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Effect of a Novel Macrophage-Regulating Drug on Wound Healing in Patients With Diabetic Foot Ulcers

A Randomized Clinical Trial

Yu-Yao Huang, MD, PhD; Ching-Wen Lin, PhD; Nai-Chen Cheng, MD, PhD; Shawn M. Cazzell, DPM; Hsin-Han Chen, MD; Kuo-Feng Huang, MD; Kwang-Yi Tung, MD; Hsuan-Li Huang, MD; Pao-Yuan Lin, MD; Cherng-Kang Perng, MD, PhD; Bimin Shi, MD; Chang Liu, MD; Yujin Ma, MD; Yemin Cao, MD; Yanbing Li, MD; Yaoming Xue, MD; Li Yan, MD; Qiu Li, MD; Guang Ning, MD, PhD; Shun-Cheng Chang, MD

Abstract

IMPORTANCE Delayed healing of diabetic foot ulcers (DFUs) is known to be caused by dysregulated M1/M2-type macrophages, and restoring the balance between these macrophage types plays a critical role in healing. However, drugs used to regulate M1/M2 macrophages have not yet been studied in large randomized clinical trials.

OBJECTIVE To compare the topical application of ON101 cream with use of an absorbent dressing (Hydrofiber; ConvaTec Ltd) when treating DFUs.

DESIGN, SETTING, AND PARTICIPANTS This multicenter, evaluator-blinded, phase 3 randomized clinical trial was performed in 21 clinical and medical centers across the US, China, and Taiwan from November 23, 2012, to May 11, 2020. Eligible patients with debrided DFUs of 1 to 25 cm^2 present for at least 4 weeks and with Wagner grade 1 or 2 were randomized 1:1 to receive ON101 or control absorbent dressings.

INTERVENTIONS Twice-daily applications of ON101 or a absorbent dressing changed once daily or 2 to 3 times a week for 16 weeks, with a 12-week follow-up.

MAIN OUTCOMES AND MEASURES The primary outcome was the incidence of complete healing, defined as complete re-epithelialization at 2 consecutive visits during the treatment period assessed on the full-analysis set (FAS) of all participants with postrandomization data collected. Safety outcomes included assessment of the incidences of adverse events, clinical laboratory values, and vital signs.

RESULTS In the FAS, 236 eligible patients (175 men [74.2%]; mean [SD] age, 57.0 [10.9] years; mean [SD] glycated hemoglobin level, 8.1% [1.6%]) with DFUs classified as Wagner grade 1 or 2 (mean [SD] ulcer area, 4.8 [4.4] cm^2) were randomized to receive either the ON101 cream (n = 122) or the absorbent dressing (n = 114) for as long as 16 weeks. The incidence of complete healing in the FAS included 74 patients (60.7%) in the ON101 group and 40 (35.1%) in the comparator group during the 16-week treatment period (difference, 25.6 percentage points; odds ratio, 2.84; 95% CI, 1.66-4.84; $P < .001$). A total of 7 (5.7%) treatment-emergent adverse events occurred in the ON101 group vs 5 (4.4%) in the comparator group. No treatment-related serious adverse events occurred in the ON101 group vs 1 (0.9%) in the comparator group.

CONCLUSIONS AND RELEVANCE In this multicenter randomized clinical trial, ON101 exhibited better healing efficacy than absorbent dressing alone in the treatment of DFUs and showed

(continued)

Key Points

Question Can the topical application of ON101 cream demonstrate a superior therapeutic benefit in wound healing among patients with diabetic foot ulcers (DFUs) compared with standard care?

Findings In this randomized phase 3 clinical trial of 236 patients with DFUs, topical application of ON101 with gauze immediately after debridement demonstrated significant healing efficacy compared with an absorbent dressing in all patients, including those with DFU-related risk factors.

Meaning Topical treatment with ON101 resulted in improved healing of DFUs.

+ Visual Abstract

+ Supplemental content

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Abstract (continued)

consistent efficacy among all patients, including those with DFU-related risk factors (glycated hemoglobin level, ≥9%; ulcer area, >5 cm^2; and DFU duration, ≥6 months).

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Introduction

Approximately 80% of lower limb amputations are preceded by chronic diabetic foot ulcers (DFUs), resulting in a heavy burden of medical care and expenditure.[1,2] The current treatment for DFUs in clinical practice focuses primarily on local wound care, including debridement, off-loading, infection control, and maintaining a moist environment with dressings,[3,4] whereas adjunctive therapies such as the use of growth factors, tissue engineering products, hyperbaric oxygen, and negative pressure wound therapies are applied if the DFUs worsen.[5] Although current treatments featuring tissue repair or the use of anti-inflammatory agents might help in closing or controlling the progression of DFUs, most of these treatments are not well supported by clinical evidence or are not recommended for routine care by the International Working Group on the Diabetic Foot.[6] In addition, the annual increase in amputations also suggests that treatment improvement is needed.[7] Diabetic foot ulcers are pathologically complex mostly because the ulceration is undermined by the existence of multiple risk factors, such as poor patient adherence to treatment, severity of the ulcer, ulcer location and duration, vascular condition, control of glycated hemoglobin (HbA_{1c}) levels, smoking habits, and kidney dysfunction.[8,9] These factors impose a significant clinical need for novel and effective interventions to tackle this life-debilitating and life-threatening disease.

Accumulating scientific evidence has revealed that targeting macrophage phenotypes might be a potentially effective therapy in DFUs because hyperglycemia increases the ratio of proinflammatory M1 to proregenerative M2 macrophages.[9-14] ON101 (supplied by Oneness Biotech Co, Ltd; previously given the research code WH-1) exerts its therapeutic effect through regulation of the balance between M1 and M2 macrophages. ON101 is composed of 2 active pharmaceutical ingredients: PA-F4 from an extract of *Plectranthus amboinicus* and S1 from an extract of *Centella asiatica*, 2 medicinal plants reported to have significant pharmacological activities in wound healing.[10-12] With 48 in vitro and in vivo studies performed, these 2 ingredients, which contribute to a synergistic effect on regulation of the M1:M2 macrophage ratio, have been defined and formulated in a cream base using a proprietary formula. One of these ingredients, PA-F4, significantly attenuates M1 macrophages by suppressing the NLRP3-mediated inflammasome pathway and the production of downstream inflammatory cytokines such as interleukin 1β and interleukin 6,[13] which arrest the inflammation phase. On the other hand, the extract of *C asiatica* has been reported to activate M2 macrophages by increasing collagen synthesis and by stimulating fibroblast proliferation and the migration of keratinocytes.[14,15] ON101 has been further demonstrated to accelerate wound healing efficiently in a db/db mouse model of diabetes, obesity, and dyslipidemia by decreasing inflammatory M1 macrophage activity and enriching M2 macrophage populations through granulocyte colony-stimulating factor–mediated M2 polarization, which changed the ulcer status from the inflammatory phase to the proliferation and remodeling stages (eFigure 1 in Supplement 2).

A clinical pharmacokinetic study on 12 patients with DFUs showed that topical administration of ON101 twice daily in single and multiple doses yielded very limited systemic exposure (Kai-Min Chu, MD, PhD, oral communication, September 4, 2017). Thus, the maximum body concentrations from days 1 and 14 were similar, demonstrating that topical ON101 has no obvious accumulation in the body. No treatment-related adverse events were observed. In a clinical research trial conducted in 24 patients with chronic DFUs classified as grade 3 according to the Wagner system,[10] treatment with ON101 for 2 weeks resulted in an approximately 20% reduction in wound size, and no serious

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adverse events were reported. Of the 21 patients with evaluable data, the mean wound size at baseline was 359 (range, 20-2352) mm^2, decreasing to 293 mm^2 after 2 weeks of ON101 treatment.[10] Another clinical trial was performed with 30 patients with Wagner grade 1 chronic DFUs treated with ON101 for as long as 12 weeks (Yu-Yao Huang, MD, PhD, oral communication, August 22, 2011). The final incidence of healing was 50%. The mean wound area at baseline was 577 (range, 303-1225) mm^2, decreasing to 163 mm^2 after 12 weeks of ON101 treatment.

The topical use of ON101 is supported with a safety profile from the manufacturer and has clear therapeutic potential in promoting wound healing based on previous studies.[10] This multicenter, phase 3 randomized clinical trial was designed to evaluate whether ON101 could treat chronic DFUs by comparing it with a standard primary wound care absorbent dressing.

Methods

We followed adequate and well-controlled studies as categorized by the US Food and Drug Administration[16] to design a randomized, controlled, evaluator-blinded phase 3 trial to evaluate the efficacy of ON101 applied topically twice daily for treating chronic DFUs (the trial protocol is available in Supplement 1). This treatment was compared with an absorbent dressing (Hydrofiber; ConvaTec Ltd) as a comparator in the control group for treating chronic DFUs. This multicenter study was performed with institutional review board approval from 21 medical/clinical centers (eTable 1 in Supplement 2) with wound care specialty across the US, China, and Taiwan, where these investigational new drug programs were initiated; all patients provided written informed consent at enrollment. The study followed the International Council on Harmonization guideline[17] and the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

From November 23, 2012, to May 11, 2020, we enrolled outpatients with type 1 or 2 diabetes (as defined by World Health Organization criteria) aged 20 to 80 years, with a baseline HbA_{1c} level of less than 12% measured during screening or within 3 months before randomization (to convert to proportion of total hemoglobin, multiply by 0.01). The target ulcer classified as grade 1 or 2 based on the Wagner system on the foot (below the ankle) needed to measure from 1 to 25 cm^2 after debridement, without active infection, and present for at least 4 weeks despite receiving standard of care (according to the International Working Group on the Diabetic Foot guidelines[18]) before randomization. To avoid possible premature discontinuation of the patient treatments during the trial, we excluded patients with an ankle-brachial before randomization; those with necrosis, purulence, or sinus tracts in the target ulcer not removable by debridement during the screening visit; or those with acute Charcot neuroarthropathy as defined by the American Diabetes Association and the American Podiatric Medical Association, which indicates perturbations of bone metabolism.[19] In addition, revascularization procedures aimed at increasing blood flow in the target limb must have been performed at least 4 weeks before randomization.

Eligible participants judged by the principal investigators (Y.-Y.H., N.-C.C., H.-H.C., K.-F.H., K.-Y.T., H.-L.H., P.-Y.L., C.-K.P., B.S., C.L., Y.M., Y.C., Y.L., Y.X., Q.L., G.N., and S.-C.C.) on completion of the screening period (≤7 days) were assigned to receive ON101 or absorbent dressing for as long as 16 weeks in a 1:1 allocation by a computer-generated block randomization scheme (eMethods 1 in Supplement 2).[20] Individual investigators and research staff were blinded to the size of the block and remained blinded to the treatment assignment before randomization, eliminating the possibility of predetermining the prospective participant's treatment assignment. The investigator was informed of the randomized treatment assignment in a sealed envelope containing the individual treatment code at the baseline visit.

The end-of-treatment visit (visit 10) was the visit in the 16th week after randomization or the visit in which complete wound closure was confirmed, whichever happened first. The independent evaluator assessed the degree of wound closure. The independent evaluator and the study statistician were blinded to the participants' treatment throughout the study until the clinical database had been locked. To ensure masking throughout the trial, a standardized procedure was

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established including camera settings, photographing and image-encoding, image delivery to the independent evaluator, and outcome assessment based on the digitally encoded images to delink the patients' identification, treatment groups, visits, or site information. The detailed blinding procedure is described in eMethods 2 in Supplement 2.

Interventions

Demographic data, medical history, disease status, radiography, and eligibility were evaluated during the screening period (before randomization). Participants were scheduled for return visits every 2 weeks to receive wound cleansing and debridement with an assessment of wound status, wound size measurement, physical examination results, and concomitant medication records throughout the 16 weeks of the study period once the interventions were administered. The principal investigators and nurses were trained to use standardized study materials, ON101 or absorbent dressings, camera setting, and off-loading recommendations. The instruction for use of off-loading devices was given to the patients with plantar ulcers as assessed by the clinical investigators. All adverse events were recorded at every visit once the intervention was applied. Blood samples for laboratory tests (including hematologic and biochemical analysis) were collected at the screening visit, then every 4 weeks during the treatment period and at the last visit of the follow-up period to detect the levels of factors such as alanine aminotransferase and aspartate aminotransferase to measure liver status, creatinine and blood urea nitrogen to measure kidney status, and albumin to measure nutritional status. Levels of HbA_{1c} and blood glucose were measured to monitor diabetes-related safety concerns.

ON101, a topical cream composed of PA-F4 and S1, was supplied by Oneness Biotech Co, Ltd, and manufactured in Taiwan in a facility in compliance with Good Manufacturing Practice certified by the Pharmaceutical Inspection Cooperation Scheme. Participants in the ON101 treatment group were shown how to self-administer the cream twice daily in an amount to cover the target ulcer fully without exceeding 2 mm in thickness at each visit. The absorbent dressing containing sodium carboxymethylcellulose (Aquacel; ConvaTec Ltd) needed to be changed daily or 2 to 3 times weekly subject to exudate level following the product's instructions or the investigators' discretion. The only secondary dressing allowed was sterile gauze for both groups. The amount of ON101 used or the frequency of absorbent dressing changes for each patient was recorded at every visit during the treatment period. No systemic prescriptions were contraindicated during the treatment period, whereas topical antimicrobials and antiseptic agents were not allowed.

In cases where the target ulcer worsened (defined as Wagner grade 3), the investigators could determine whether to terminate treatment. If the ulcer was judged by the blinded evaluator as having undergone complete epithelialization for 2 consecutive visits during the treatment period (at or before visit 10), the intervention (ON101 or absorbent dressing) was stopped, and a visit 10 was scheduled after this judgment. If the patients were confirmed to have an unhealed target ulcer at visit 10, continual standard of care with the absorbent dressings was provided to them regardless of the allocated group during the 12-week follow-up period.

Data Collection and Outcome Measures

The primary efficacy outcome was to compare the incidence of complete healing between the 2 groups at the end of the 16-week treatment period. Complete healing, defined as complete epithelialization maintained without drainage or requirement of dressings for at least 2 consecutive visits, was determined by an independent evaluator blinded to the patient's information and treatment allocation. Secondary ulcer-related outcomes included time to complete ulcer healing (from baseline visit to first 100% re-epithelialization visit), percentage of change in ulcer surface area from baseline (to the latest treatment visit or complete wound closure), percentage of patients with a 50% reduction in ulcer surface area, and incidence of infection of the target ulcer. The exploratory, ulcer-related outcome data included any incidence of ulcer recurrence during the 12-week follow-up period. Target wound size was measured by an investigator using digital planimetry at every visit

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after any necessary debridement. In addition, efficacy variables were further assessed for subgroups for the incidence of complete healing, characterized according to the prior duration of ulcers recorded at the baseline visit (6 months as a cutoff),[21] ulcer size (5 cm^2 as a cutoff),[22] and HbA_{1c} level (9% as a cutoff regarded as poor glycemic control according to the definition of the American Diabetes Association). Safety outcomes were used to assess adverse events and clinical laboratory values.

Statistical Analysis

The sample size was calculated based on the results of ON101 in the previous trial by hypothesizing a 20% superiority in the incidence of wound closure compared with the efficacy of the absorbent dressing (Yu-Yao Huang, MD, PhD, oral communication, August 22, 2011). With a 1:1 randomization ratio in the 2 groups, 236 participants were required to be enrolled to ensure that at least 212 had evaluable data for achieving 80% power with a 2-sided α value of 5% nominal significance. All analyses were performed using SAS software, version 9.4 (SAS Institute Inc). The intention-to-treat (ITT) principle was applied to the full-analysis set (FAS), which included all randomized patients irrespective of the actual receipt of study intervention and adherence to the protocol or the occurrence of adverse events. The FAS was used to analyze all efficacy and safety data. A modified ITT (mITT) protocol was applied to exclude patients in the FAS with ineligible target ulcers at baseline. The mITT was used for supportive analysis of efficacy data as appropriate.

For the primary end point, we used a χ^2 test and a logistic regression model with intervention as a fixed factor, with the baseline ulcer size and Wagner grade adjusted as covariates. The results of the logistic regression model are presented in terms of the odds ratio (OR), with *P* values and associated 95% CIs. Some outcomes are expressed as the hazard ratio (HR). Exploratory post hoc analyses of pertinent variables, such as ulcer duration, ulcer size, and patients' HbA_{1c} levels, were also performed. The time to complete ulcer healing was calculated using the Kaplan-Meier method with a log-rank test. The HRs and 95% CIs were estimated using a Cox proportional hazards regression model. The percentile changes in ulcer surface area and ulcer surface area change from baseline were subjected to regression analysis adjusted by baseline ulcer area and Wagner grades. The incidences of infection of target ulcers and of recurrence were evaluated using the Fisher exact test.

The adverse events were regarded as treatment emergent if they occurred after the intervention started. Adverse events, treatment-emergent adverse events, and serious adverse events were summarized by frequency and proportion of total patients by system organ class and by preferred terms. All adverse event–related comparisons between the 2 groups were performed using the Fisher exact test. The clinical laboratory test data were used to tabulate the change in values from baseline and were compared between groups using analysis of covariance. All tests were 2 tailed, and $P < .05$ was considered statistically significant.

For possible early study termination, an independent data monitoring committee was established to monitor data when the patient numbers reached approximately 50% and 90% of the planned enrollment. The futility or superiority of ON101 cream was assessed by the independent data monitoring committee using the Lan-DeMets alpha-spending approach, in which the boundaries were determined by the type of O'Brien-Fleming spending function.[23] The superiority of ON101 was confirmed by the independent data monitoring committee ($P < .001$, much less than the boundary of 0.03476) on achieving 90% of the planned enrollment (212 participants with evaluable data) so the interim analysis could proceed. The trial was not terminated despite ON101 achieving superiority in the interim analysis because the 236th patient with evaluable data was already enrolled before this point.

Results

A total of 236 patients were included in the FAS (175 men [74.2%]; 61 women [25.8%]; mean [SD] age, 57.0 [10.9] years). The mean (SD) HbA_{1c} level was 8.1% (1.6%) at baseline and did not change

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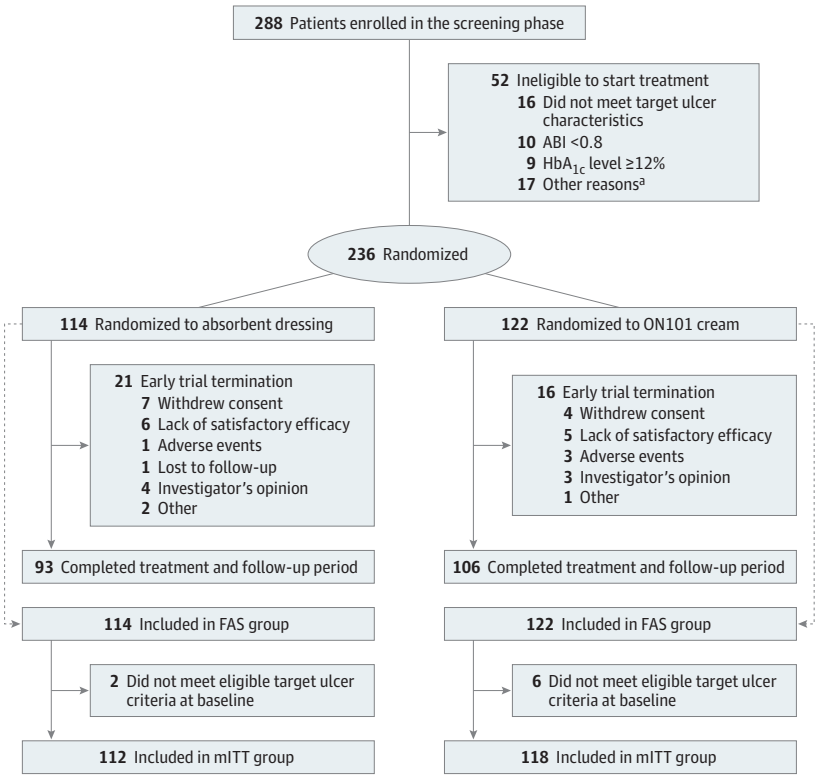
significantly at the end of treatment (mean [SD] HbA_{1c} of 8.0% [1.8%] in the ON101 group vs 7.9% [1.6%] in the comparator group), and 144 patients (61.0%) were diagnosed as having had diabetes for more than 10 years. Patients in the FAS were randomly allocated to treatment: 114 (48.3%) to the comparator group and 122 (51.7%) to the ON101 group. Sixteen patients (13.1%) in the ON101 group vs 21 (18.4%) in the comparator group had an early termination (total of 37) (**Figure 1**). The instructions for using off-loading devices were given to the patients who were assessed by the clinical investigators. Some patients did not follow the suggestion because of the humidity in Taiwan (**Table 1**). Among the 236 patients in the FAS, 184 (78.0%) were classified as having Wagner grade 2 ulcers, 117 (49.6%) had ulcers in the plantar region, and 64 (27.1%) had a baseline HbA_{1c} level of at least 9%. The mean (SD) ulcer size was 4.8 (4.4) cm^2, and the mean (SD) prior duration of the target ulcer was 7.2 (13.4) months at entry (Table 1).

Primary Outcome

Seventy-four patients (60.7%) in the ON101 group vs 40 (35.1%) in the comparator group achieved ulcer closure within 16 weeks (OR, 2.84; 95% CI, 1.66-4.84; $P < .001$) (**Table 2**). Similar results were also noted in the mITT population, where 73 of 118 patients (61.9%) in the ON101 group and 38 of 112 (33.9%) in the comparator group had ulcer closure (OR, 3.15; 95% CI, 1.82-5.43; $P < .001$) (Table 2 and eTable 2 in Supplement 2). The independent evaluator assessed the degree of wound closure.

Ulcer duration, ulcer size, and HbA_{1c} levels are known to be associated with poor prognosis of DFUs.[9,24,25] Therefore, a subgroup analysis was conducted on baseline ulcer duration (6 months as a cutoff), baseline ulcer area (5 cm^2 as a cutoff size), and baseline HbA_{1c} level (9% as a cutoff). The subgroup analysis displayed a significant OR in favor of the ON101 group compared with the comparator group (OR, 3.14 [95% CI, 1.04-9.50; $P = .04$] for HbA_{1c} level ≥9%; OR, 3.99 [95% CI, 1.09-14.63; $P = .04$] for ulcer duration ≥6 months; OR, 4.09 [95% CI, 1.42-11.80; $P = .009$] for ulcer size >5 cm^2) (Table 2). In addition, we subgrouped patients with an ulcer reduction of less than 10%

Figure 1. CONSORT Diagram of Study Flow



A total of 236 patients were randomized. Absorbent dressing was Hydrofiber (ConvaTec Ltd). To convert glycated hemoglobin (HbA_{1c}) to proportion of total hemoglobin, multiply by 0.01. ABI indicates ankle-brachial index; FAS, full-analysis set; and mITT, modified intention to treat.

[a] Judged by the investigator to be unsuitable for the study for any other reason.

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Table 1. Baseline Patient Characteristics and Intervention During the Study

Characteristic	Patient group[a]		
	ON101 (n = 122)	Absorbent dressing (n = 114)	All (N = 236)
Baseline patient characteristics			
Age, mean (SD), y	57.4 (10.6)	56.6 (11.3)	57.0 (10.9)
Sex			
Male	93 (76.2)	82 (71.9)	175 (74.2)
Female	29 (23.8)	32 (28.1)	61 (25.8)
Type 2 diabetes	121 (99.2)	113 (99.1)	234 (99.2)
Diabetes duration, y			
≤10	55 (45.1)	37 (32.5)	92 (39.0)
>10	67 (54.9)	77 (67.5)	144 (61.0)
HbA_{1c} level, %			
Mean (SD)	8.1 (1.5)	8.1 (1.8)	8.1 (1.6)
<9	90 (73.8)	82 (71.9)	172 (72.9)
≥9	32 (26.2)	32 (28.1)	64 (27.1)
BMI			
<25	59 (48.4)	50 (43.9)	109 (46.2)
≥25	63 (51.6)	64 (56.1)	127 (53.8)
Hypertension	78 (63.9)	73 (64.0)	151 (64.0)
CVD history[b]	25 (20.5)	23 (20.2)	48 (20.3)
Kidney status			
eGFR, mL/min/1.73 m^2			
≥60	90 (73.8)	81 (71.1)	171 (72.5)
<60	32 (26.2)	33 (28.9)	65 (27.5)
ABI, mean (SD)	1.1 (0.2)	1.1 (0.1)	1.11 (0.1)
Amputation history[c]	56 (45.9)	60 (52.6)	116 (49.2)
Wound conditions, Wagner grade			
1	29 (23.8)	23 (20.2)	52 (22.0)
2	93 (76.2)	91 (79.8)	184 (78.0)
Ulcer size, cm^2			
Mean (SD)	5.0 (4.4)	5.1 (4.7)	4.8 (4.4)
1-5	88 (72.1)	77 (67.5)	165 (69.9)
>5	33 (27.0)	36 (31.6)	69 (29.2)
Ulcer duration, mo			
Mean (SD)	7.2 (13.0)	7.3 (13.9)	7.15 (13.4)
<6	86 (70.5)	79 (69.3)	165 (69.9)
≥6	36 (29.5)	35 (30.7)	71 (30.1)
Plantar ulcers	64 (52.5)	53 (46.5)	117 (49.6)
Intervention during the study			
Off-loading in plantar ulcer[d]			
Use	33 (51.6)	34 (64.2)	67 (57.3)
No use	15 (23.4)	9 (17.0)	24 (20.5)
Not specified	16 (25.0)	10 (18.9)	26 (22.2)
Diabetes medication prescribed			
Metformin	62 (50.8)	51 (44.7)	113 (47.9)
Insulin	67 (54.9)	67 (58.8)	134 (56.8)
Any oral hypoglycemic agent	84 (68.9)	81 (71.1)	165 (69.9)
Use of antibiotics	30 (24.6)	26 (22.8)	56 (23.7)

Abbreviations: ABI, ankle-brachial index; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycated hemoglobin.

SI conversion factor: To convert HbA_{1c} to proportion of total hemoglobin, multiply by 0.01.

[a] Unless otherwise indicated, data are expressed as number (%) of patients. Owing to missing data, numbers may not total column headings or percentages may not total 100. Absorbent dressing was Hydrofiber (ConvaTec Ltd).

[b] Includes ischemic heart disease, coronary artery disease, or cerebral vascular accident with embolic, ischemic, or hemorrhagic stroke.

[c] Due to previous diabetic foot ulcers.

[d] Includes only patients with plantar ulcer.

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during the screening period and analyzed the primary efficacy variable. The result also favored the ON101 treatment (32 of 64 [50.0%] vs 18 of 66 [27.3%]; *P* = .02) (eTable 6 in Supplement 2).

Secondary Outcome

Patients in the ON101 group had a better healing rate than those in the comparator group (HR, 1.80 [95% CI, 1.23-2.65; *P* = .002]) (**Figure 2**) in the FAS as well as the mITT population (HR, 1.91 [95% CI, 1.29-2.83; *P* = .001]) (eFigure 2 in Supplement 2). The cumulative incidence of complete healing at each week also reflected the continual higher probability in the ON101 group for reaching complete wound closure from week 4 onward. The time to reach median population healing was 98 days in the ON101 group, whereas it was undeterminable in the comparator group because ulcers of only 40 patients (35.1%) healed in this group during the treatment period (Figure 2). The mean reduction in ulcer surface area (from the last treatment visit to baseline) was 78.0% in both groups (SDs, 42.6% for the ON101 group and 34.9% for the comparator group; *P* = .89), and the incidence of a 50% reduction in ulcer surface area was not significantly different between both groups (101 of 122 [82.8%] vs 98 of 114 [86.0%]) (Table 2). Only a few episodes of target ulcer infection occurred in both groups during the treatment period (6 in the ON101 group and 7 in the comparator group; *P* = .78) (Table 2). The incidence of recurrence in completely healed wounds during the follow-up

Table 2. Primary and Secondary Outcomes[a]

Outcome	Patient group		OR (95% CI)	*P* value
	ON101 (n = 122)	Absorbent dressing (n = 114)		
Complete healing, No. (%)				
FAS	74 (60.7)	40 (35.1)	2.84 (1.66-4.84)	<.001[b]
mITT	73 (61.9)	38 (33.9)	3,15 (1.82-5.43)	<.001[b]
Secondary				
Change in WSA from baseline to visit 10, mean (SD), %	−78.0 (42.6)	−78.0 (34.9)	NA	.89
Incidence of patients with 50% reduction in WSA on visit 10, No. (%)	101 (82.8)	98 (86.0)	0.80 (0.39-1.62)	.53[b]
Incidence of wound infection	6 (4.9)	7 (6.1)	NA	.78
Ulcer recurrence, No. (%)[c]	15 (20.3)	7 (17.5)	NA	.81
Safety				
Patients with TEAEs, No. (%)	76 (62.3)	77 (67.5)	NA	.42
No. of TEAEs	207	235	NA	
Related TEAEs				
Patients, No. (%)	7 (5.7)	5 (4.4)	NA	.77
No. of events	11	5	NA	NA
Serious TEAEs				
Patients, No. (%)	14 (11.5)	9 (7.9)	NA	.39
No. of events	24	14	NA	NA
Related serious TEAEs in events, No. (%)	0	1 (0.9)	NA	<.48
TEAE leading to death, No.	0	0	NA	NA
Subgroup analysis				
Wound closure, No./total No. (%)				
HbA_{1c} level				
<9%	59/90 (65.6)	33/82 (40.2)	2.81 (1.50-5.26)	<.001[b]
≥9%	15/32 (46.9)	7/32 (21.9)	3.14 (1.04-9.50)	.04[b]
Ulcer size, cm^2				
1-5	55/88 (62.5)	31/77 (40.3)	2.46 (1.31-4.61)	.005[b]
>5	18/33 (54.5)	8/36 (22.2)	4.09 (1.42-11.80)	.009[b]
Ulcer duration, mo				
<6 mo	62/86 (72.1)	36/79 (45.6)	3.07 (1.59-5.95)	<.001[b]
≥6 mo	12/36 (33.3)	4/35 (11.4)	3.99 (1.09-14.63)	.04[b]

Abbreviations: FAS, full-analysis set; HbA_{1c}, glycated hemoglobin; mITT, modified intent-to-treat; NA, not applicable; OR, odds ratio; TEAEs, treatment-emergent adverse events; WSA, wound (ulcer) surface area.

[a] The absorbent dressing used was Hydrofiber (ConvaTec Ltd).

[b] Calculated using a logistic regression model. Treatment was the main exposure variable; the baseline wound size in cm^2 and Wagner grade were covariates.

[c] Ulcer recurrence was recorded once the ulcer had healed completely and was observed during the follow-up period.

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phase was 15 of 74 (20.3%) in the ON101 group and 7 of 40 (17.5%) in the comparator group without statistical significance (*P* = .81) (Table 2).

Adverse Events

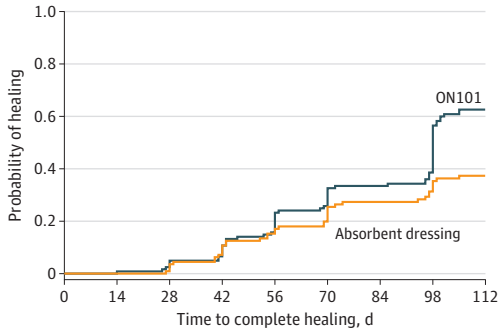
In terms of safety, there were no clinically significant changes or differences between the 2 treatment groups in hematology, biochemistry (including HbA_{1c} and fasting glucose levels), or vital signs (Table 2 and eTable 3 in Supplement 2). Treatment-emergent adverse events were reported in 76 patients in the ON101 group and 77 in the comparator group, of whom 7 of 122 (5.7%) in the ON101 group and 5 of 114 (4.4%) from the comparator group were considered related to the treatments (Table 2 and eTable 4 in Supplement 2). None of the serious adverse events was related to ON101 treatment, whereas there was 1 case of osteomyelitis reported to be linked to the comparator group in which 1 patient (0.8%) assigned to ON101 died of septic shock, acute kidney injury, and acute respiratory failure, which were not considered to be related to treatment or to ulcer progression (eTable 5 in Supplement 2).

Discussion

To our knowledge, this study is the first international phase 3 randomized clinical trial of an investigational drug able to regulate M1/M2 macrophage activities in patients with DFUs. ON101 exhibited better efficacy in facilitating the complete healing of DFUs. Hyperglycemia is an underlying cause of chronic DFUs in which the M1-to-M2 macrophage transition is delayed and the inflammatory stage is prolonged.[26,27] ON101 can restore the balance of M1/M2 macrophages caused by hyperglycemia. The robust efficacy in patients with high-risk factors suggests that ON101 might provide multiple and proactive ways to improve wound healing by promoting the M1-to-M2 transition and thereby accelerating wound healing for ulcers not only in terms of early formation but also with high-risk factors including ulcer duration of at least 6 months, ulcer size greater than 5 cm^2, and an HbA_{1c} level of at least 9%.

The design of this study followed US Food and Drug Administration guidelines.[16] The complete healing rate of the comparator group at 16 weeks (35.1%) was consistent with the 28.2% shown by ITT analysis at week 12 disclosed in a previous trial by Jeffcoate et al.[28] This finding verifies the suitability of the design and implementation of this study in conforming to other randomized clinical

Figure 2. Kaplan-Meier Plot of Time to Complete Healing in the Full-Analysis Set Population



No. at risk

ON101	122	122	119	113	101	87	78	72	34
Absorbent dressing	114	112	111	104	93	86	76	69	47

The survival curve indicates the incidence of ulcers healed at each visit in the full-analysis set population. Complete healing was defined as epithelialization without drainage observed at 2 consecutive visits. A full-analysis set cohort randomly assigned to the absorbent dressing (Hydrofiber; ConvaTec Ltd) group (n = 114) or ON101 group (n = 122) was used for Kaplan-Meier analysis.

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trials. The application of ON101 after debridement—which can be self-administered at home—indicated the same level of convenience of use as for the absorbent dressing.

Despite the statistically significant wound closure and healing rates provided by ON101, the ulcer reduction outcomes, including changes in ulcer area from baseline and rate of 50% reduction in the wound area, were not statistically significant between the 2 groups during the treatment period. This discrepancy possibly arose from the use of 2-dimensional measurements on the wound area without considering the wound depth. In this study, 78.0% of the ulcers were Wagner grade 2, meaning that they extended into tendon, bone, or capsule. Thus, the measurement of wound area instead of volume might not reflect the actual volumetric change. Similar outcomes were also noted in the pivotal study (study 92-22120-K) of becaplermin (Regranex; Smith & Nephew plc). The use of 3-dimensional measurement tools should be considered in future studies.

Limitations

This study has some limitations. The first was the open-label design, which did not allow us to mask the interventions to patients or clinical investigators; therefore, blinded evaluation was implemented to minimize any possible bias. Second, the inclusion and exclusion criteria ruled out patients requiring dialysis, which, to a certain extent, reflects some types of patients with DFUs. Using the ankle-brachial index as the sole criterion in judging blood perfusion could not exclude patients with ischemia completely. Last, the lack of a 2-week run-in period was a potential flaw in the design, because possible rapid healers might not have been excluded in the study. To assess whether this factor affected the trial results, a separate analysis of the complete ulcer healing rate was performed by excluding those patients with an ulcer reduction of at least 10% during the screening period, the results of which favored ON101 treatment (32 of 64 [50.0%] vs 18 of 66 [27.3%]; $P = .02$) (eTable 6 in Supplement 2).

Conclusions

The results of this randomized clinical trial demonstrate a clinically and statistically superior therapeutic efficacy of ON101 in the treatment of DFUs in both FAS and mITT populations in terms of complete healing rate and time to complete healing compared with absorbent dressing. For chronic wounds in patients with high-risk factors, the therapeutic efficacy of ON101 could be sustained in ulcers that last for more than 6 months or measure greater than 5 cm^2 or in patients with high HbA_{1c} levels. The findings of this study suggest that ON101, a macrophage regulator that behaves differently from moisture-retaining dressings, represents an active-healing alternative for home and primary care of patients with chronic DFUs.

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Corresponding Author: Shun-Cheng Chang, MD, Division of Plastic Surgery, Department of Surgery, Integrated Burn and Wound Care Center, Taipei Medical University-Shuang Ho Hospital, Ministry of Health and Welfare, No. 291 Zhongzheng Rd, Zhonghe Dist, New Taipei City 235, Taiwan (csc901515@gmail.com).

Author Affiliations: Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospital, Taoyuan City, Taiwan (Y.-Y. Huang); Department of Medical Nutritional Therapy, Chang Gung Memorial Hospital, Taoyuan City, Taiwan (Y.-Y. Huang); Oneness Biotech Company Limited, Taipei, Taiwan (C.-W. Lin); Division of Plastic Surgery, Department of Surgery, National Taiwan University Hospital and College of Medicine, Taipei, Taiwan (Cheng); Limb Preservation Platform Inc, Fresno, California (Cazzell); Plastic and Reconstruction Surgery, China Medical University Hospital, Taichung, Taiwan (Chen); Plastic Surgery Center,

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Chi-Mei Medical Center, Tainan, Taiwan (K.-F. Huang); Department of Surgery, Mackay Memorial Hospital, Taipei, Taiwan (Tung); Division of Cardiology, Buddhist Tzu Chi General Hospital, Taipei, Taiwan (H.-L. Huang); Department of Plastic and Reconstructive Surgery, Chang Gung Memorial Hospital, Kaohsiung, Taiwan (P.-Y. Lin); Division of Plastic and Reconstructive Surgery, Taipei Veterans General Hospital, Taipei, Taiwan (Perng); Department of Surgery, National Yang Ming Chiao Tung University School of Medicine, Taipei, Taiwan (Perng); Department of Endocrinology, The First Affiliated Hospital of Soochow University, Jiangsu, China (Shi); Department of Burn and Plastic Surgery, Affiliated Hospital of Jiangsu University, Jiangsu, China (Liu); Department of Endocrinology, The First Affiliated Hospital of Henan Science and Technology University, Henan, China (Ma); Department of Vascular Anomalies, Shanghai TCM (Traditional Chinese Medicine)–Integrated Hospital, Shanghai, China (Cao); Department of Endocrinology, The First Affiliated Hospital of Sun Yat-sen University, Guangdong, China (Y. Li); Department of Endocrinology, Nanfang Hospital of Southern Medical University, Guangdong, China (Xue); Department of Endocrinology, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangdong, China (Yan); Department of Endocrinology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Shandong, China (Q. Li); Shanghai National Clinical Research Center for Endocrine and Metabolic Diseases, Shanghai, China (Ning); Key Laboratory for Endocrine and Metabolic Diseases of the National Health Commission of the People's Republic of China, Shanghai, China (Ning); Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai, China (Ning); Shanghai Jiao Tong University School of Medicine, Shanghai, China (Ning); Division of Plastic Surgery, Department of Surgery, Integrated Burn and Wound Care Center, Taipei Medical University-Shuang Ho Hospital, Ministry of Health and Welfare, New Taipei City, Taiwan (Chang); Division of Plastic Surgery, Department of Surgery, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan (Chang).

Author Contributions: Drs Y.-Y. Huang and Chang had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Chen, H.-L. Huang, P.-Y. Lin, Chang.

Acquisition, analysis, or interpretation of data: Y.-Y. Huang, C.-W. Lin, Cheng, Cazzell, K.-F. Huang, Tung, H.-L. Huang, Perng, Shi, Liu, Ma, Cao, Y. Li, Xue, Yan, Q. Li, Ning.

Drafting of the manuscript: C.-W. Lin, Chen, P.-Y. Lin, Perng, Liu.

Critical revision of the manuscript for important intellectual content: Y.-Y. Huang, Cheng, Cazzell, K.-F. Huang, Tung, H.-L. Huang, Shi, Ma, Cao, Y. Li, Xue, Yan, Q. Li, Ning, Chang.

Statistical analysis: C.-W. Lin.

Administrative, technical, or material support: Y.-Y. Huang, Cheng, Chen, K.-F. Huang, Tung, P.-Y. Lin, Shi, Liu, Ma, Cao, Y. Li, Xue, Yan, Q. Li, Ning, Chang.

Supervision: Chang.

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SUPPLEMENT 1.

Trial Protocol

SUPPLEMENT 2.

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SUPPLEMENT 3.

Data Sharing Statement

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