 1 error rate, the secondary endpoints were analyzed in hierarchical order following analyzing the primary endpoint as defined using a step-down procedure. Any statistical tests performed on efficacy endpoints after a p-value exceeding 0.05 was encountered were considered exploratory for informational purposes and planning of future research. For both endpoints of changes in pain score and wound area, descriptive statistics with raw change from baseline are presented. Handling of missing data was as follows: For incidence of complete debridement; EX-02 and Gel Vehicle patients for whom the debridement status was partially or fully missing, and complete debridement was never achieved within the period of up to 8 applications, imputed "failure" for the primary and for the covariate analyses; For all 'time-to' analyses; Patients who did not achieve complete debridement were censored at the last available time where no debridement was confirmed, thus, all data was considered in the analysis and no imputation was needed; For continuous outcomes of NPRS and wound size; Missing values were not imputed, i.e. only observed

measurements were used. All statistical analyses were carried out using SAS Version 9.4 under Windows 2016 Terminal.

2.9 Role of the funding source

This study was planned and funded by MediWound Ltd., with oversight by an independent Data Safety Monitoring Board (DSMB). The study was conducted by a contact research organization (CRO) in the United States (IQVIA Inc., Durham, NC, USA), and by MediWound Ltd (Yavne, Israel) in Switzerland and Israel. MediWound employees (YKL, KDZ, EK, and RJS) made substantial contributions to conception or design of the study, the acquisition, analysis, and interpretation of the data, drafted and revised the manuscript and provided final approval of the manuscript version to be published.

3. RESULTS

A total of 196 patients were enrolled in 20 medical centers and wound care clinics in the United States, Switzerland and Israel. The first patient was enrolled on November 12th, 2019, and the last patient completed follow up on February 15th, 2022 (the study was terminated as planned). Of these, 119 were found eligible and randomized, 46 to the EX-02 arm, 43 to the GV arm, and 30 to the NSSOC arm. Eight patients (6.7%) terminated early from the study, while 111 patients (93.3%) completed the study visits per protocol. No deaths were reported during the study. A summary of the trial profile / flow diagram can be seen in Figure 1.

3.1 Patients and wounds baseline characteristics

Baseline characteristics of patients and wounds were similar in the 3 treatment arms (p=NS, Table 1). The average age of patients in the study was 64.0±12.5 years, with a 64/55 male to female ratio. Most patients were white (79%) with an average BMI of 32.8±7.5 kg/m^2. Despite no significant differences between the arms, patients in the GV arm had somewhat older and larger wounds with a larger percentage of nonviable tissue.

3.2 Incidence of complete debridement EX-02 vs. GV (primary endpoint)

The incidence of complete debridement during the daily visits debridement period (up to 8 applications within up to 14 days) was significantly higher in the EX-02 arm, 63% (29/46 patients) as compared to 30.2% (13/43 patients) in the GV arm (p=0.004), thus achieving the primary endpoint of the study. The absolute risk reduction between the EX-02 and GV arms was 32.8% (95% CI=9.78-51.67).

3.3 Time to complete debridement EX-02 vs. NSSOC and GV

The Kaplan-Meier median estimates for time to complete debridement were significantly shorter in the EX-02 arm, 9 (5-15, 95% CI) days as compared to 59 (30-85, 95% CI) days in the NSSOC arm (p=0.016) and 63 (21-93, 95% CI) days in the GV arm (p=0.004, see Figure 2).

3.4 Change in pain from baseline to end of twice weekly period EX-02 vs. GV

The average NPRS reduction at the end of the twice weekly period (2 weeks after end of debridement) was 1.3±2.7 and 1.4±2.7 for the EX-02 and GV arms, respectively (p=NS).

3.5 Change in wound area from baseline to end of twice weekly period EX-02 vs. GV

The average reduction at the end of the twice weekly period (percent change from baseline to 2 weeks after end of debridement) as assessed by eKare inSight™ was 2.9 ± 11.4 cm^2 (21.5%) and 2.7 ± 12.5 cm^2 (25.3%) for the EX-02 and GV arms, respectively (p=NS).

3.6 Incidence of >75% granulation tissue at end of debridement period EX-02 vs. GV

The incidence of >75% granulation tissue assessed clinically at the end of the daily visits period was 93% and 56% for the EX-02 and GV arms, respectively (p <0.0001).

3.7 Change in Wound -QoL from baseline to end of twice weekly period EX-02 vs. GV

The average total Wound-QoL scores improved from 1.9±1.0 to 1.3±1.0 for the EX-02 arm and from 2.1±1.0 to 1.5±1.0 for the GV arm. The mean change was 0.5±0.8 and 0.5±0.6 for the EX-02 and GV arms, respectfully (p=NS).

A summary of the primary and secondary endpoints results can be seen in table 2.

3.8 Exploratory and post-hoc analyses

The incidence of complete debridement at the end of the daily visits debridement period was 63% (29/46 patients) and 13.3% (4/30 patients) in the EX-02 and NSSOC arms, respectively (p<0.001). The incidence of 100% granulation tissue at the end of the daily visits debridement period (post hoc analysis) was 50% (23/46 patients) in the EX-02 arm, which was significantly higher than the 25.6% (n=11/43 patients) in the GV arm (p=0.01) and the 10% (3/30 patients) in the NSSOC arm (p=0.0002).

3.9 Safety

Sixty-five (54.6%) patients suffered treatment emergent adverse events (TEAE) throughout the study, distributed similarly among treatment arms; 24 (52.2%), 27 (62.8%) and 14 (46.7%) patients in the EX-02, GV and NSSOC arms, respectfully. Of these, a total of 5 (5.2%)

patients experienced serious TEAEs throughout the study: 1 (2.2%) in the EX-02 arm, 3 (7.0%) in the GV arm, and 1 (3.3%) in the NSSOC arm. Thirteen (10.9%) patients experienced TEAEs that were assessed by investigators as having reasonable possibility relationships to study treatment; 9 (19.6%) in the EX-02 arm, 3 (7.0%) in the GV arm, and 1 (3.3%) in the NSSOC arm. Most TEAEs were mild, with moderate severity TEAEs reported in 12 (10.1%) patients. Severe TEAEs were reported in 1 (2.2%) patient in the EX-02 arm (increased blood pressure), 2 (4.7%) patients in the GV arm (skin graft infection and cellulitis), and none in the NSSOC arm. Two patients (1.7%) discontinued the study treatment due to TEAEs; 1 (2.2%) due to pain in the EX-02 arm (this was the only case of application site pain reported as a TEAE in the study) and 1 (2.3%) due to cellulitis in the GV arm. Throughout the study, the use of analgesics was reported by 16 (34.8%) patients in the EX-02 arm, 17 (39.5%) in the GV arm, and 8 (26.7%) in the NSSOC arm (NS). During the daily visits period analgesics were reported by 16 (34.8%) patients in the EX-02 arm, 17 (39.5%) in the GV arm, and 4 (13.3%) in the NSSOC arm. Opioids use was also similar in all arms (13.0% in EX-02; 18.6% in GV, 13.3% in NSSOC). No deaths were reported during the study. There were no significant differences in the incidence of (EX-02=32.6%, GV=27.9%, NSSOC=26.7%) or time to complete wound closure (25^{th} percentile: EX-02=64 days, GV=63 days, NSSOC=78 days). A representative case of a VLU treated with EX-02 during the study can be seen in figure 3. Figure 4 shows all adverse events throughout the study that had a frequency higher than 4% in any of the treatment arms. In general, there were no significant differences in adverse event rates between the arms.

4. DISCUSSION

In this RCT enzymatic debridement of VLUs with EX-02 lead to a significantly higher incidence of complete debridement as compared to GV, thus achieving the primary endpoint of the study. Additionally, EX-02 lead to a significantly shorter time to complete debridement as compared to both GV and NSSOC. These findings, together with the significantly higher rate of achieving complete granulation at the end of the daily visits period, demonstrate the wound bed preparation efficacy of EX-02 as compared to both NSSOC and GV. While GV played the role of a double blinded placebo in this trial (in accordance with FDA guidance), as a hydrogel it should also be considered an autolytic debridement agent. Therefore, the significantly better results of EX-02 as compared to GV should be interpreted accordingly. The efficacy of EX-02 demonstrated in this study is in line with the results of a previous RCT with an older generation Bromelain based enzymatic debridement product (EX-01, MediWound Ltd, Yavne, Israel), where 73 patients suffering from lower extremity ulcers (venous, diabetic and post-surgical/traumatic etiology) were randomized to debridement with either EX-01 or its gel vehicle. Patients were treated with up to 10 daily 4-hour topical applications, and then continued follow-up for up to 6 months. The EX-01 arm achieved a significantly higher incidence of complete debridement over gel vehicle (55% vs. 29%, p=0.047).[15] Additionally, as in our current study with EX-02, there were no significant differences in wound closure rates and no significant safety issues were encountered throughout the study. A clear benefit of EX-02 over EX-01 is the longer application time (24 hours versus 4 hours).

Wound bed preparation is considered the first step in the treatment of chronic wounds. As surgical debridement has some drawbacks and is not always available or feasible, the use of non-surgical debridement agents remains common despite their limited efficacy. Thus, there is an unmet need for an efficient non-surgical debridement agent. Gethin et al reviewed 10

RCTs (including a total of 715 patients) comparing different debridement methods of VLUs.[10] They presented study results in a narrative form, as meta-analysis was not possible, and noted that most trials were at high risk of bias. They concluded that their review failed to identify an optimal debridement method or duration of treatment. We summarized the main results of their review in a panel, together with the results of our study (Table 3). As one of these RCTs reporting the results of honey vs. hydrogel has since been retracted due to errors in the data analysis we did not include it in the panel and discussion.[19] Obviously, when comparing our study results to those of these studies, differences in patient cohorts must be considered. Nevertheless, we believe the comparison to other VLU debridement studies is important. Six RCTs evaluated different methods of autolytic debridement including biocellulose wound dressing (BWD), non-adherent dressing, hydrogel, hydrofiber dressing, hydrocolloid dressings, dextranomer beads, Edinburgh University Solution of Lime (EUSOL) and paraffin gauze. Two RCTs evaluated enzymatic preparations including collagenase and one evaluated bio-surgical debridement with maggots. No RCTs evaluated surgical, sharp or mechanical methods of debridement, or debridement versus no debridement. Only 2 of the studies stated a 50% minimum amount of slough required in the wound bed for inclusion, the other studies indicated that people with necrotic or sloughy venous ulcers were included but did not state the baseline percentage of slough. Study durations varied between 1-12 weeks. The use of compression therapy was relatively consistent, with 8 of the 10 studies using compression during the treatment period. The Gethin et al review focuses on 6 main outcomes, including incidence of complete debridement, time to achieve debridement, wound healing rate, reduction in wound size, pain reduction and adverse events.[10]

Four studies reported the number of wounds completely debrided (Groenewald 1980,[20] Jasiel 1996,[21] Alvarez 2012,[22] Wayman 2000[23]), and five studies reported the time to achieve debridement (Groenewald 1996,[20] Jasiel 1996,[21] Westerhof 1990,[24] Konig 2005,[25] Wayman

2000[23]). The only agent reported to have achieved a higher incidence of complete debridement within a shorter time of the current study results of EX-02 is Dextranomer beads.[20] However, this study did not define a minimum amount of slough as an inclusion criteria so the amount of non-viable tissue debrided is unclear. Additionally, this study was conducted over 40 years ago and this product is no longer available. Larvae were also demonstrated as rapid and efficient, however, due to a small sample size, superiority over hydrogel was only demonstrated in the time to achieve debridement. Additionally, larvae therapy has significant drawbacks, such as significantly higher pain levels, cost and treatment complexity as compared to hydrogel.[26] In contrast to these studies, our study demonstrated superiority over GV in addition to other debridement agents. While GV was included as a placebo, it is in fact a hydrogel (missing the enzymes that when added to it constitute EX-02), that completely debrided 30.2% of the wounds within 14 days. Therefore, it is reasonable to attribute the statistically significant difference between the debridement rate of EX-02 and GV to the added efficacy of the enzymes, over the performance of a standard of care autolytic debridement agent.

Only one study reported the number of wounds healed (Alvarez 2012).[22] The wound closure rate reported is slightly higher than the results of our study, however 13 patients withdrew from that study and their wound closure status is unknown. In any case, both studies did not demonstrate a significantly higher wound closure rate vs. a comparator, despite a significantly higher rate of complete debridement. Five studies reported reduction in wound size (Skog 1983,[27] Hansson 1998,[28] Wild 2010,[29] Alvarez 2012,[22] Westerhof 1990[24]). Only two studies demonstrated superior results of cadexomer iodine and hydrocolloid vs. paraffin/saline soaked dressings within 4-6 weeks, however wound healing rates were not reported for these studies.[27,28] Our study did not demonstrate significant differences in wound size reduction vs. a comparator, similar to the findings of the other 3 studies. This may be attributed to the fact that our study was not powered to demonstrate decrease in

wound size or healing. An additional factor may be the variety of dressings used after the end of the debridement period. Perhaps a larger sample size together with a more standardized regimen of allowed treatments towards wound closure could help link between debridement and wound healing rates in a significant manner.

Six studies reported pain as an outcome (Groenewald 1980,[20] Skog 1983,[27] Hansson 1998,[28] Wild 2010,[29] Alvarez 2012,[22] Westerhof 1990).[24] There was an overall reduction in pain when wounds were debrided.[10] Our study also demonstrates reduction in pain, however the differences between the groups were not significant. It is worth noting that there is usually significant pain associated with debriding VLUs, however EX-02 was able to debride a significant majority of these wounds without significant pain.

Only 3 studies reported adverse events (Jasiel 1996,[21] Alvarez 2012,[22] Hansson 1998).[28] Overall, the reporting of adverse events was poor, most probably under-reported.[10] Our study reported a higher incidence of adverse events, however, there were no significant differences in their incidence between the study groups and the safety profile of EX-02 seems acceptable.

One of the most common enzymatic debridement agents in chronic wound care is collagenase. However, a systematic review and meta-analysis of RCTs on the use of collagenase in wounds and ulcers by Patry et al[30] did not find sufficient evidence for its use in VLU (only 1 RCT performed in VLU, Konig 2005),[25] and concluded that there is very limited data on the effect of collagenase as an enzymatic debridement technique on venous wounds. Additionally, this meta-analysis demonstrated that patients treated with collagenase have an increased risk of adverse events compared to alternative treatments.

An important additional aspect in treatment of chronic wounds is the focus on microbial barriers that impair or delay wound healing. Recently published results of a small pilot study (12 patients) demonstrated that Bromelain-based enzymatic debridement of VLU with

EscharEx® lead to reductions in both biofilm and bacterial autofluorescence values, suggesting a favorable effect on reduction of bioburden, in addition to its debridement efficacy.[31]The strengths of this study include a comparison to both a blinded assessment compared to placebo and to a standardized set of unblinded NSSOC methods of debridement, with an adequate sample size allowing for achieving statistically significant superiority for the primary endpoint. Additionally, this study addresses all 6 main outcomes mentioned in the review above, while using pre-defined eligibility criteria including a minimum amount of necrotic tissue.

Our study has several limitations. While there were no significant differences in baseline characteristics between the study arms, The GV arm had larger wounds as compared to the EX-02 arm (NS). This difference in size may be explained by the fact that study randomization was stratified by wound size categories. The stratification levels were defined as ≤40cm2 and >40cm2. Each arm had a similar percentage of subjects within each category. Perhaps using more than 2 size categories in randomization schemes may improve stratification in future studies. Another limitation is that while EX-02 was applied once daily, a treatment regimen similar to most of the VLU RCTs mentioned above, it is reasonable to assume that a more infrequent dressing change (such as once in 48 hours) would lead to higher patient and caregiver compliance, and to experiencing less pain. Although other (prospective) studies have also not been able to link debridement to wound closure rates in VLU, we believe the fact that EX-02 did not lead to a higher incidence of or a shorter time to wound closure during the study period despite its superior complete debridement efficacy, is also a study limitation. Better standardization strategies and larger sample sizes may assist in overcoming this limitation in future studies. Better standardization strategies in the future may also allow for better interpretation of the results of the NSSOC arm, that included a range of products commonly employed in the clinical practice of the participating investigators. This is also a study limitation, although this reflects the pragmatic, real-world

NSSOC. Another limitation is that only 3 nations participated in this multicenter study, representing North America, Europe and the Middle East, while other regions were not represented.

In conclusion, the superior debridement efficacy of EX-02 vs. both a double-blinded placebo and NSSOC, combined with the acceptable safety profile demonstrated in this RCT, may provide a solution to the unmet need for a safe and efficient non-surgical debridement agent for VLU. This would lend itself to earlier application of cellular, acellular, matrix-like products or spilt thickness skin graft to close the VLU, hopefully leading to a shorter time to complete wound closure. This, along with the efficacy, safety and ease of use demonstrated in this trial potentially allowing for home use by the patient or a caregiver, may prove to be substantial benefits for patients and health care professionals. Additional studies are needed to further demonstrate the efficacy and potential benefits in VLU, including improved size category stratification and a more uniform dressing regimen towards wound closure. Clinical trials in other chronic wound etiologies should also be considered.

CONTRIBUTORS

Study design and protocol development: YS, JCL, KDZ, YKL, EK, MK and LR.

Data collection: YKL, CRD, RA, AR, FS, GT, ES, MH, FPC, JCL, SMC, YSD, CAC, DE, DV, AJS, RCG, JRH, CM, AS, and LTT.

Data curation: YKL and KDZ.

Data analysis: YKL, KDZ, EK, MK, RJS, LR and YS.

First manuscript draft: YS, YKL, KDZ, EK, RJS, LR and JCL.

All authors had full access to the study data and critically reviewed the manuscript. YS, KDZ and YKL directly accessed and verified the underlying data. All authors take responsibility for the accuracy of the analysis and had authority over manuscript preparation and the decision to submit the manuscript for publication. All authors approved the manuscript for submission.

DATA SHARING

Deidentified individual participant data will not be made available at this stage. Data may be made available upon reasonable request after product approval in the US or after product development is discontinued.

DECLARATION OF INTERESTS

YS is a consultant for MediWound Ltd and Vericel Corp. LR, JCL and CD are consultants for MediWound Ltd. RJS, EK, KDZ, MK and YKL are MedWound's Chief Medical Officer, Chief R&D Officer, Director of Clinical Affairs, Medical Director and Director of Clinical Development. AR served as an advisory board member for MediWound Ltd. AR and LTT report receiving study funding. The other authors report no competing interests beyond funding to their institutions for the current project.

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REFERENCES

1. Tatsioni A, Balk E, O'Donnell T, Lau J. Usual care in the management of chronic wounds: a review of the recent literature. *J Am Coll Surg* 2007; **205**(4): 617-24e57.
2. O'Donnell TF, Jr., Passman MA, Marston WA, et al. Management of venous leg ulcers: clinical practice guidelines of the Society for Vascular Surgery ® and the American Venous Forum. *J Vasc Surg* 2014; **60**(2 Suppl): 3s-59s.
3. Wicke C, Bachinger A, Coerper S, Beckert S, Witte MB, Königsrainer A. Aging influences wound healing in patients with chronic lower extremity wounds treated in a specialized Wound Care Center. *Wound Repair Regen* 2009; **17**(1): 25-33.
4. Raffetto JD, Ligi D, Maniscalco R, Khalil RA, Mannello F. Why Venous Leg Ulcers Have Difficulty Healing: Overview on Pathophysiology, Clinical Consequences, and Treatment. *J Clin Med* 2020; **10**(1).
5. Probst S, Saini C, Gschwind G, et al. Prevalence and incidence of venous leg ulcers-A systematic review and meta-analysis. *Int Wound J* 2023; Nov;20(9):3906-3921.
6. Bradley M, Cullum N, Sheldon T. The debridement of chronic wounds: a systematic review. *Health Technol Assess* 1999; **3**(17 Pt 1): iii-iv, 1-78.
7. Werdin F, Tenenhaus M, Rennekampff HO. Chronic wound care. *Lancet* 2008; **372**(9653): 1860-2.
8. Strohal R, Dissemond J, Jordan O'Brien J, et al. EWMA document: Debridement. An updated overview and clarification of the principle role of debridement. *J Wound Care* 2013; **22**(1): 5.
9. Cardinal M, Eisenbud DE, Armstrong DG, et al. Serial surgical debridement: a retrospective study on clinical outcomes in chronic lower extremity wounds. *Wound Repair Regen* 2009; **17**(3): 306-11.
10. Gethin G, Cowman S, Kolbach DN. Debridement for venous leg ulcers. *Cochrane Database Syst Rev* 2015; **2015**(9): Cd008599.
11. Rosenberg L, Krieger Y, Bogdanov-Berezovski A, Silberstein E, Shoham Y, Singer AJ. A novel rapid and selective enzymatic debridement agent for burn wound management: a multi-center RCT. *Burns* 2014; **40**(3): 466-74.
12. Rosenberg L, Shoham Y, Krieger Y, et al. Minimally invasive burn care: a review of seven clinical studies of rapid and selective debridement using a bromelain-based debriding enzyme (Nexobrid®). *Ann Burns Fire Disasters* 2015; **28**(4): 264-74.
13. Shoham Y, Gasteratos K, Singer AJ, Krieger Y, Silberstein E, Goverman J. Bromelain-based enzymatic burn debridement: A systematic review of clinical studies on patient safety, efficacy and long-term outcomes. *Int Wound J* 2023.
14. Shoham Y, Krieger Y, Tamir E, et al. Bromelain-based enzymatic debridement of chronic wounds: a preliminary report. *International Wound Journal* 2018; **15**(5): 769-75.
15. Shoham Y, Shapira E, Haik J, et al. Bromelain-based enzymatic debridement of chronic wounds: Results of a multicentre randomized controlled trial. *Wound Repair Regen* 2021; **29**(6): 899-907.
16. Gould L, Li WW. Defining complete wound closure: Closing the gap in clinical trials and practice. *Wound Repair Regen* 2019; **27**(3): 201-24.
17. Farrar JT, Young JP, Jr., LaMoreaux L, Werth JL, Poole MR. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain* 2001; **94**(2): 149-58.
18. Augustin M, Conde Montero E, Zander N, et al. Validity and feasibility of the wound-QoL questionnaire on health-related quality of life in chronic wounds. *Wound Repair Regen* 2017; **25**(5): 852-7.

19. Gethin G, Cowman S. Manuka honey vs. hydrogel--a prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing outcomes in venous ulcers. *J Clin Nurs* 2009; **18**(3): 466-74.
20. Groenewald JH. An evaluation of dextranomer as a cleansing agent in the treatment of the post-phlebitic stasis ulcer. *S Afr Med J* 1980; **57**(20): 809-15.
21. Jasiel E, Nowak A, Gwiezdzinski Z, et al. A randomised, controlled, parallel group clinical trial of a new debriding agent (Sterigel) versus paraffin gauze in the treatment of necrotic venous leg ulcers. Proceedings of 6th European Conference on Advances in Wound Management. 1996:81-3.
22. Alvarez OM, Phillips TJ, Menzoian JO, Patel M, Andriessen A. An RCT to compare a bio-cellulose wound dressing with a non-adherent dressing in VLUs. *J Wound Care* 2012; **21**(9): 448-53.
23. Wayman J, Nirojogi V, Walker A, Sowinski A, Walker MA. The cost effectiveness of larval therapy in venous ulcers. *J Tissue Viability* 2000; **10**(3): 91-4.
24. Westerhof W, van Ginkel CJ, Cohen EB, Mekkes JR. Prospective randomized study comparing the debriding effect of krill enzymes and a non-enzymatic treatment in venous leg ulcers. *Dermatologica* 1990; **181**(4): 293-7.
25. König M, Vanscheidt W, Augustin M, Kapp H. Enzymatic versus autolytic debridement of chronic leg ulcers: a prospective randomised trial. *J Wound Care* 2005; **14**(7): 320-3.
26. Dumville JC, Worthy G, Bland JM, et al. Larval therapy for leg ulcers (VenUS II): randomised controlled trial. *BMJ* 2009; **338**: b773.
27. Skog E, Arnesjö B, Troëng T, et al. A randomized trial comparing cadexomer iodine and standard treatment in the out-patient management of chronic venous ulcers. *Br J Dermatol* 1983; **109**(1): 77-83.
28. Hansson C. The effects of cadexomer iodine paste in the treatment of venous leg ulcers compared with hydrocolloid dressing and paraffin gauze dressing. Cadexomer Iodine Study Group. *Int J Dermatol* 1998; **37**(5): 390-6.
29. Wild T ET, Andriessen A. Wound cleansing efficacy of two cellulose-based dressings. Wounds UK 2010;6(3):14-21.
30. Patry J, Blanchette V. Enzymatic debridement with collagenase in wounds and ulcers: a systematic review and meta-analysis. *Int Wound J* 2017; **14**(6): 1055-65.
31. Snyder RJ, Singer AJ, Dove CR, et al. An open-label, proof-of-concept study assessing the effects of bromelain-based enzymatic debridement on biofilm and microbial loads in patients with venous leg ulcers and diabetic foot ulcers. *Wounds* 2023; **35**(12): E414-E419.

TABLES

Table 1: Patient and wound baseline characteristics (p=NS; BMI=Body Mass Index; EX-02=EshcarEx; NSSOC=Non-Surgical Standard of Care; % Non-viable tissue* was assessed clinically)

Characteristic	EX-02 (n=46)	Gel Vehicle (n=43)	NSSOC (n=30)
Female, *n (%)*	20 (43.5)	16 (37.2)	19 (63.3)
Male, *n (%)*	26 (56.5)	27 (62.8)	11 (36.7)
Age (years), *mean (±SD)*	65.5 (±12.2)	61.4 (±12.6)	65.5 (±12.7)
Ethnic origin, *n (%)*			
White	38 (82.6)	36 (83.7)	20 (66.7)
Black/African American	7 (15.2)	6 (14.0)	9 (30.0)
Asian	1 (2.2)	0 (0.0)	1 (3.3)
Pacific Islands	0 (0.0)	1 (2.3)	0 (0.0)
BMI (kg/m^2), *mean (±SD)*	31.8 (±7.4)	34.0 (±7.9)	32.6 (±7.1)
Wound age (weeks), *mean (±SD)*	26.8 (±20.5)	39.5 (±27.6)	26.1 (±20.5)
Wound size (cm^2), *mean (±SD)*	13.1 (±20.5)	18.8 (±18.1)	14.4 (±19.6)
Wound size ≤ 40cm², n (%)	42 (91.3)	39 (90.7)	28 (93.3)
Wound size > 40cm², n (%)	4 (8.7)	4 (9.3)	2 (6.7)
% Non-viable tissue*, *mean (±SD)*	72% (±13%)	78% (±15%)	68% (±17%)

Table 2: Summary of primary and secondary endpoint results

Endpoints	Outcomes			Treatment effect (95% CI)	p-value
	EX-02 N=46	**Gel Vehicle N=43**	**NSSOC N=30**		
Primary					
Incidence of complete debridement during the daily visit period	63.0% (n=29)	30.2% (n=13)	13.3% [1] (n=4)	Risk difference = 32.8% (9.78,51.67)	P = 0.004 [2]
Secondary					
Time to complete debridement	9 (5,15) [3] days	63 (21,93) [1,3] days	59 (30,85) [3] days	Hazard ratio = 1.9 (1.1, 3.26)	P = 0.016 [4]
Change in pain from baseline to the end of the twice-weekly period, as assessed by Numeric Pain Rating Scale	1.3 (2.7) [5]	1.4 (2.7) [5]	1.6 (1.9) [1,5]	Adjusted mean difference [6] = 0.45 (-0.56,1.5)	P = 0.4 [6]
Change in wound area from baseline to the end of the twice-weekly period, assessed by eKare inSight™	2.9 (11.4) [5] cm^2	2.7 (12.5) [5] cm^2	4.7 (12.4) [1,5] cm^2	Adjusted mean difference [6] = 0.96 (-4.33,6.25)	P = 0.72 [6]
Incidence of >75% granulation tissue at the end of the daily visits period, assessed clinically	93.3% (n=42)	55.8% (n=24)	37.9% [1] (n=11)	Risk difference = 37.5% (18.12,54.15)	P < 0.0001[7]
Change in Wound-QoL score from baseline to end of follow-up period	0.5 (0.8) [5]	0.5 (0.6) [5]	0.6 (0.6) [1,5]	Adjusted mean difference [8] = 0.04 (-0.26,0.34)	P = 0.789 [8]

Primary and secondary endpoints were predefined between the EX-02 and GV arms except for the first secondary endpoint which was predefined between the EX-02 and NSSOC arms. Treatment effect and p-values shown in the table relate only to the two predefined endpoint arms' results. [1] Data shown in light grey font are for the arm that was not predefined for this endpoint, therefore treatment effect and p-value shown do not relate to this arm's data.[2] P-value was calculated using Chi Square test adjusted for continuity.[3] Median and 95% CI estimated from Kaplan Meier. [4] P-value was calculated using Log-Rank test. [5] Mean reduction (SD). [6] Adjusted LS Means difference; 95% CI and p-value calculated using mixed model with repeated measures. [7] P-value was calculated using 2-sides Fisher's exact test. [8] Adjusted LS Means difference; 95% CI and p-value calculated using one-way analysis of covariance.

Table 3: Review panel of VLU debridement methods randomized controlled trials (based on Gethin et al).[10]

Study	Agent (patients included in analysis)	Complete debridement rate	Time to complete debridement	Wound healing rate	Wound size reduction	Pain reduction	Adverse events
Groenewald 1980[20]	Dextranomer beads (n=50) EUSOL soaks (n=50)	80% vs. 14% after 1 week (P< 0.0001)	Mean 5.9 days vs. 15.4 days (p<0.001)	NR	NR	All dextranomer beads patients had a reduction in pain within 24 hours of treatments, no reported pain after 10 days. Four EUSOL patients had increase in pain that did not improve. At 21 days two participants continued to have pain.	NR
Jasiel 1996[21]	Hydrogel (n=46) Paraffin gauze (n=40)	76% vs. 45% within 3 weeks (p=0.05)	No differences, but precise figures were not reported	NR	NR	NR	Two adverse events in hydrogel, 1 possibly related to treatment (erysipelas). One adverse event in paraffin gauze (maceration and infection). One participant withdrawn from each groups (thrombophlebitis, infection).
Alvarez 2012[22]	Biocellulose wound dressing (Suprasorb®, n=25) Nonadherent dressing (Adaptic®, n=23)	84% vs. 26% in 12 weeks (p<0.0001) **(75%-100% clean)*	NR	39% vs. 47% in 12 weeks, but 13 pts withdrew during follow up (NS)	74% vs. 54% in 6 weeks (NS)	Over 12-week period, a larger proportion of BWD patients had no/mild pain compared to control group, and at week seven there was a significant difference between the groups however no pain scores were provided at any time point.	14 adverse events attributed to study treatment; Clinically-infected ulcer (3 BWD group, 5 non-adherent group), cellulitis (2 BWD group, 1 non-adherent group), dermatitis (1 BWD group, 2 non-adherent group). None reported not related to study treatment.
Hansson 1998[28]	Cadexomer iodine (n=56) Hydrocolloid (n=48) Paraffin gauze (n=49)	NR	NR	NR	Mean 35.5% vs. 34.4% vs. 10.6% in 4 weeks[a]	Overall reduction by week 12 was 66% to 29% in cadexomer-iodine, 73% to 57% in hydrocolloid, and 57% to 15% in paraffin	12 participants in the iodine group, 7 in the hydrocolloid group and 9 in the paraffin gauze group were withdrawn due to allergic reactions, dermatitis, pain or poor compliance (stated these were not due to the study treatments).
Wild 2010[29]	Biocellulose wound dressing (Suprasorb®, n=20) Hydrofibre dressing (AquaCel®, n=20)	NR	NR	NR	Mean 43.5% vs. 17.9% in 4 weeks (NS)	Pain at dressing changes on days 7, 14 and 28 were 2.25, 2.7 and 1.3 for BWD vs. 3.73, 5.25 and 3.2 for hydrofibre dressing, however no baseline scores were provided	NR
Skog 1983[27]	Cadexomer iodine (n=38) Non adherent dressings (n=36)	NR	NR	NR	Mean 34% vs. 5% in 6 weeks (P< 0.02)	VAS scores before treatment, at week 1 and at 6 weeks were 32, 27 and 10 for cadexomer iodine vs. 33, 29 and 23 for non-adherent dressings (P<0.05)	NR (while withdrawals from the study were noted, there were no adverse events reported).
Westerhof 1990[24]	Krill enzymes (n=16) Standard autolytic protocol[b] (n=15)	NR	Mean 7 days vs. 10 days (NS)	NR	13% vs. 3% in 1 week, however 12 patients excluded from analysis	Both treatments caused a similar reduction in pain, no figures reported	There were no signs of side effects in either group.
	Collagenase (n=27)	NR		NR	NR	NR	NR

Konig 2005[25]	Moist dressing (Tender-Wet®, n=15)		8.5% vs 18.7% slough reduction after 14 days (NS)				
Wayman 2000[23]	Larvae (n=6)	100% vs 33% in 1 month (p=0.065)	Mean 3 days vs. 22 days (p=0.003)	NR	NR	NR	NR
	Hydrogel (n=6)						
ChronEx	EX-02 (n=46)	63% vs. 30.2% vs 13.3% within 14 days (p<0.01)	Median 9 days for EX-02 vs. 59 days for NSSOC (p=0.016), vs. 63 days for GV (p=0.004)	32.6% vs. 27.9% vs. 26.7% within 12 weeks (non-inferiority, p<0.01))	Mean size reduction of 21.5% vs. 25.3% vs. 33.2% from baseline to 2 weeks after debridement (NS)	1.3±2.7 vs. 1.4±2.7 vs 1.6±1.9 mean NPRS reduction from baseline to 2 weeks after debridement for EX-02 vs. Gel vs. NSSOC (NS)	Incidence of adverse events 52.2% vs. 62.8% vs. 46.7% (NS). There were 9 vs. 3 vs. 1 adverse events with possible relationship to study treatment. Two patients discontinued study due to adverse events; 1 in EX-02 and 1 in Gel.
	Gel vehicle (n=43)						
	NSSOC (n=30)						

NR= not reported, BWD= Biocellulose wound dressing, EUSOL= Edinburgh University solution of lime. NPRS= Numeric Pain Rating Scale.

[a]cadexomer iodine vs. paraffin gauze (P= 0.006); hydrocolloid vs. paraffin gauze (P=0.01); cadexomer iodine vs. hydrocolloid (NS).

[b]Acetic acid for 2 days, followed by povidine iodine for 2 days, followed by 3 days of saline soaking.

FIGURES / LEGEND TO FIGURES

Figure 1: Patient flow diagram

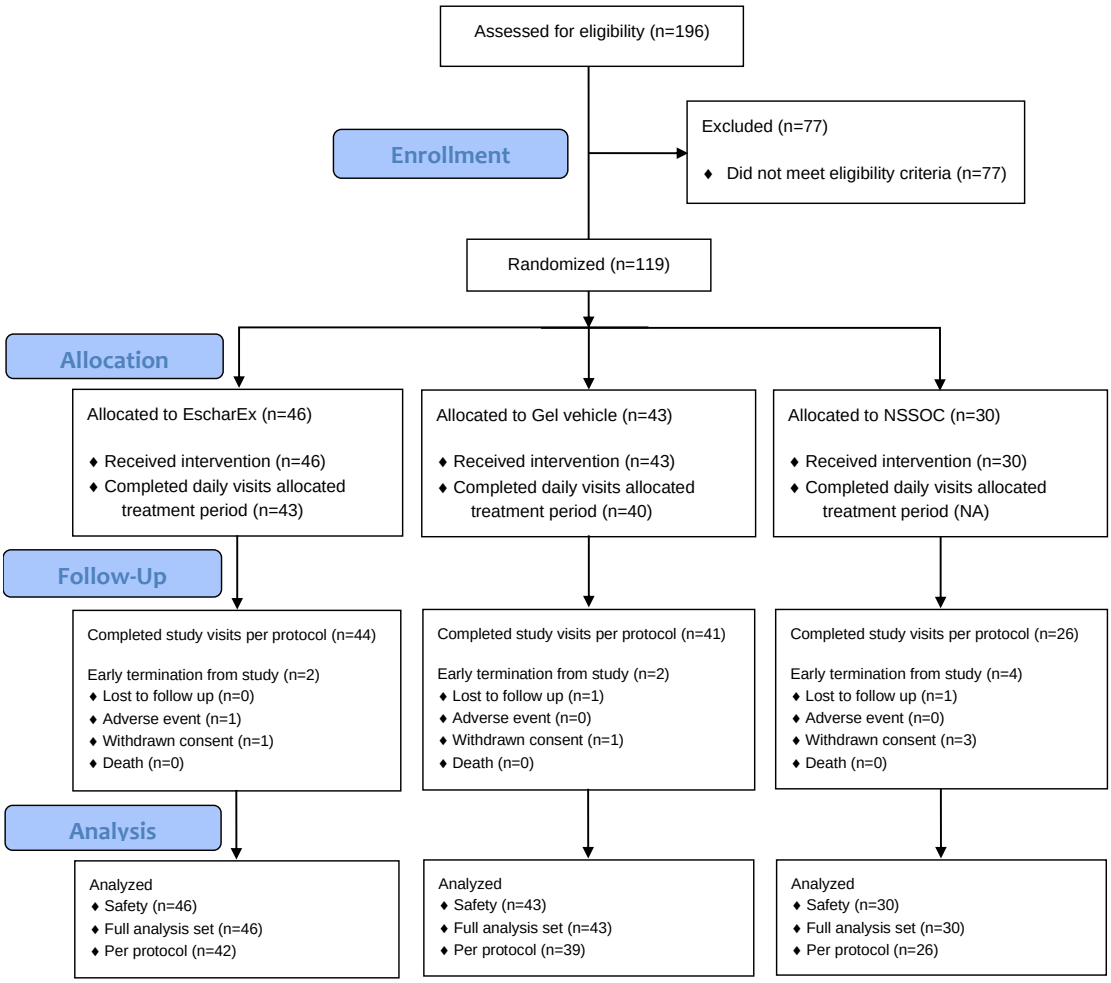
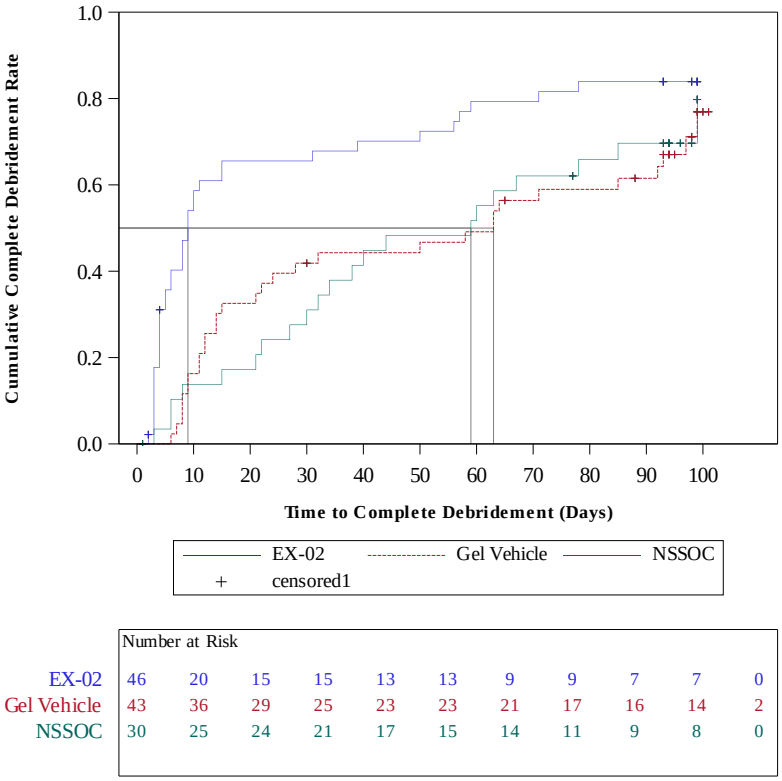


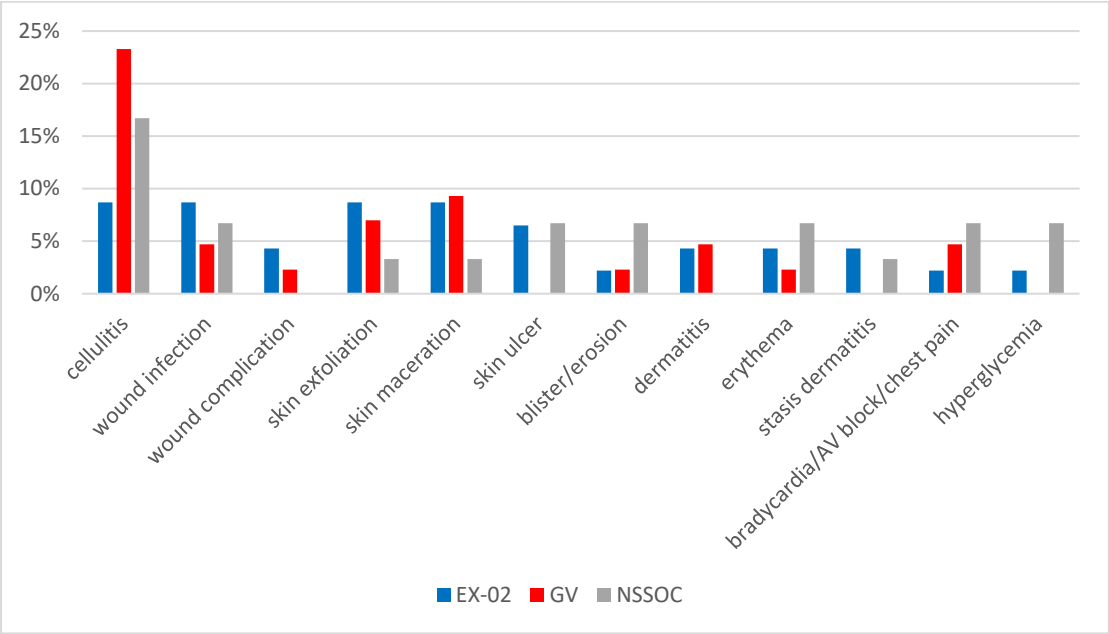
Figure 2: Kaplan-Meier estimates for time to complete debridement for EscharEx (EX-02), Gel Vehicle (GV) and Non-Surgical Standard of Care (NSSOC) arms. The medians depicted by the gray lines (and 95% CI) were 9 (5-15) days for EX-02, 59 (30-85) days for GV, and 63 (21-93) days for NSSOC.



Number at Risk											
EX-02	46	20	15	15	13	13	9	9	7	7	0
Gel Vehicle	43	36	29	25	23	23	21	17	16	14	2
NSSOC	30	25	24	21	17	15	14	11	9	8	0

Figure 3: Serial photographs of a VLU (pre-existing for 32 weeks, wound area 8cm^2) in a 59-year-old woman (BMI=35) treated with EX-02 during the study: A) Baseline; B) After 1st application of EX-02; C) After 2nd application of EX-02 (complete debridement); D) Wound closure confirmation 53 days after 1st application

Figure 4: Treatment emergent adverse events throughout the study (reported in >4% of patients in any of the study arms).



Revised manuscript (with marked changes)

Click here to view linked References

Once daily Bromelain- based enzymatic debridement of venous leg ulcers versus gel vehicle (placebo) and non-surgical standard of care: a three-arm multicenter, double blinded, randomized controlled study

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SUMMARY

Background: Debridement is considered the first step in treatment of chronic wounds, however, current enzymatic and autolytic debridement agents are slow or ineffective. Previous studies have shown positive initial results with EscharEx® (EX-02 formulation), a Bromelain- based enzymatic debridement agent in development for chronic wounds. The main objective of this study was to assess its efficacy in debriding venous leg ulcers (VLU), compared to gel-vehicle (GV) as a placebo control and to non-surgical standard of care (NSSOC).

Methods: A prospective, randomized, multicenter, placebo-controlled trial in patients with VLU from 20 medical centers and clinics in the United States, Switzerland and Israel was undertaken. Patients were treated with daily topical applications of either EX-02, GV, or NSSOC (in a 3:3:2 ratio), until reaching complete debridement or up to 8 daily treatments (within 2 weeks), and then followed-up for up to 14 weeks. The primary efficacy endpoint was the incidence of complete debridement. This study is registered with ClinicalTrials.gov (NCT03588130) and EudraCT (number 2020-004861-38).

Findings: A total of 196 patients were enrolled, and 119 randomized (between November 12th, 2019, and February 15th, 2022); 46 to the EX-02 arm, 43 to the GV arm, and 30 to the NSSOC arm. Eight patients dropped out of the study (2 in EX-02, 2 in GV, 4 in NSSOC). The incidence of complete debridement within 8 daily treatments was 63% (29/46 patients) in the EX-02 arm as compared to 30.2% (13/43 patients) in the GV arm (p=0.004) and 13.3% (4/30 patients) in the NSSOC arm (p<0.001). Sixty-five patients reported wound related adverse events throughout the study; 24 (52.2%), 27 (62.8%) and 14 (46.7%) patients in the EX-02, GV and NSSOC arms (p=NS). No deaths occurred during the study.

Interpretation: EX-02 lead to a significantly higher incidence of complete debridement as compared to GV and NSSOC, without significant safety issues. Additional studies are needed to explore the benefits of EX-02 in VLU and other chronic wound etiologies.

Funding: MediWound Ltd.

Keywords: VLU, venous leg ulcer, enzymatic debridement, EscharEx, RCT, chronic wounds

RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed from database inception to July 1st, 2023, for papers published in English including the following terms: “venous leg ulcer” OR “VLU” AND “debridement”. We identified an article reviewing 10 randomized controlled trials comparing different non-surgical debridement methods of venous leg ulcers, which failed to demonstrate an optimal debridement method or duration of treatment. A recent multi-center, assessor blinded RCT, demonstrated that a Bromelain based enzymatic debridement agent achieved a significantly higher incidence of complete debridement of venous, diabetic and post-surgical/traumatic ulcers as compared to its gel vehicle. ~~However, this was done with 4-hour applications whose applicability for home use, especially in an aging population, seems questionable.~~

Added value of this study

Once daily Bromelain-based enzymatic debridement led to a significantly higher incidence of complete debridement of venous leg ulcers within two weeks as compared to gel vehicle, and to a significantly shorter time to complete debridement as compared to both gel vehicle and various non-surgical standard of care debridement agents. Additionally, it led to a significantly higher incidence of >75% granulation tissue within two weeks as compared to gel vehicle. The efficacy of these 24-hour applications, combined with an acceptable safety profile demonstrated in this study, may provide a solution to the unmet need for a safe and efficient non-surgical debridement agent for venous leg ulcers.

Implications of all the available evidence

Non-surgical debridement of venous leg ulcers has thus far not been demonstrated to lead to earlier wound closure. A faster debridement as seen in this study may lend itself to earlier application of cellular, acellular, matrix-like products or spilt thickness skin graft to close the

wound, hopefully leading to a shorter time to complete wound closure. This, along with the efficacy, safety and ease of use demonstrated in this trial potentially allowing for home use by the patient or a caregiver, may prove to be substantial benefits for patients and health care professionals. Additional studies are needed to further demonstrate the efficacy and potential benefits of Bromelain based enzymatic debridement in venous leg ulcers and other chronic wounds.

1. INTRODUCTION

Venous leg ulcers (VLU) are the most common ulceration on the lower extremity and account for 70% of all leg ulcers.[1] The prevalence of VLU has been estimated to range up to 2% of the population based on observational studies, with an overall cost of treatment approaching 1% of the health care budget of some western European countries.[2] The constant increase in the aging population is expected to increase these numbers as age negatively affects healing and recurrence rates.[3-5]

Eschar or fibrinous debris, consisting of devitalized tissue or slough that may harden by desiccation, commonly exists on the surface of chronic wound-beds.[6] Therefore, wound bed preparation (WBP) is the first step in treatment of these wounds, which is initiated by debridement, that removes the local impediments to healing by eliminating devascularized tissue, necrotic material, and excessive bacterial burden.[7] The aims of debridement are to create a healthy wound bed, edges and peri-wound skin, with the objective of promoting and accelerating healing.[8] Previous studies have shown the importance of adequate debridement in VLU. Cardinal et al demonstrated a 47% closure rate in VLUs that were surgically debrided serially versus a 30% closure rate in those that were not.[9] Several debridement methods exist, including surgical (or sharp in case of a minor bedside procedure), enzymatic, autolytic, osmotic (i.e. honey), mechanical (i.e. wet to dry), biologic (larvae), and technical (i.e. jet lavage, ultrasound).[8] However, according to the clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum, surgical debridement is recommended over other methods for VLUs that contain slough, nonviable tissue, or eschar.[2] This is in line with the fact that there is little evidence to suggest that the use of non-surgical debridement agents is beneficial for VLU healing.[10] Nevertheless, as surgical debridement has its drawbacks such as associated pain and bleeding, and requires the availability of a clinician trained in surgical debridement, the use

of currently available slow acting non-surgical debridement agents remains common. EscharEx® (MediWound Ltd, Yavne, Israel), is a Bromelain- based enzymatic topical agent currently in development for debridement of chronic wounds. Bromelain-based enzymatic debridement has been shown to provide an efficient, rapid, selective and safe non-surgical debridement in burns (NexoBrid®, MediWound Ltd, Yavne, Israel).[11-13] The positive experience in burns lead to an initial proof-of-concept study, demonstrating efficacy in debridement of chronic wounds of various etiologies.[14] This study was followed by a multi-center, assessor-blinded RCT, that demonstrated a significantly higher incidence of complete debridement of lower extremity ulcers treated with EscharEx as compared to those treated with its gel vehicle.[15] However, these studies utilized 4 hour topical applications, whose applicability for home use, and in an aging population, seemed questionable. Therefore, a new EscharEx formulation (EX-02) was developed to allow for longer safe treatment times (24 hours).

The main objective of this study was to assess the efficacy of EX-02 compared to its gel vehicle and to current non-surgical standard of care (NSSOC) debridement agents in debridement of VLU.

2. METHODS

2.1 Study design

The ChronEx study was an international, multicenter, prospective, randomized, placebo-controlled trial conducted across 20 medical centers and clinics in the United States, Switzerland and Israel, under the ethical rules for human experimentation that are stated in the 1975 Declaration of Helsinki, including approval by the institutional review boards of all participating centers (clinicaltrials.gov identifier NCT03588130, EudraCT number 2020-004861-38). After giving written informed consent (to a study investigator) and completing a screening period of 1-week, patients suffering from VLUs were randomized (using a computer-generated randomization stratified by region, pain level and wound size) to daily treatments with either EX-02, its gel vehicle (GV) as a placebo control, or NSSOC (in a 3:3:2 ratio), until reaching complete debridement or up to 8 daily treatments (within 2 weeks). Following the debridement period, patients were followed-up for up to 14 weeks. The overall duration of the study for patients in all arms was up to 17 weeks (See supplementary material figure 1). EX-02 and GV arm randomizations were double-blinded. The NSSOC arm was not blinded for both patients and assessors due to the different appearance of topical agents in this arm. The use of standardized compression therapy was indicated throughout the study for all patients. The trial protocol can be found in the supplemental material.

2.2 Inclusion and exclusion criteria

Inclusion criteria: Adult patients between 18 and 90 years of age; Suffering from a VLU present 4 weeks to 2 years (determined by medical history, physical examination, and an ultrasound scan demonstrating venous insufficiency); nonviable tissue (necrotic/slough/fibrin) covering at least 50% of the wound area (assessed by clinical evaluation); wound surface area between 2-100 cm2 (assessed by inSight™, eKare Inc.,

Fairfax, VA, USA); patient understood the nature of the procedure, was able to adhere to the protocol regimen, and provided a written informed consent prior to any study procedure.

Exclusion criteria: Patients with more than one leg ulcer (area ≥ $2cm^2$) on the leg of the target wound; wound area decreased by >20% after one week of standard-of-care-only period (screening period); signs of clinically significant infection including purulent discharge, deep-tissue abscess, erysipelas, cellulitis, etc; severely damaged skin (e.g., abrasion, exfoliation) extending >2 cm around the wound edge; presence of gangrene, signs of systemic infection, sepsis, or osteomyelitis during screening phase; clinical suspicion of skin cancer not ruled out by biopsy; skin disorders unrelated to the wound that are present adjacent to the wound; chronic skin disorders (e.g., idiopathic pruritus, psoriasis, panniculitis, pyoderma gangrenosum) that may deteriorate as a result of local trauma or debridement; wound with sinus tracts or tunnels extending under healthy tissue or penetrating into joint capsule; vascular operations in proximity to the wound in the last month; patients with primary lymphatic edema; significant decrease in the arterial blood flow of the extremity, as demonstrated by either Toe-Brachial Index (TBI) ≤ 0.50, Ankle-Brachial Index (ABI) ≤ 0.70, Skin Perfusion Pressure (SPP) ≤ 40 mmhg, or Transcutaneous oximetry (TCOM) ≤ 40 mmhg; pre-enrollment wounds which were covered by eschar heavily saturated with iodine or silver sulfadiazine; history of allergy or atopic disease or a known sensitivity to pineapples, papaya, bromelain, or papain, as well as known sensitivity to latex proteins, bee venom or olive tree pollen; poorly controlled diabetes mellitus (Hba1c >12%); Hemoglobin<8 g/dl) or WBC<3,800/µl or >15000/µl or Platelets<100,000/µl; abnormal liver function (aspartate transaminase, alanine transaminase >2 × upper limit of normal range); BMI>48 or albumin <2.5 g/dl; patients undergoing renal/peritoneal dialysis or creatinine >2.5 mg/dl; any condition that would preclude safe participation in the study (e.g., evidence of significant or unstable cardiovascular, pulmonary, liver, hematological, immunological, positive for COVID-19 or neoplastic disease, or any immediate life-threatening condition);

recent history (<6 months) of myocardial infarction; concurrent acute injury or disease that might compromise patient welfare; patient receiving (or received at any time within 3 months prior to enrollment) any medications or treatments known to affect the wound healing processes, including chronic systemic steroid intake with topical skin changes (i.e., thin, fragile skin with multiple hematomas, or previous laceration history), immunosuppressive drugs, radiation therapy, immunomodulating medications and chemotherapy; mentally incapacitated adults who were incapable of giving informed consent; concurrent use of nonapproved drugs or alcohol abuse; pregnant women or nursing mothers; exposure to investigational intervention within 3 months prior to enrollment, or anticipated participation in any other interventional study.

2.3 Screening period

Study procedures performed during this period (6-9 days) included recording of demographics and medical history, concomitant medications, vital signs, physical examination, clinical laboratory tests, pain assessment, wound assessments and cultures, and wound photograph assessment. Additionally, arterial insufficiency was ruled out by either; measurement of ankle-brachial index (ABI), toe-brachial index (TBI), skin perfusion pressure (SPP), or transcutaneous oximetry (TCOM). During this week, the wounds were treated by non-surgical standard treatment according to the investigators' choice, and standardized compression therapy with the Coban™ 2 Two-Layer Compression System (3M, St Paul, MN, USA). Patients whose wound surface area decreased by more than 20% as assessed by the eKare inSight™ digital wound management system (eKare Inc., Fairfax, VA, USA) during this period were excluded.

2.4 Debridement period

This was a period of up to 2 weeks, where patients arrived for up to 8 daily visits, and were treated according to their randomization.

2.4.1 EscharEx and Gel Vehicle Arms

EX-02 is a biological product that consists of a concentrate of proteolytic enzymes enriched in Bromelain, extracted from the stem of pineapples (MediWound Ltd, Yavne, Israel). The mechanism of action is mediated by the proteolytic activity of the enzymes. The powder of EX-02 or GV (8.1 grams in a 50 milliliter type II glass vial) was reconstituted with 20 grams sterile water for injection to obtain 5% EX-02 or the gel vehicle. In order to maintain blinding, the appearance of the vials and powders of EX-02 and GV was identical, and so was their appearance and consistency after reconstituting with water for injection. Patients, study investigators, and the study sponsor were all blinded. The content of the mixed product was sufficient for a wound area of up to 80 cm^2. For wounds larger than 80 cm^2, 2 vials of IMP (investigational medicinal product) were used. EX-02 or GV were topically applied on the wound surface for 24±3 hours, up to 8 consecutive applications (4 treatments a week), with 3 days of IMP holiday during the weekends, or until a complete debridement was achieved, whichever occurred first. A single additional IMP holiday of up to 24±3 hours was allowed in case of medical needs (e.g., in case of tolerability issues). During IMP holiday, a non-active dressing such as a hydrocolloid, hydrogel, or foam, was applied. At all times, the peri-wound area was protected by Calmoseptine® ointment (Calmoseptine Inc, Huntington Beach, CA, USA) and analgesics were provided as needed. Between dressings the wounds were cleaned with either sterile saline solution or water and mild soap. A flow chart describing the method for EX-02 / GV application during the study can be seen in Supplementary material figure 2.

2.4.2 NSSOC Arm

The NSSOC arm consisted of a standardized selection of dressings (including enzymatic agents, alginates, foams, highly absorbents, honey, hydrocolloids, hydrofibers, hydrogels, and silver products) that were applied on the wound surface according to their approved label, instructions for use, and investigator discretion in accordance with ulcer status, until complete debridement was achieved.

2.4.3 Additional study treatments

The Coban™ 2 Two-Layer Compression System (3M, St Paul, MN, USA) was used continuously throughout the study in all patients, reapplied after each dressing change, and limb elevation was instructed 3 times a day. All active methods of debridement including surgical, mechanical (wet-to-dry), biological (maggots), and the use of negative pressure wound therapy were prohibited during the debridement (daily visits) and follow-up periods. Hyperbaric oxygen therapy was not allowed throughout the entire study period.

2.5 Follow-up period

This period consisted of 4 twice-weekly visits for 2 weeks, followed by once-weekly visits until achieving complete wound closure or reaching a total of 10 weeks. During this period a similar variety of NSSOC treatments could be used in all arms. Complete wound closure was defined as complete re-epithelialization of the wound surface without drainage or dressing, confirmed at two visits 2 weeks apart, in accordance with FDA definitions.[16] Therefore, a wound closure confirmation visit was performed 2 weeks after wound closure if applicable. Thus, the maximal duration of the follow-up period was up to 14 weeks.

2.6 Endpoints

The primary efficacy endpoint of the study was the incidence of complete debridement in the EX-02 and GV arms, scored dichotomously (yes/no), assessed clinically after each application during the daily visits period (up to 8 applications within 14 days).

Secondary endpoints included the time to complete debridement in the EX-02 and NSSOC arms (from the initiation of study treatments, throughout the entire study); change in pain from baseline to the end of the twice weekly period in the EX-02 and GV arms, as assessed by Numeric Pain Rating Scale (NPRS);[17] change in wound area from baseline to the end of the twice weekly period in the EX-02 and GV arms, as assessed by eKare inSight™; incidence of >75% granulation tissue at the end of the daily visits period in the EX-02 and GV arms

(clinical assessment); change in quality of life (QoL) from baseline to the end of the twice weekly period in the EX-02 and GV arms, as assessed by the Wound-QoL questionnaire (ranging from 0 to 4, higher values describe larger restriction).[18] *Safety outcome measures* included the incidence and to time to complete wound closure in the EX-02 and GV arms, and the incidence and severity of adverse events and tolerability issues, including the proportion of patients discontinuing treatment due to adverse events, assessed over the entire course of the study.

2.7 Sample size calculation

Sample size calculation was based on demonstrating superiority of EX-02 over GV on the primary endpoint. Based on the assumption of a complete debridement rate of 60% for EX-02 and 30% for GV, a sample size of 45 patients in the EX-02 arm and 45 patients in the GV arm were to provide a minimum of 80% power using a two-sided test, based on the asymptotic normal approximation for a difference in proportions with α=0.05. With 30 additional patients in the NSSOC arm (based on the 3:3:2 randomization scheme), the total minimal planned sample size was 120 patients. The final sample size for this study was determined at an interim sample size re-assessment planned after 80 subjects (67% of the subjects) had completed the daily visits period (thus, producing the results of primary endpoint). The final sample size was determined based on Mehta and Pocock's promising zone approach. The design criterion for this approach was predicated on achieving a final power of at least 80% for the primary efficacy endpoint.

2.8 Statistical analysis

Data was collected by investigators at the participating medical centers and wound care clinics in the United States, Switzerland and Israel, and were transferred to a central database. Numerical variables were tabulated using number of observations, mean, standard deviation,

minimum, median, and maximum. Categorical variables were tabulated using number of observations and percentages. Statistical tests were conducted against a two-sided alternative hypothesis, employing a significance level of 0.05. Fisher's exact test was used to compare rates and incidences between the study groups. The incidence of complete debridement, which was the primary endpoint of the study, was conducted on the full analysis set and compared the incidence of complete debridement between EX-02 and GV. The hypothesis was evaluated as follows: $H0$: $\pi EX\text{–}02 = \pi Gel\ Vehicle$; $H1$: $\pi EX\text{–}02 \neq \pi Gel\ Vehicle$ (where π was the proportion of subjects who achieved complete debridement during the daily visits period). This hypothesis was tested using the normal approximation with 2-sided α=0.05. The normal approximation (rather than Fisher's exact test) was used to match the calculation of conditional power done for sample size re-assessment. Treatment effect is represented by absolute risk reduction with its 95% confidence intervals. Kaplan–Meier's survival function curve was used to compare the time to complete debridement and time to wound closure. To avoid inflation of type 1 error rate, the secondary endpoints were analyzed in hierarchical order following analyzing the primary endpoint as defined using a step-down procedure. Any statistical tests performed on efficacy endpoints after a p-value exceeding 0.05 was encountered were considered exploratory for informational purposes and planning of future research. For both endpoints of changes in pain score and wound area, descriptive statistics with raw change from baseline are presented. Handling of missing data was as follows: For incidence of complete debridement; EX-02 and Gel Vehicle patients for whom the debridement status was partially or fully missing, and complete debridement was never achieved within the period of up to 8 applications, imputed "failure" for the primary and for the covariate analyses; For all 'time-to' analyses; Patients who did not achieve complete debridement were censored at the last available time where no debridement was confirmed, thus, all data was considered in the analysis and no imputation was needed; For continuous outcomes of NPRS and wound size; Missing values were not imputed, i.e. only observed

measurements were used. All statistical analyses were carried out using SAS Version 9.4 under Windows 2016 Terminal.

2.9 Role of the funding source

This study was planned and funded by MediWound Ltd., with oversight by an independent Data Safety Monitoring Board (DSMB). The study was conducted by a contact research organization (CRO) in the United States (IQVIA Inc., Durham, NC, USA), and by MediWound Ltd (Yavne, Israel) in Switzerland and Israel. MediWound employees (YKL, KDZ, EK, and RJS) made substantial contributions to conception or design of the study, the acquisition, analysis, and interpretation of the data, drafted and revised the manuscript and provided final approval of the manuscript version to be published.

3. RESULTS

A total of 196 patients were enrolled in 20 medical centers and wound care clinics in the United States, Switzerland and Israel. The first patient was enrolled on November 12th, 2019, and the last patient completed follow up on February 15th, 2022 (the study was terminated as planned). Of these, 119 were found eligible and randomized, 46 to the EX-02 arm, 43 to the GV arm, and 30 to the NSSOC arm. Eight patients (6.7%) terminated early from the study, while 111 patients (93.3%) completed the study visits per protocol. No deaths were reported during the study. A summary of the trial profile / flow diagram can be seen in Figure 1.

3.1 Patients and wounds baseline characteristics

Baseline characteristics of patients and wounds were similar in the 3 treatment arms (p=NS, Table 1). The average age of patients in the study was 64.0±12.5 years, with a 64/55 male to female ratio. Most patients were white (79%) with an average BMI of 32.8±7.5 kg/m^2. Despite no significant differences between the arms, patients in the GV arm had somewhat older and larger wounds with a larger percentage of nonviable tissue.

3.2 Incidence of complete debridement EX-02 vs. GV (primary endpoint)

The incidence of complete debridement during the daily visits debridement period (up to 8 applications within up to 14 days) was significantly higher in the EX-02 arm, 63% (29/46 patients) as compared to 30.2% (13/43 patients) in the GV arm (p=0.004), thus achieving the primary endpoint of the study. The absolute risk reduction between the EX-02 and GV arms was 32.8% (95% CI=9.78-51.67).

3.3 Time to complete debridement EX-02 vs. NSSOC and GV

The Kaplan-Meier median estimates for time to complete debridement were significantly shorter in the EX-02 arm, 9 (5-15, 95% CI) days as compared to 59 (30-85, 95% CI) days in the NSSOC arm (p=0.016) and 63 (21-93, 95% CI) days in the GV arm (p=0.004, see Figure 2).

3.4 Change in pain from baseline to end of twice weekly period EX-02 vs. GV

The average NPRS reduction at the end of the twice weekly period (2 weeks after end of debridement) was 1.3±2.7 and 1.4±2.7 for the EX-02 and GV arms, respectively (p=NS).

3.5 Change in wound area from baseline to end of twice weekly period EX-02 vs. GV

The average reduction at the end of the twice weekly period (percent change from baseline to 2 weeks after end of debridement) as assessed by eKare inSight™ was 2.9 ± 11.4 cm^2 (21.5%) and 2.7 ± 12.5 cm^2 (25.3%) for the EX-02 and GV arms, respectively (p=NS).

3.6 Incidence of >75% granulation tissue at end of debridement period EX-02 vs. GV

The incidence of >75% granulation tissue assessed clinically at the end of the daily visits period was 93% and 56% for the EX-02 and GV arms, respectively (p <0.0001).

3.7 Change in Wound -QoL from baseline to end of twice weekly period EX-02 vs. GV

The average total Wound-QoL scores improved from 1.9±1.0 to 1.3±1.0 for the EX-02 arm and from 2.1±1.0 to 1.5±1.0 for the GV arm. The mean change was 0.5±0.8 and 0.5±0.6 for the EX-02 and GV arms, respectfully (p=NS).

A summary of the primary and secondary endpoints results can be seen in table 2.

3.8 Exploratory and post-hoc analyses

The incidence of complete debridement at the end of the daily visits debridement period was 63% (29/46 patients) and 13.3% (4/30 patients) in the EX-02 and NSSOC arms, respectively (p<0.001). The incidence of 100% granulation tissue at the end of the daily visits debridement period (post hoc analysis) was 50% (23/46 patients) in the EX-02 arm, which was significantly higher than the 25.6% (n=11/43 patients) in the GV arm (p=0.01) and the 10% (3/30 patients) in the NSSOC arm (p=0.0002).

3.9 Safety

Sixty-five (54.6%) patients suffered treatment emergent adverse events (TEAE) throughout the study, distributed similarly among treatment arms; 24 (52.2%), 27 (62.8%) and 14 (46.7%) patients in the EX-02, GV and NSSOC arms, respectfully. Of these, a total of 5 (5.2%)

patients experienced serious TEAEs throughout the study: 1 (2.2%) in the EX-02 arm, 3 (7.0%) in the GV arm, and 1 (3.3%) in the NSSOC arm. Thirteen (10.9%) patients experienced TEAEs that were assessed by investigators as having reasonable possibility relationships to study treatment; 9 (19.6%) in the EX-02 arm, 3 (7.0%) in the GV arm, and 1 (3.3%) in the NSSOC arm. Most TEAEs were mild, with moderate severity TEAEs reported in 12 (10.1%) patients. Severe TEAEs were reported in 1 (2.2%) patient in the EX-02 arm (increased blood pressure), 2 (4.7%) patients in the GV arm (skin graft infection and cellulitis), and none in the NSSOC arm. Two patients (1.7%) discontinued the study treatment due to TEAEs; 1 (2.2%) due to pain in the EX-02 arm (this was the only case of application site pain reported as a TEAE in the study) and 1 (2.3%) due to cellulitis in the GV arm. Throughout the study, the use of analgesics was reported by 16 (34.8%) patients in the EX-02 arm, 17 (39.5%) in the GV arm, and 8 (26.7%) in the NSSOC arm (NS). During the daily visits period analgesics were reported by 16 (34.8%) patients in the EX-02 arm, 17 (39.5%) in the GV arm, and 4 (13.3%) in the NSSOC arm. Opioids use was also similar in all arms (13.0% in EX-02; 18.6% in GV, 13.3% in NSSOC). No deaths were reported during the study. There were no significant differences in the incidence of (EX-02=32.6%, GV=27.9%, NSSOC=26.7%) or time to complete wound closure (25^{th} percentile: EX-02=64 days, GV=63 days, NSSOC=78 days). A representative case of a VLU treated with EX-02 during the study can be seen in figure 3. Figure 4 shows all adverse events throughout the study that had a frequency higher than 4% in any of the treatment arms. In general, there were no significant differences in adverse event rates between the arms.

4. DISCUSSION

In this RCT enzymatic debridement of VLUs with EX-02 lead to a significantly higher incidence of complete debridement as compared to GV, thus achieving the primary endpoint of the study. Additionally, EX-02 lead to a significantly shorter time to complete debridement as compared to both GV and NSSOC. These findings, together with the significantly higher rate of achieving complete granulation at the end of the daily visits period, demonstrate the wound bed preparation efficacy of EX-02 as compared to both NSSOC and GV. While GV played the role of a double blinded placebo in this trial (in accordance with FDA guidance), as a hydrogel it should also be considered an autolytic debridement agent. Therefore, the significantly better results of EX-02 as compared to GV should be interpreted accordingly. The efficacy of EX-02 demonstrated in this study is in line with the results of a previous RCT with an older generation Bromelain based enzymatic debridement product (EX-01, MediWound Ltd, Yavne, Israel), where 73 patients suffering from lower extremity ulcers (venous, diabetic and post-surgical/traumatic etiology) were randomized to debridement with either EX-01 or its gel vehicle. Patients were treated with up to 10 daily 4-hour topical applications, and then continued follow-up for up to 6 months. The EX-01 arm achieved a significantly higher incidence of complete debridement over gel vehicle (55% vs. 29%, p=0.047).[15] Additionally, as in our current study with EX-02, there were no significant differences in wound closure rates and no significant safety issues were encountered throughout the study. A clear benefit of EX-02 over EX-01 is the longer application time (24 hours versus 4 hours).

Wound bed preparation is considered the first step in the treatment of chronic wounds. As surgical debridement has some drawbacks and is not always available or feasible, the use of non-surgical debridement agents remains common despite their limited efficacy. Thus, there is an unmet need for an efficient non-surgical debridement agent. Gethin et al reviewed 10

RCTs (including a total of 715 patients) comparing different debridement methods of VLUs.[10] They presented study results in a narrative form, as meta-analysis was not possible, and noted that most trials were at high risk of bias. They concluded that their review failed to identify an optimal debridement method or duration of treatment. We summarized the main results of their review in a panel, together with the results of our study (Table 3). As one of these RCTs reporting the results of honey vs. hydrogel has since been retracted due to errors in the data analysis we did not include it in the panel and discussion.[19] Obviously, when comparing our study results to those of these studies, differences in patient cohorts must be considered. Nevertheless, we believe the comparison to other VLU debridement studies is important. Six RCTs evaluated different methods of autolytic debridement including biocellulose wound dressing (BWD), non-adherent dressing, hydrogel, hydrofiber dressing, hydrocolloid dressings, dextranomer beads, Edinburgh University Solution of Lime (EUSOL) and paraffin gauze. Two RCTs evaluated enzymatic preparations including collagenase and one evaluated bio-surgical debridement with maggots. No RCTs evaluated surgical, sharp or mechanical methods of debridement, or debridement versus no debridement. Only 2 of the studies stated a 50% minimum amount of slough required in the wound bed for inclusion, the other studies indicated that people with necrotic or sloughy venous ulcers were included but did not state the baseline percentage of slough. Study durations varied between 1-12 weeks. The use of compression therapy was relatively consistent, with 8 of the 10 studies using compression during the treatment period. The Gethin et al review focuses on 6 main outcomes, including incidence of complete debridement, time to achieve debridement, wound healing rate, reduction in wound size, pain reduction and adverse events.[10]

Four studies reported the number of wounds completely debrided (Groenewald 1980,[20] Jasiel 1996,[21] Alvarez 2012,[22] Wayman 2000[23]), and five studies reported the time to achieve debridement (Groenewald 1996,[20] Jasiel 1996,[21] Westerhof 1990,[24] Konig 2005,[25] Wayman

2000[23]). The only agent reported to have achieved a higher incidence of complete debridement within a shorter time of the current study results of EX-02 is Dextranomer beads.[20] However, this study did not define a minimum amount of slough as an inclusion criteria so the amount of non-viable tissue debrided is unclear. Additionally, this study was conducted over 40 years ago and this product is no longer available. Larvae were also demonstrated as rapid and efficient, however, due to a small sample size, superiority over hydrogel was only demonstrated in the time to achieve debridement. Additionally, larvae therapy has significant drawbacks, such as significantly higher pain levels, cost and treatment complexity as compared to hydrogel.[26] In contrast to these studies, our study demonstrated superiority over GV in addition to other debridement agents. While GV was included as a placebo, it is in fact a hydrogel (missing the enzymes that when added to it constitute EX-02), that completely debrided 30.2% of the wounds within 14 days. Therefore, it is reasonable to attribute the statistically significant difference between the debridement rate of EX-02 and GV to the added efficacy of the enzymes, over the performance of a standard of care autolytic debridement agent.

Only one study reported the number of wounds healed (Alvarez 2012).[22] The wound closure rate reported is slightly higher than the results of our study, however 13 patients withdrew from that study and their wound closure status is unknown. In any case, both studies did not demonstrate a significantly higher wound closure rate vs. a comparator, despite a significantly higher rate of complete debridement. Five studies reported reduction in wound size (Skog 1983,[27] Hansson 1998,[28] Wild 2010,[29] Alvarez 2012,[22] Westerhof 1990[24]). Only two studies demonstrated superior results of cadexomer iodine and hydrocolloid vs. paraffin/saline soaked dressings within 4-6 weeks, however wound healing rates were not reported for these studies.[27,28] Our study did not demonstrate significant differences in wound size reduction vs. a comparator, similar to the findings of the other 3 studies. This may be attributed to the fact that our study was not powered to demonstrate decrease in

wound size or healing. An additional factor may be the variety of dressings used after the end of the debridement period. Perhaps a larger sample size together with a more standardized regimen of allowed treatments towards wound closure could help link between debridement and wound healing rates in a significant manner.

Six studies reported pain as an outcome (Groenewald 1980,[20] Skog 1983,[27] Hansson 1998,[28] Wild 2010,[29] Alvarez 2012,[22] Westerhof 1990).[24] There was an overall reduction in pain when wounds were debrided.[10] Our study also demonstrates reduction in pain, however the differences between the groups were not significant. It is worth noting that there is usually significant pain associated with debriding VLUs, however EX-02 was able to debride a significant majority of these wounds without significant pain.

Only 3 studies reported adverse events (Jasiel 1996,[21] Alvarez 2012,[22] Hansson 1998).[28] Overall, the reporting of adverse events was poor, most probably under-reported.[10] Our study reported a higher incidence of adverse events, however, there were no significant differences in their incidence between the study groups and the safety profile of EX-02 seems acceptable.

One of the most common enzymatic debridement agents in chronic wound care is collagenase. However, a systematic review and meta-analysis of RCTs on the use of collagenase in wounds and ulcers by Patry et al[30] did not find sufficient evidence for its use in VLU (only 1 RCT performed in VLU, Konig 2005),[25] and concluded that there is very limited data on the effect of collagenase as an enzymatic debridement technique on venous wounds. Additionally, this meta-analysis demonstrated that patients treated with collagenase have an increased risk of adverse events compared to alternative treatments.

An important additional aspect in treatment of chronic wounds is the focus on microbial barriers that impair or delay wound healing. Recently published results of a small pilot study (12 patients) demonstrated that Bromelain-based enzymatic debridement of VLU with

EscharEx® lead to reductions in both biofilm and bacterial autofluorescence values, suggesting a favorable effect on reduction of bioburden, in addition to its debridement efficacy.[31]The strengths of this study include a comparison to both a blinded assessment compared to placebo and to a standardized set of unblinded NSSOC methods of debridement, with an adequate sample size allowing for achieving statistically significant superiority for the primary endpoint. Additionally, this study addresses all 6 main outcomes mentioned in the review above, while using pre-defined eligibility criteria including a minimum amount of necrotic tissue.

Our study has several limitations. While there were no significant differences in baseline characteristics between the study arms, The GV arm had larger wounds as compared to the EX-02 arm (NS). This difference in size may be explained by the fact that study randomization was stratified by wound size categories. The stratification levels were defined as ≤40cm2 and >40cm2. Each arm had a similar percentage of subjects within each category. Perhaps using more than 2 size categories in randomization schemes may improve stratification in future studies. Another limitation is that while EX-02 was applied once daily, a treatment regimen similar to most of the VLU RCTs mentioned above, it is reasonable to assume that a more infrequent dressing change (such as once in 48 hours) would lead to higher patient and caregiver compliance, and to experiencing less pain. Although other (prospective) studies have also not been able to link debridement to wound closure rates in VLU, we believe the fact that EX-02 did not lead to a higher incidence of or a shorter time to wound closure during the study period despite its superior complete debridement efficacy, is also a study limitation. Better standardization strategies and larger sample sizes may assist in overcoming this limitation in future studies. Better standardization strategies in the future may also allow for better interpretation of the results of the NSSOC arm, that included a range of products commonly employed in the clinical practice of the participating investigators. This is also a study limitation, although this reflects the pragmatic, real-world

NSSOC. Another limitation is that only 3 nations participated in this multicenter study, representing North America, Europe and the Middle East, while other regions were not represented.

In conclusion, the superior debridement efficacy of EX-02 vs. both a double-blinded placebo and NSSOC, combined with the acceptable safety profile demonstrated in this RCT, may provide a solution to the unmet need for a safe and efficient non-surgical debridement agent for VLU. This would lend itself to earlier application of cellular, acellular, matrix-like products or spilt thickness skin graft to close the VLU, hopefully leading to a shorter time to complete wound closure. This, along with the efficacy, safety and ease of use demonstrated in this trial potentially allowing for home use by the patient or a caregiver, may prove to be substantial benefits for patients and health care professionals. Additional studies are needed to further demonstrate the efficacy and potential benefits in VLU, including improved size category stratification and a more uniform dressing regimen towards wound closure. Clinical trials in other chronic wound etiologies should also be considered.

CONTRIBUTORS

Study design and protocol development: YS, JCL, KDZ, YKL, EK, MK and LR.

Data collection: YKL, CRD, RA, AR, FS, GT, ES, MH, FPC, JCL, SMC, YSD, CAC, DE, DV, AJS, RCG, JRH, CM, AS, and LTT.

Data curation: YKL and KDZ.

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All authors had full access to the study data and critically reviewed the manuscript. YS, KDZ and YKL directly accessed and verified the underlying data. All authors take responsibility for the accuracy of the analysis and had authority over manuscript preparation and the decision to submit the manuscript for publication. All authors approved the manuscript for submission.

DATA SHARING

Deidentified individual participant data will not be made available at this stage. Data may be made available upon reasonable request after product approval in the US or after product development is discontinued.

DECLARATION OF INTERESTS

YS is a consultant for MediWound Ltd and Vericel Corp. LR, JCL and CD are consultants for MediWound Ltd. RJS, EK, KDZ, MK and YKL are MedWound's Chief Medical Officer, Chief R&D Officer, Director of Clinical Affairs, Medical Director and Director of Clinical Development. AR served as an advisory board member for MediWound Ltd. AR and LTT report receiving study funding. The other authors report no competing interests beyond funding to their institutions for the current project.

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REFERENCES

1. Tatsioni A, Balk E, O'Donnell T, Lau J. Usual care in the management of chronic wounds: a review of the recent literature. *J Am Coll Surg* 2007; **205**(4): 617-24e57.
2. O'Donnell TF, Jr., Passman MA, Marston WA, et al. Management of venous leg ulcers: clinical practice guidelines of the Society for Vascular Surgery ® and the American Venous Forum. *J Vasc Surg* 2014; **60**(2 Suppl): 3s-59s.
3. Wicke C, Bachinger A, Coerper S, Beckert S, Witte MB, Königsrainer A. Aging influences wound healing in patients with chronic lower extremity wounds treated in a specialized Wound Care Center. *Wound Repair Regen* 2009; **17**(1): 25-33.
4. Raffetto JD, Ligi D, Maniscalco R, Khalil RA, Mannello F. Why Venous Leg Ulcers Have Difficulty Healing: Overview on Pathophysiology, Clinical Consequences, and Treatment. *J Clin Med* 2020; **10**(1).
5. Probst S, Saini C, Gschwind G, et al. Prevalence and incidence of venous leg ulcers-A systematic review and meta-analysis. *Int Wound J* 2023; Nov;20(9):3906-3921.
6. Bradley M, Cullum N, Sheldon T. The debridement of chronic wounds: a systematic review. *Health Technol Assess* 1999; **3**(17 Pt 1): iii-iv, 1-78.
7. Werdin F, Tenenhaus M, Rennekampff HO. Chronic wound care. *Lancet* 2008; **372**(9653): 1860-2.
8. Strohal R, Dissemond J, Jordan O'Brien J, et al. EWMA document: Debridement. An updated overview and clarification of the principle role of debridement. *J Wound Care* 2013; **22**(1): 5.
9. Cardinal M, Eisenbud DE, Armstrong DG, et al. Serial surgical debridement: a retrospective study on clinical outcomes in chronic lower extremity wounds. *Wound Repair Regen* 2009; **17**(3): 306-11.
10. Gethin G, Cowman S, Kolbach DN. Debridement for venous leg ulcers. *Cochrane Database Syst Rev* 2015; **2015**(9): Cd008599.
11. Rosenberg L, Krieger Y, Bogdanov-Berezovski A, Silberstein E, Shoham Y, Singer AJ. A novel rapid and selective enzymatic debridement agent for burn wound management: a multi-center RCT. *Burns* 2014; **40**(3): 466-74.
12. Rosenberg L, Shoham Y, Krieger Y, et al. Minimally invasive burn care: a review of seven clinical studies of rapid and selective debridement using a bromelain-based debriding enzyme (Nexobrid®). *Ann Burns Fire Disasters* 2015; **28**(4): 264-74.
13. Shoham Y, Gasteratos K, Singer AJ, Krieger Y, Silberstein E, Goverman J. Bromelain-based enzymatic burn debridement: A systematic review of clinical studies on patient safety, efficacy and long-term outcomes. *Int Wound J* 2023.
14. Shoham Y, Krieger Y, Tamir E, et al. Bromelain-based enzymatic debridement of chronic wounds: a preliminary report. *International Wound Journal* 2018; **15**(5): 769-75.
15. Shoham Y, Shapira E, Haik J, et al. Bromelain-based enzymatic debridement of chronic wounds: Results of a multicentre randomized controlled trial. *Wound Repair Regen* 2021; **29**(6): 899-907.
16. Gould L, Li WW. Defining complete wound closure: Closing the gap in clinical trials and practice. *Wound Repair Regen* 2019; **27**(3): 201-24.
17. Farrar JT, Young JP, Jr., LaMoreaux L, Werth JL, Poole MR. Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. *Pain* 2001; **94**(2): 149-58.
18. Augustin M, Conde Montero E, Zander N, et al. Validity and feasibility of the wound-QoL questionnaire on health-related quality of life in chronic wounds. *Wound Repair Regen* 2017; **25**(5): 852-7.

19. Gethin G, Cowman S. Manuka honey vs. hydrogel--a prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing outcomes in venous ulcers. *J Clin Nurs* 2009; **18**(3): 466-74.
20. Groenewald JH. An evaluation of dextranomer as a cleansing agent in the treatment of the post-phlebitic stasis ulcer. *S Afr Med J* 1980; **57**(20): 809-15.
21. Jasiel E, Nowak A, Gwiezdzinski Z, et al. A randomised, controlled, parallel group clinical trial of a new debriding agent (Sterigel) versus paraffin gauze in the treatment of necrotic venous leg ulcers. Proceedings of 6th European Conference on Advances in Wound Management. 1996:81-3.
22. Alvarez OM, Phillips TJ, Menzoian JO, Patel M, Andriessen A. An RCT to compare a bio-cellulose wound dressing with a non-adherent dressing in VLUs. *J Wound Care* 2012; **21**(9): 448-53.
23. Wayman J, Nirojogi V, Walker A, Sowinski A, Walker MA. The cost effectiveness of larval therapy in venous ulcers. *J Tissue Viability* 2000; **10**(3): 91-4.
24. Westerhof W, van Ginkel CJ, Cohen EB, Mekkes JR. Prospective randomized study comparing the debriding effect of krill enzymes and a non-enzymatic treatment in venous leg ulcers. *Dermatologica* 1990; **181**(4): 293-7.
25. König M, Vanscheidt W, Augustin M, Kapp H. Enzymatic versus autolytic debridement of chronic leg ulcers: a prospective randomised trial. *J Wound Care* 2005; **14**(7): 320-3.
26. Dumville JC, Worthy G, Bland JM, et al. Larval therapy for leg ulcers (VenUS II): randomised controlled trial. *BMJ* 2009; **338**: b773.
27. Skog E, Arnesjö B, Troëng T, et al. A randomized trial comparing cadexomer iodine and standard treatment in the out-patient management of chronic venous ulcers. *Br J Dermatol* 1983; **109**(1): 77-83.
28. Hansson C. The effects of cadexomer iodine paste in the treatment of venous leg ulcers compared with hydrocolloid dressing and paraffin gauze dressing. Cadexomer Iodine Study Group. *Int J Dermatol* 1998; **37**(5): 390-6.
29. Wild T ET, Andriessen A. Wound cleansing efficacy of two cellulose-based dressings. Wounds UK 2010;6(3):14-21.
30. Patry J, Blanchette V. Enzymatic debridement with collagenase in wounds and ulcers: a systematic review and meta-analysis. *Int Wound J* 2017; **14**(6): 1055-65.
31. Snyder RJ, Singer AJ, Dove CR, et al. An open-label, proof-of-concept study assessing the effects of bromelain-based enzymatic debridement on biofilm and microbial loads in patients with venous leg ulcers and diabetic foot ulcers. *Wounds* 2023; **35**(12): E414-E419.

TABLES

Table 1: Patient and wound baseline characteristics (p=NS; BMI=Body Mass Index; EX-02=EshcarEx; NSSOC=Non-Surgical Standard of Care; % Non-viable tissue* was assessed clinically)

Characteristic	EX-02 (n=46)	Gel Vehicle (n=43)	NSSOC (n=30)
Female, *n (%)*	20 (43.5)	16 (37.2)	19 (63.3)
Male, *n (%)*	26 (56.5)	27 (62.8)	11 (36.7)
Age (years), *mean (±SD)*	65.5 (±12.2)	61.4 (±12.6)	65.5 (±12.7)
Ethnic origin, *n (%)*			
White	38 (82.6)	36 (83.7)	20 (66.7)
Black/African American	7 (15.2)	6 (14.0)	9 (30.0)
Asian	1 (2.2)	0 (0.0)	1 (3.3)
Pacific Islands	0 (0.0)	1 (2.3)	0 (0.0)
BMI (kg/m^2), *mean (±SD)*	31.8 (±7.4)	34.0 (±7.9)	32.6 (±7.1)
Wound age (weeks), *mean (±SD)*	26.8 (±20.5)	39.5 (±27.6)	26.1 (±20.5)
Wound size (cm^2), *mean (±SD)*	13.1 (±20.5)	18.8 (±18.1)	14.4 (±19.6)
Wound size ≤ 40cm², n (%)	42 (91.3)	39 (90.7)	28 (93.3)
Wound size > 40cm², n (%)	4 (8.7)	4 (9.3)	2 (6.7)
% Non-viable tissue*, *mean (±SD)*	72% (±13%)	78% (±15%)	68% (±17%)

Table 2: Summary of primary and secondary endpoint results

Endpoints	Outcomes			Treatment effect (95% CI)	p-value
	EX-02 N=46	**Gel Vehicle N=43**	**NSSOC N=30**		
Primary					
Incidence of complete debridement during the daily visit period	63.0% (n=29)	30.2% (n=13)	13.3% [1] (n=4)	Risk difference = 32.8% (9.78,51.67)	P = 0.004 [2]
Secondary					
Time to complete debridement	9 (5,15) [3] days	63 (21,93) [1,3] days	59 (30,85) [3] days	Hazard ratio = 1.9 (1.1, 3.26)	P = 0.016 [4]
Change in pain from baseline to the end of the twice-weekly period, as assessed by Numeric Pain Rating Scale	1.3 (2.7) [5]	1.4 (2.7) [5]	1.6 (1.9) [1,5]	Adjusted mean difference [6] = 0.45 (-0.56,1.5)	P = 0.4 [6]
Change in wound area from baseline to the end of the twice-weekly period, assessed by eKare inSight™	2.9 (11.4) [5] cm^2	2.7 (12.5) [5] cm^2	4.7 (12.4) [1,5] cm^2	Adjusted mean difference [6] = 0.96 (-4.33,6.25)	P = 0.72 [6]
Incidence of >75% granulation tissue at the end of the daily visits period, assessed clinically	93.3% (n=42)	55.8% (n=24)	37.9% [1] (n=11)	Risk difference = 37.5% (18.12,54.15)	P < 0.0001[7]
Change in Wound-QoL score from baseline to end of follow-up period	0.5 (0.8) [5]	0.5 (0.6) [5]	0.6 (0.6) [1,5]	Adjusted mean difference [8] = 0.04 (-0.26,0.34)	P = 0.789 [8]

Primary and secondary endpoints were predefined between the EX-02 and GV arms except for the first secondary endpoint which was predefined between the EX-02 and NSSOC arms. Treatment effect and p-values shown in the table relate only to the two predefined endpoint arms' results. [1] Data shown in light grey font are for the arm that was not predefined for this endpoint, therefore treatment effect and p-value shown do not relate to this arm's data.[2] P-value was calculated using Chi Square test adjusted for continuity.[3] Median and 95% CI estimated from Kaplan Meier. [4] P-value was calculated using Log-Rank test. [5] Mean reduction (SD). [6] Adjusted LS Means difference; 95% CI and p-value calculated using mixed model with repeated measures. [7] P-value was calculated using 2-sides Fisher's exact test. [8] Adjusted LS Means difference; 95% CI and p-value calculated using one-way analysis of covariance.

Table 3: Review panel of VLU debridement methods randomized controlled trials (based on Gethin et al).[10]

Study	Agent (patients included in analysis)	Complete debridement rate	Time to complete debridement	Wound healing rate	Wound size reduction	Pain reduction	Adverse events
Groenewald 1980[20]	Dextranomer beads (n=50) EUSOL soaks (n=50)	80% vs. 14% after 1 week (P< 0.0001)	Mean 5.9 days vs. 15.4 days (p<0.001)	NR	NR	All dextranomer beads patients had a reduction in pain within 24 hours of treatments, no reported pain after 10 days. Four EUSOL patients had increase in pain that did not improve. At 21 days two participants continued to have pain.	NR
Jasiel 1996[21]	Hydrogel (n=46) Paraffin gauze (n=40)	76% vs. 45% within 3 weeks (p=0.05)	No differences, but precise figures were not reported	NR	NR	NR	Two adverse events in hydrogel, 1 possibly related to treatment (erysipelas). One adverse event in paraffin gauze (maceration and infection). One participant withdrawn from each groups (thrombophlebitis, infection).
Alvarez 2012[22]	Biocellulose wound dressing (Suprasorb®, n=25) Nonadherent dressing (Adaptic®, n=23)	84% vs. 26% in 12 weeks (p<0.0001) **(75%-100% clean)*	NR	39% vs. 47% in 12 weeks, but 13 pts withdrew during follow up (NS)	74% vs. 54% in 6 weeks (NS)	Over 12-week period, a larger proportion of BWD patients had no/mild pain compared to control group, and at week seven there was a significant difference between the groups however no pain scores were provided at any time point.	14 adverse events attributed to study treatment; Clinically-infected ulcer (3 BWD group, 5 non-adherent group), cellulitis (2 BWD group, 1 non-adherent group), dermatitis (1 BWD group, 2 non-adherent group). None reported not related to study treatment.
Hansson 1998[28]	Cadexomer iodine (n=56) Hydrocolloid (n=48) Paraffin gauze (n=49)	NR	NR	NR	Mean 35.5% vs. 34.4% vs. 10.6% in 4 weeks[a]	Overall reduction by week 12 was 66% to 29% in cadexomer-iodine, 73% to 57% in hydrocolloid, and 57% to 15% in paraffin	12 participants in the iodine group, 7 in the hydrocolloid group and 9 in the paraffin gauze group were withdrawn due to allergic reactions, dermatitis, pain or poor compliance (stated these were not due to the study treatments).
Wild 2010[29]	Biocellulose wound dressing (Suprasorb®, n=20) Hydrofibre dressing (AquaCel®, n=20)	NR	NR	NR	Mean 43.5% vs. 17.9% in 4 weeks (NS)	Pain at dressing changes on days 7, 14 and 28 were 2.25, 2.7 and 1.3 for BWD vs. 3.73, 5.25 and 3.2 for hydrofibre dressing, however no baseline scores were provided	NR
Skog 1983[27]	Cadexomer iodine (n=38) Non adherent dressings (n=36)	NR	NR	NR	Mean 34% vs. 5% in 6 weeks (P< 0.02)	VAS scores before treatment, at week 1 and at 6 weeks were 32, 27 and 10 for cadexomer iodine vs. 33, 29 and 23 for non-adherent dressings (P<0.05)	NR (while withdrawals from the study were noted, there were no adverse events reported).
Westerhof 1990[24]	Krill enzymes (n=16) Standard autolytic protocol[b] (n=15)	NR	Mean 7 days vs. 10 days (NS)	NR	13% vs. 3% in 1 week, however 12 patients excluded from analysis	Both treatments caused a similar reduction in pain, no figures reported	There were no signs of side effects in either group.
	Collagenase (n=27)	NR		NR	NR	NR	NR

Konig 2005[25]	Moist dressing (Tender-Wet®, n=15)		8.5% vs 18.7% slough reduction after 14 days (NS)				
Wayman 2000[23]	Larvae (n=6)	100% vs 33% in 1 month (p=0.065)	Mean 3 days vs. 22 days (p=0.003)	NR	NR	NR	NR
	Hydrogel (n=6)						
ChronEx	EX-02 (n=46)	63% vs. 30.2% vs 13.3% within 14 days (p<0.01)	Median 9 days for EX-02 vs. 59 days for NSSOC (p=0.016), vs. 63 days for GV (p=0.004)	32.6% vs. 27.9% vs. 26.7% within 12 weeks (non-inferiority, p<0.01))	Mean size reduction of 21.5% vs. 25.3% vs. 33.2% from baseline to 2 weeks after debridement (NS)	1.3±2.7 vs. 1.4±2.7 vs 1.6±1.9 mean NPRS reduction from baseline to 2 weeks after debridement for EX-02 vs. Gel vs. NSSOC (NS)	Incidence of adverse events 52.2% vs. 62.8% vs. 46.7% (NS). There were 9 vs. 3 vs. 1 adverse events with possible relationship to study treatment. Two patients discontinued study due to adverse events; 1 in EX-02 and 1 in Gel.
	Gel vehicle (n=43)						
	NSSOC (n=30)						

NR= not reported, BWD= Biocellulose wound dressing, EUSOL= Edinburgh University solution of lime. NPRS= Numeric Pain Rating Scale.

[a]cadexomer iodine vs. paraffin gauze (P= 0.006); hydrocolloid vs. paraffin gauze (P=0.01); cadexomer iodine vs. hydrocolloid (NS).

[b]Acetic acid for 2 days, followed by povidine iodine for 2 days, followed by 3 days of saline soaking.

FIGURES / LEGEND TO FIGURES

Figure 1: Patient flow diagram

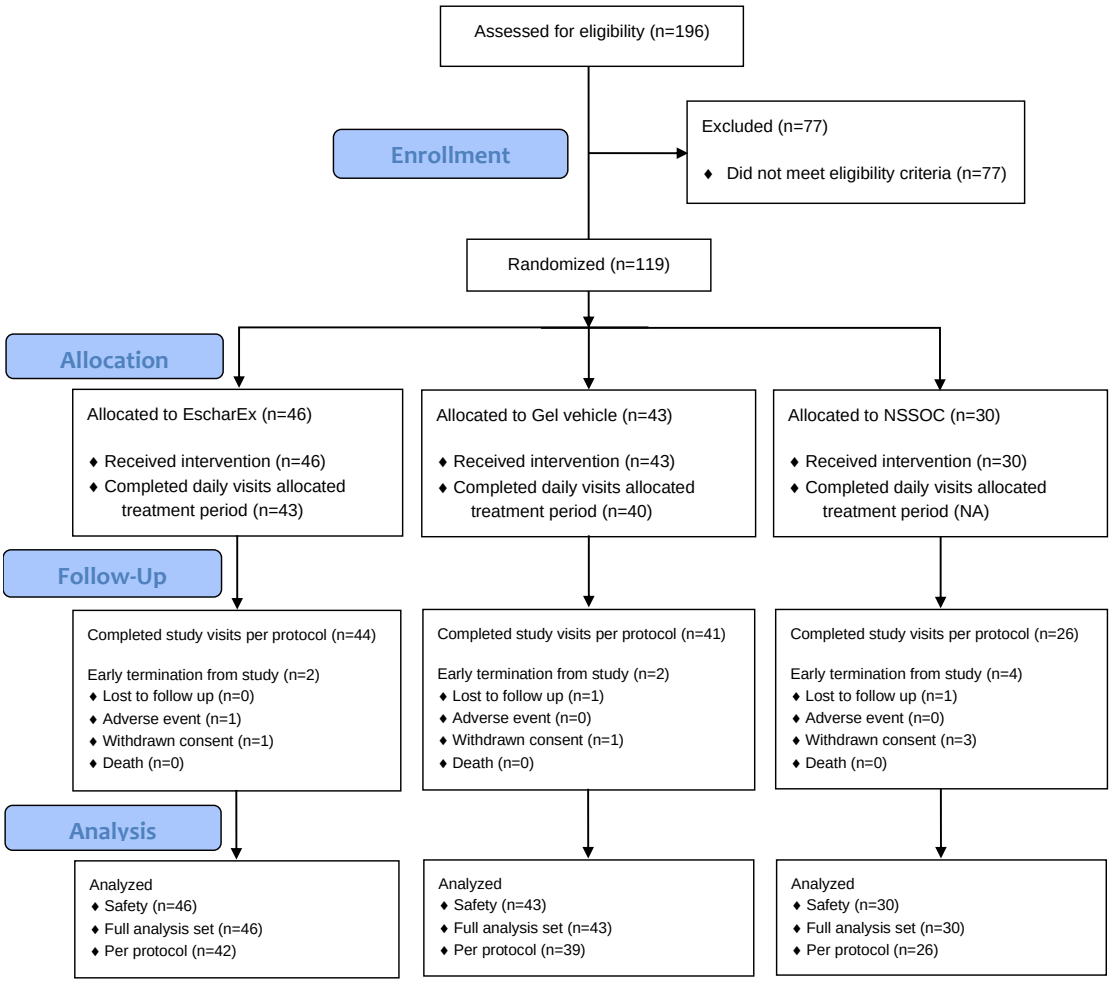
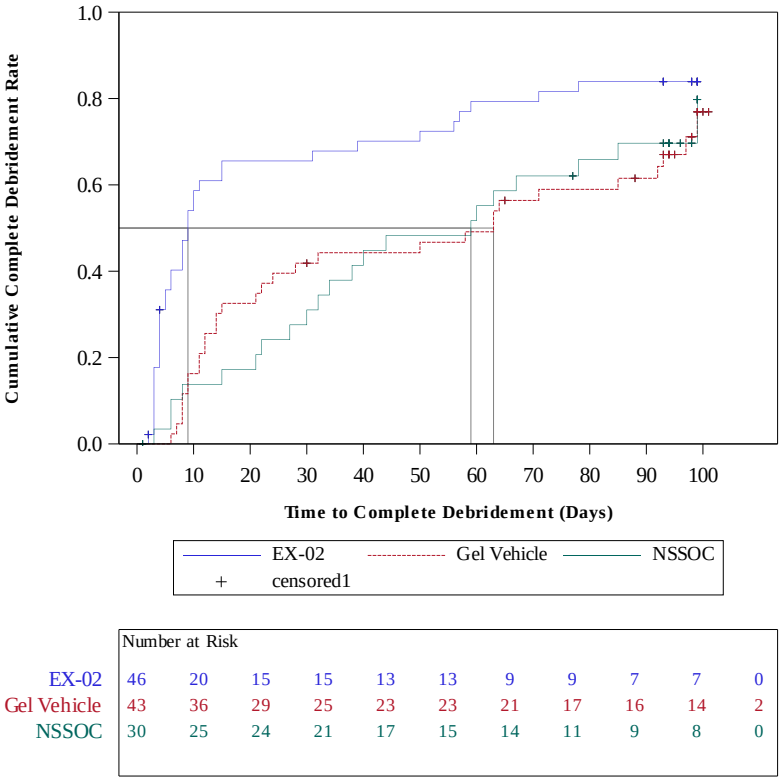


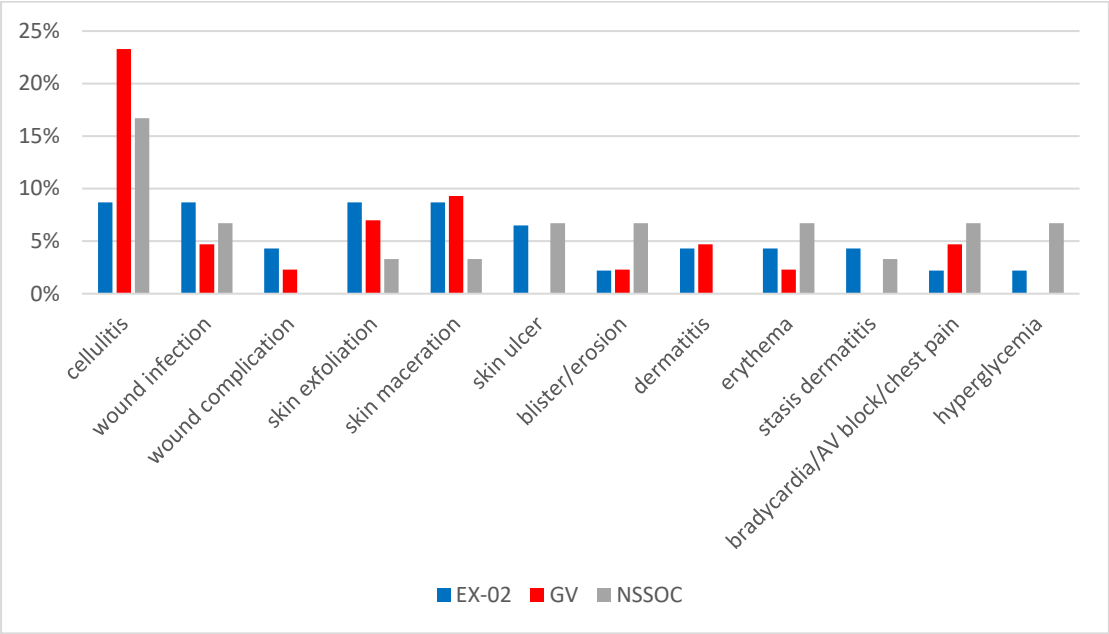
Figure 2: Kaplan-Meier estimates for time to complete debridement for EscharEx (EX-02), Gel Vehicle (GV) and Non-Surgical Standard of Care (NSSOC) arms. The medians depicted by the gray lines (and 95% CI) were 9 (5-15) days for EX-02, 59 (30-85) days for GV, and 63 (21-93) days for NSSOC.



Number at Risk											
EX-02	46	20	15	15	13	13	9	9	7	7	0
Gel Vehicle	43	36	29	25	23	23	21	17	16	14	2
NSSOC	30	25	24	21	17	15	14	11	9	8	0

Figure 3: Serial photographs of a VLU (pre-existing for 32 weeks, wound area 8cm^2) in a 59-year-old woman (BMI=35) treated with EX-02 during the study: A) Baseline; B) After 1st application of EX-02; C) After 2nd application of EX-02 (complete debridement); D) Wound closure confirmation 53 days after 1st application

Figure 4: Treatment emergent adverse events throughout the study (reported in >4% of patients in any of the study arms).



RESEARCH IN CONTEXT

Evidence before this study

We searched PubMed from database inception to July 1st, 2023, for papers published in English including the following terms: “venous leg ulcer” OR “VLU” AND “debridement”. We identified an article reviewing 10 randomized controlled trials comparing different non-surgical debridement methods of venous leg ulcers, which failed to demonstrate an optimal debridement method or duration of treatment. A recent multi-center, assessor blinded RCT, demonstrated that a Bromelain based enzymatic debridement agent achieved a significantly higher incidence of complete debridement of venous, diabetic and post-surgical/traumatic ulcers as compared to its gel vehicle.

Added value of this study

Once daily Bromelain-based enzymatic debridement led to a significantly higher incidence of complete debridement of venous leg ulcers within two weeks as compared to gel vehicle, and to a significantly shorter time to complete debridement as compared to both gel vehicle and various non-surgical standard of care debridement agents. Additionally, it led to a significantly higher incidence of >75% granulation tissue within two weeks as compared to gel vehicle. The efficacy of these 24-hour applications, combined with an acceptable safety profile demonstrated in this study, may provide a solution to the unmet need for a safe and efficient non-surgical debridement agent for venous leg ulcers.

Implications of all the available evidence

Non-surgical debridement of venous leg ulcers has thus far not been demonstrated to lead to earlier wound closure. A faster debridement as seen in this study may lend itself to earlier

application of cellular, acellular, matrix-like products or spilt thickness skin graft to close the wound, hopefully leading to a shorter time to complete wound closure. This, along with the efficacy, safety and ease of use demonstrated in this trial potentially allowing for home use by the patient or a caregiver, may prove to be substantial benefits for patients and health care professionals. Additional studies are needed to further demonstrate the efficacy and potential benefits of Bromelain based enzymatic debridement in venous leg ulcers and other chronic wounds.

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