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Multicenter, randomized controlled, observer-blinded study of a nitric oxide generating treatment in foot ulcers of patients with diabetes—ProNOx1 study

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ABSTRACT

The aim of this multicenter, prospective, observer-blinded, parallel group, randomized controlled trial was to assess the safety and efficacy of EDX110, a nitric oxide generating medical device, in the treatment of diabetic foot ulcers in a patient group reflecting “real world” clinical practice compared against optimal standard care. Participants were recruited from ten hospital sites in multidisciplinary foot ulcer clinics. The ulcers were full thickness, with an area of 25–2,500 mm² and either a palpable pedal pulse or ankle brachial pressure index > 0.5. Infected ulcers were included. Treatment lasted 12 weeks, or until healed, with a 12-week follow-up period. Both arms were given optimal debridement, offloading and antimicrobial treatment, the only difference being the fixed used of EDX110 as the wound dressing in the EDX110 group. 135 participants were recruited with 148 ulcers (EDX110—75; Control—73), 30% of which were clinically infected at baseline. EDX110 achieved its primary endpoint by attaining a median Percentage Area Reduction of 88.6% compared to 46.9% for the control group ($p = 0.016$) at 12 weeks in the intention-to-treat population. There was no significant difference between wound size reduction achieved by EDX110 after 4 weeks and the wound size reduction achieved in the control group after 12 weeks. EDX110 was well tolerated. Thirty serious adverse events were reported (12 in the EDX110 group, of which 4 were related to the ulcer; 18 in the control group, of which 10 were related and 1 possibly related to the ulcer), with significant reduction in serious adverse events related

AATCC	American Association of Textile Chemists and Colorists
ABPI	Ankle brachial pressure index
AE	Adverse event
CRF	Case report forms
DFU	Diabetic foot ulcer
FDA	US Food and Drug Administration
IQR	Interquartile range
ITT	Intention to treat
NO	Nitric oxide
PAR	Percentage area reduction from baseline

PHMB	Polyhexamethylene biguanide
PI	Principal Investigator
PP	Per protocol
RCT	Randomized controlled trial
SAE	Serious adverse event
SD	Standard deviation
SOC	Standard of care
UK	United Kingdom
US	United States
VAS	Visual analog score
VRS	Verbal rating score

to the ulcer in EDX group. There was no significant difference in adverse events. This study, in a real world clinical foot ulcer population, demonstrates the ability of EDX110 to improve healing, as measured by significantly reducing the ulcer area, compared to current best clinical practice.

Patients with diabetes have a lifetime incidence of up to 25% for developing diabetic foot ulcers (DFUs).¹ These notoriously hard-to-heal ulcers are associated with poor clinical outcomes and a long-term impact on morbidity, mortality, and quality of life. DFUs account for almost 50% of diabetes-specific hospital admissions and precede more than 80% of amputations in people with diabetes,^{2,3} the rate of which is 20 times that of people without diabetes. The latest figures show that, for 2014–15, the annual cost to the National Health Service in England for DFUs was between US\$1.31bn and \$1.53bn.⁴

Prior to this study, the lack of an evidence base for advanced wound dressings to manage DFUs was widely acknowledged.^{5,6} Each of the available guidelines for the treatment of DFU recommends debridement, pressure off-loading, and an “appropriate” dressing,^{5,7} and it is commonly stated there is little difference in efficacy between the many dressings in current use and a lack of robust data.^{3,5–8} Of note, some RCTs, although useful in establishing efficacy under ideal circumstances for a variety of wound-healing interventions, do not provide the necessary information to establish effectiveness in the “usual” compromised wound center patient.⁹

The critical factors determining nonhealing status of DFUs are ischemia and/or infection.¹⁰ Nitric oxide (NO) plays a crucial role in maintaining the microvascular supply and infection control in the skin, and its absence in diabetes is a compounding factor in poor ulcer healing.^{11,12} The role of NO in ulcer healing involves three recognized elements: vascular, as NO influences blood vessel vasodilatation and stimulates angiogenesis;^{13,14} inflammatory, as NO influences the host immune response;¹⁵ and antimicrobial as NO demonstrates potent, broad spectrum antimicrobial activity.¹⁶ EDX110 (Edixomed, London, UK) is a two-layer system designed to generate NO in situ. EDX110 provides a moist wound environment, absorbs exudate, triggers autolytic debridement and, when the two layers are placed in contact and applied to the wound, nitric oxide (NO) is generated as an ancillary function.

In addition, the antimicrobial action of EDX110 has been assessed in the laboratory via the AATCC Test Method 100, required by the FDA to claim antimicrobial activity, where it demonstrated microbicidal action against strains of gram-positive and gram-negative bacteria, mold and yeast.¹⁷ EDX110 has also demonstrated significant activity against biofilms.¹⁸ EDX110 prevented biofilms from forming and was bactericidal to established biofilms, including gram-positive and gram-negative bacteria, to multidrug resistant strains and polymicrobial biofilms.

The aim of this study, ProNOx1, was to assess the safety and efficacy of EDX110, a Class III medical device, compared to standard of care (SOC) dressings—the control group—in the treatment of diabetic foot ulcers with a wide inclusion criteria to reflect the population in which

EDX110 would inevitably be used if the study was successful.

METHODS

Study design

The ProNOx1 study was a multicenter, prospective, observer-blinded, parallel group, randomized controlled trial of a NO-generating wound treatment versus current standard of care dressings in DFUs. Patients were recruited at ten established expert multidisciplinary clinics for the management of diabetic foot ulcers.

The study was approved by the National Research Ethics Committee and the Medicines and Healthcare Products Regulatory Agency in the UK.

Participants were aged 18 or over, with type 1 or type 2 diabetes and a chronic, (present for at least 6 weeks) full-thickness foot ulcer (on or below the malleoli) not penetrating to tendon, periosteum, or bone, and with a cross-sectional area between 25 and 2,500 mm². Participants were required to have either a palpable pedal pulse, or an ankle-brachial pressure index (ABPI) of >0.5. Notably, participants with ulcer infection were not excluded.

There was one relevant in-study substantial protocol amendment: inclusion was amended to allow entry to participants with ulcers >14 days duration reduced from 6 weeks to improve recruitment in February 2014. Duration of >14 days was chosen as a reasonable indicator of the chronicity of ulceration (8 ulcers of less than 4 weeks duration were included in the EDX110 group and 6 in the control group).

Procedures

All participants received the normal DFU management procedures for each study site, with debridement, infection management including antibiotics when indicated, and off-loading as clinically appropriate across both study arms. Following randomization, dressings were first applied to both study groups at the clinical centers. Participants were shown how to change and apply new dressings and subsequently, for both groups, dressing changes were primarily undertaken in the home.

The EDX110 group used the EDX110 2-layer, NO-generating wound treatment. The primary layer of EDX110 is a moist mesh which is placed centrally over the ulcer. The secondary layer is then applied on top of the mesh, positioned so there is a similar sized border surrounding the primary layer, which retains the primary layer in place, see Figure 1. Participants were directed to change EDX110 every 48 hours.

The investigators were instructed to treat the control group with whichever available dressing they considered best clinical practice for their participants at that time. These participants were directed to change the dressing as specifically

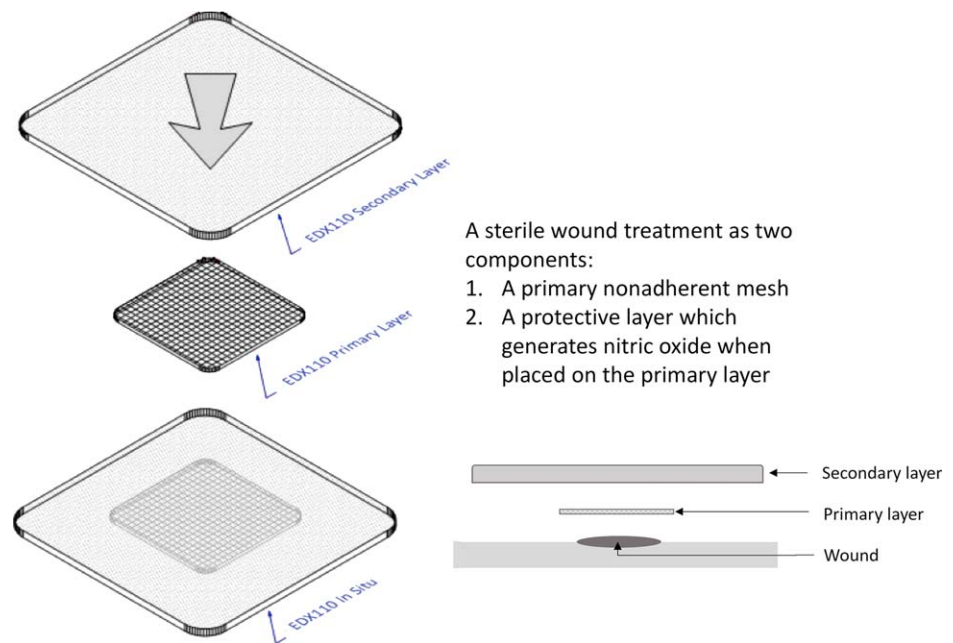


Figure 1. EDX110. The image shows the 2 layers. Once the layers are placed together on the wound the system generates nitric oxide. [Color figure can be viewed at wileyonlinelibrary.com]

indicated for the dressing being used. The investigators could alter the dressing type as clinically required, including using antimicrobial dressings as appropriate.

The duration of treatment was 12 weeks, with clinical visits weekly for the first four weeks, and every 2 weeks thereafter. Between study visits, dressings were changed either by participants, their carers or by community health-care professionals. A follow-up visit occurred for participants either at 12 weeks posthealing, or at week 24, whichever came first. Ulcer recurrence was recorded if a “new” ulcer was present at the same site as the original.

At each clinical visit, images of the ulcers were recorded using the Silhouette system (Aranz Medical, Christchurch, New Zealand). The Silhouette system is a proven wound imaging, 3D measurement, and documentation system for clinical practice and clinical research allowing accurate quantitative data collection.¹⁹

With the Silhouette system it is possible to:

1. calculate accurate wound surface area, depth, perimeter and volume
2. combine imaging and measurement with assessment information to generate comprehensive reports
3. immediately store wound information in a secure environment

Critically, point 3 enabled every image of the study ulcers to be assessed and measured remotely by one wound care expert. This enabled completely blind assessment of the ulcer progression and removed any subjectivity providing a completely unbiased measure of the outcome.

Outcomes

The co-primary outcomes were the efficacy of EDX110 as assessed by the percentage ulcer area reduction from

baseline (PAR) compared with the control group and the safety and tolerability of EDX110. PAR at 12 weeks was the primary efficacy measure. The percentage of ulcers healed at 12 weeks and ulcer recurrence were secondary outcomes.

PAR was measured by the blinded assessor using the Silhouette system.¹⁹ Final healing status was determined by the center investigators, with healing defined as complete epithelialization of the ulcer, without drainage, and the blinded assessor confirmed complete epithelialization using the Silhouette system. There were eight missing healed ulcer images where only the PI reported the healed ulcer. Infection was diagnosed by the investigator based on the presence of two classical signs of infection, including purulent discharge, erythema, pain, and swelling, as per standard clinical practice.²⁰

Serious adverse events (SAEs) and adverse events (AEs) were defined in the study protocol and complied with good clinical practice. Each event was classified by the local principal investigator for relatedness to the index ulcer and/or dressing.

For the EDX110 group only, tolerability (at change of EDX110 and with EDX110 in situ) was recorded at each visit. At change, a validated verbal rating score (VRS) was used, ranging from 1 to 6, where 1 = comfortable/pleasant and 6 = severe discomfort. For tolerability in situ, a visual analog scale (VAS) ranging from 1 to 10, where 1 = no sensation and 10 = severe discomfort, was utilized.

Statistical analysis

Following power calculations with an 80% power to detect a 25–30% difference in healing between the 2 randomly assigned groups with 1:1 distribution, with a 20% attrition rate, based on a current standard of care healing rate of 25%,^{8,21} a study size of 60 participants in each arm was

chosen. The test statistic used was the two-sided z test with pooled variance and a target significance level of 0.05. PAR was the intended primary efficacy measure as it can be measured by a blinded observer. It would have been preferable to use PAR for the power calculation. However, the most robust data available for standard of care from Jeffcoate et al in 2009,⁸ only reported complete healing and this was used for the power calculation.

The study was open-label, and treatment allocation followed a computer generated, center-stratified randomization list in blocks of four—the centers were unaware of the randomization strategy. Participants were randomized 1:1 to the EDX110 group or the control group and assigned to their respective groups by the principal investigator at each center. The Silhouette Wound Imaging System was used to capture images of the ulcer, from which an observer blinded assessment was conducted of all images by an independent wound care expert.

For the efficacy endpoint, unadjusted analysis of PAR differences between the groups was conducted using Mann-Whitney U tests to account for nonnormal distribution of PAR. The rates of complete healing were compared using chi-square tests. A post hoc linear mixed effect model was also used to investigate the effects of different variables as random factors including clustering at the participant level, clustering at the site level, and ulcer area, ulcer age and infection status at randomization as fixed effects. The dependent variable was PAR at 12 weeks and 4 weeks. Separate models were conducted for outcomes at 4 and 12 weeks.

Data were analyzed by intention-to-treat (ITT) population, and per protocol (PP) population. Safety included analysis of all adverse outcomes in all participants and ulcers. The ITT population comprised all ulcers which received at least one treatment. Participants were excluded from the PP population if they withdrew consent or were withdrawn by the principal investigator or were withdrawn due to an unrelated AE/SAE, or experienced a serious protocol deviation unless they completed at least 10 weeks of treatment. Any participant with a healed ulcer or withdrawn for an ulcer-related AE/SAE remained in the PP population as these were critical outcomes.

An independent statistical group conducted analysis. Hypothesis testing was performed for selected primary and secondary endpoints. Unless specified, all statistical testing was two-sided and performed using a significance (α) level of 0.05. For continuous variables with approximately normal distribution, means and standard deviations (SD) were the preferred reporting metric while for nonnormal variables, medians and interquartile ranges (IQR) were the preferred reporting metric, particularly PAR due to its non normal distribution, but means and SDs are also included for completeness in the case of ulcer age, ulcer area and PAR. Missing data were imputed for the ITT population using the last observation carried forward principle.

An explorative analysis compared the full ITT results to a cohort where only the first randomized ulcer was included for analysis if a participant's second or subsequent randomized ulcer was being treated within the same cohort.

An adjustment for multiplicity of statistical testing was made using a complex gatekeeping strategy for the ITT analysis and the step-up Hochberg procedure for the PP

analysis for all reported p -values, with the reported p -values being the adjusted values, except where otherwise explicitly stated.²²

For the safety and tolerability endpoints, AEs and SAEs were analyzed using a chi-square test.

RESULTS

Study demographics

From October 2013, to July 2015, 217 ulcers were screened of which 147 were randomized (Figure 2). Seventy participants were excluded for either not meeting the ulcer inclusion criteria ($n = 45$) or declining to participate ($n = 15$), or other reasons ($n = 10$).

Three participants received no treatment post randomization, having withdrawn between consent and baseline and were removed from the ITT population.

There were 135 participants receiving treatment in the study and 123 of these were randomized with one ulcer only. Three participants also had an ulcer on their contralateral foot and were reentered into the study at a later date. One participant had two ulcers on the same foot treated with separate dressings. In four participants with simultaneous ulcers on both feet, the ulcer on the right was first randomized and then the ulcer on the left was automatically assigned to the other study group. In all the above cases, separate identification codes and CRFs were assigned to each DFU.

In addition, four participants were included with two ulcers treated under the same dressing labelled as ulcer A and B in the same CRF. In one of these cases the participant was reentered into the study at a later date with an ulcer on the contralateral foot, resulting in this participant having three ulcers in the study being documented in two separate CRFs.

In all, 63 participants with 71 ulcers were treated with EDX110 only, 68 participants with 69 ulcers were treated only with standard of care dressings and 4 participants had 8 bilateral ulcers, one of which was treated with EDX110 and the other treated with standard of care dressings.

In summary, 123 participants had one ulcer, 11/135 participants each had two ulcers in the study, 1/135 participants had three ulcers in the study. There was a total of 144 CRFs (EDX110-72; control-72) reporting 148 ulcers (EDX110-75; control-73) in the study. As all 148 ulcers received at least one treatment during the study, they were all included in the ITT population and safety analyses.

The PP population comprised 114 participants with a total of 124 ulcers—61 EDX110; 63 control (Figure 2).

There were no significant differences between the study groups with each having an expected higher proportion of male to female participants (Table 1). In the EDX110 group, 89% of ulcers and in the control group, 92% of ulcers had been present for at least one month. Thirty-eight different participant comorbidities were recorded, with no significant differences between the groups although of note, there were 17 (23%) prior amputations in the EDX110 group, (6 major, 11 minor) and 10 (14%) in the control group, (4 major, 6 minor).

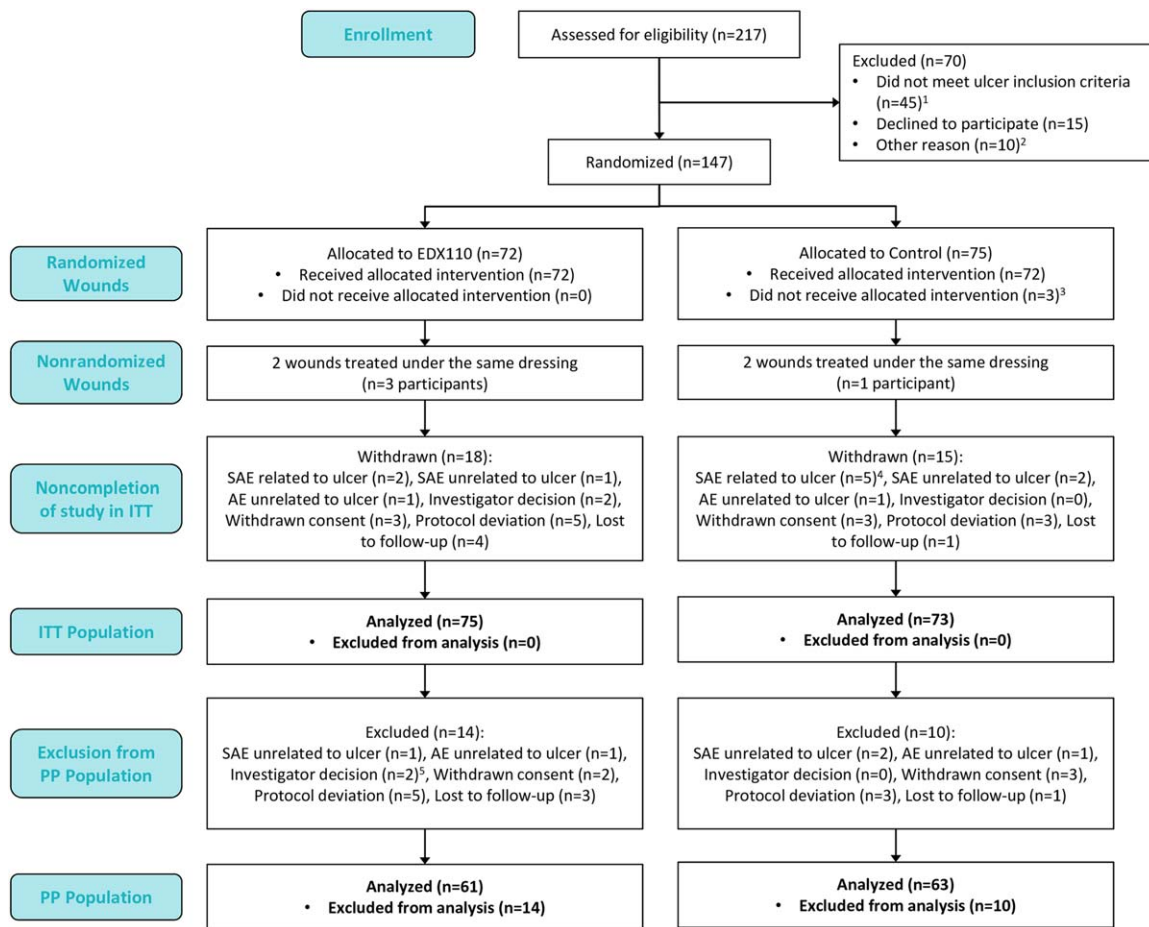


Figure 2. Disposition of ulcers in the study. (1) Too small n=15, healed/healing n=18, vascular n=4, probes to bone n=2, fistula n=1, gangrene n=1, in plaster n=1, not suitable (undefined) n=1, undefined n=2. (2) Not diabetic n=3, enrolled in other study n=2, blind n=2, dementia n=1, unwell at enrollment n=1, language n=1. (3) 3 pts were randomized and allocated to the control group. However, they were withdrawn prior to treatment due to failure to meet the inclusion criteria. (4) Includes one death. (5) PI withdrew one patient to use nonremovable total contact cast. PI withdrew one patient due to ongoing mental health issues EDX, EDX110; ITT, intention-to-treat; PP, per protocol; AE, adverse event; SAE, serious adverse event. [Color figure can be viewed at wileyonlinelibrary.com]

Efficacy analysis

There was a significant improvement in the median PAR at 12 weeks in the EDX110 group compared to control in both the ITT ($p = 0.016$) and PP ($p = 0.012$) populations (Table 2). The progression of median PAR is shown in Figure 3, indicating no significant difference in the PAR achieved by EDX110 in 4 weeks compared to that achieved by control in 12 weeks. There was also a significant improvement ($p = 0.036$) in the median PAR at 4 weeks in the EDX110 compared to control in the PP population (Table 2). A post hoc linear mixed model analysis of the full, 148 wound ITT population at 4 weeks indicated a significant difference in mean PAR between groups of 17.3 in favor of EDX110 ($p = 0.031$) and, importantly, showed that no other fixed effect variables or random effect variables generated a significant difference in outcome in the model.

In the ITT analysis, the proportion of ulcers healed at 12 weeks with EDX110 was 30/75 (40%) and in the

control group was 19/73 (26%) ($p = 0.07$). However, following a strict definition of ITT, including the 3 patients randomized but not treated, to give a control population of 76, the difference in complete healing is significant, $p = 0.049$. The complete healed rate at 12 weeks in the PP analysis was significant, with 49% for the EDX110 group (30/61) compared to 30% for the control group (19/63) ($p = 0.04$).

The percentage of DFUs demonstrating $\geq 50\%$ ulcer area reduction at 4 weeks in the ITT population, an important measure for clinicians,^{23,24} showed a significant difference in favor of EDX110 (53% vs. 36%; $p = 0.03$).

The median PAR at 12 weeks for DFUs present for ≤ 6 months, was significantly greater for EDX110 ($n = 51$; PAR = 97%) compared with the control group ($n = 44$; PAR = 55%) ($p = 0.04$). For those ulcers present for greater than 6 months there was no significant difference between the groups. (Table 2)

Table 1. Baseline demographics of the intention-to-treat population

Parameter	EDX110	Control
Age (years)*	59.3 (SD 12.41)	59.0 (SD 11.85)
Male*	63 (87%)	59 (82%)
Female*	9 (13%)	13 (18%)
Palpable foot pulse*		
Yes	68 (94%)	62 (87%)
No	4 (6%)	9 (13%)
Blood pressure (mmHg)*		
Systolic	143.9 (21.11)	143.3 (21.89)
Diastolic	81.9 (13.13)	81.2 (15.45)
Blood glucose (mmol/L)*	9.9 (5.68)	10.2 (4.11)
ABPI*	1.26 (0.55)	1.24 (0.28)
Bypass graft	3 (4)	7 (10)
DFU age (weeks) [‡]		
Mean	28.9 (35.1)	41.1 (54.7)
Median	13.0 (33.1)	19.6 (44.1)
DFU age (weeks) [‡]		
>26	24 (32%)	28 (39%)
≤26	51 (68%)	44 (61%)
Initial area (cm ²) [†]		
Mean	2.10 (3.33)	1.58 (2.22)
Median	0.86 (1.89)	0.7 (1.26)
Area (cm ²)		
<0.25	8 (11)	12 (16)
≥0.25	67 (89)	61 (84)
Area (cm ²) [†]		
<1.0	43 (57)	41 (56)
≥1.0	32 (43)	32 (44)
Plantar ulcer [†]	47 (63)	44 (60)
Location [†]		
Toe	15 (20)	14 (19)
Foot	50 (67)	46 (63)
Heel/ankle	10 (13)	13 (18)
DFU offloaded	44 (58)	42 (58)
DFU infected [¶]	20 (30)	22 (34)

Parameters are specified as mean (SD; standard deviation) and as median (interquartile range for nonnormal data) and numbers (%) for categories.

*These data are presented for each case report form, $n = 144$; there were 9 participants in the EDX110 group and 10 participants in the control group with ABPI data.

[‡]These data are presented for DFUs, $n = 148$.

[†]Duration of ulcer data was missing for 1 control ulcer.

[¶]Infection data available for 66 DFUs in the EDX110 group and 64 DFUs in the control group.

There are no significant differences in baseline parameters. APBI, ankle pressure brachial index; DFU, diabetic foot ulcer.

The median PAR at 12 weeks for DFUs that were ≥ 1 cm² at baseline, was significantly greater for EDX110 ($n = 32$; PAR = 82%) compared with the control group ($n = 32$; PAR = 29%) ($p = 0.007$). For those ulcers < 1 cm² at baseline, there was no significant difference between the groups. (Table 2)

The percentage of healed ulcers in the EDX110 group for those ulcers classified as infected at baseline was 45% (9/20) compared to 23% (5/22) in the control group ($p = 0.20$). In those ulcers classified as noninfected at baseline, EDX110 achieved complete healing of 38% (21/55) vs. 27% (14/51) in the control group ($p = 0.24$).

The explorative analysis, including only a participant's first randomized ulcer if their second or subsequent ulcer was treated with the same dressing, showed negligible difference from the ITT population. The mean PAR for EDX110 and control group remained the same as the ITT population at 59% and 37%, respectively. The complete healing rate also remained the same as for the full ITT ulcer population, EDX110—40% vs. control group—26%.

Ulcer recurrence at 12 weeks post healing was 1/23 (4%) in EDX110 and 4/16 (25%) in the control group, which did not reach statistical significance.

Safety analysis

Thirty SAEs were recorded (EDX110—12 SAEs in 12 participants; control group—18 SAEs in 15 participants) of which 14 were considered related and one possibly related to the study ulcer, (EDX110—4; control group—11) but no SAE was reported as related to EDX110 or the SOC dressings. The fewer occurrences of SAEs related or possibly related to the ulcer in the EDX group compared with the SOC group was significant ($p = 0.049$). There was no significant difference in the overall number of SAEs between the EDX110 and control groups. The SAEs included two major amputations, and one death in hospital from septicemia and pneumonia following osteomyelitis of the index ulcer, all in the SOC group.

There was no significant difference in AE occurrence between the study groups (EDX110—49 AEs in 32 participants; control group—54 AEs in 34 participants). The most common AE was foot infection: EDX110—17 (35%); control group: 19 (35%). Among the foot infections in the EDX110 group reported as AEs, 12 were related to the index ulcer, four (33%) of which became serious adverse events. In the control group, 14 foot infections reported as AEs were related to the index ulcer, 9 (64%) of which became serious adverse events.

EDX110 was well tolerated with a mean score of 1.7 (SD: 0.73) on a VRS at dressing change and 1.6 (SD: 1.01) on a VAS when in situ.

The median number of dressing changes for the EDX110 group per week was 3.5; (IQR: 1.8), as per instructions. The median number of dressing changes for the control group per week was 1.6; (IQR: 1.3).

Nine different types of dressing were recorded in the control group—absorbent pad, alginate, anti-microbial, foam, gauze, gel fiber, hydro-colloid, hydrofiber or hydro-gel and 32% of the dressings used were classified as anti-microbial, namely iodine, honey, polyhexamethylene biguanide (PHMB) or hydrophobic (Sor bact).

Table 2. Percentage ulcer area reductions in specific ulcer populations

	Mean (SD)		Median (IQR)		<i>p</i>
	EDX110	Control	EDX110	Control	
PAR at 12 weeks					
ITT	<i>n</i> = 75 58.7 (59.20)	<i>n</i> = 73 37.0 (80.58)	<i>n</i> = 75 88.6 (73.7)	<i>n</i> = 73 46.9 (100)	0.016*
PP	<i>n</i> = 61 71.0 (50.39)	<i>n</i> = 63 38.6 (85.66)	<i>n</i> = 61 98.5 (36.9)	<i>n</i> = 63 52.1 (98.5)	0.012*
PAR at 4 weeks					
ITT	<i>n</i> = 75 45.4 (45.96)	<i>n</i> = 73 31.7 (47.73)	<i>n</i> = 75 53.7 (60.5)	<i>n</i> = 73 34.4 (61.1)	0.097*
PP	<i>n</i> = 61 55.4 (37.89)	<i>n</i> = 63 32.9 (49.82)	<i>n</i> = 61 55.9 (53.7)	<i>n</i> = 63 36.3 (58.7)	0.036*
PAR related to ulcer duration at baseline (ITT ulcer population) [†]					
≤ 6 months	<i>n</i> = 51 67.1 (56.8) [‡]	<i>n</i> = 44 37.8 (93.0) [¶]	<i>n</i> = 51 97.0 (37.4)	<i>n</i> = 44 55 (88.7)	0.04
> 6 months	<i>n</i> = 24 40.8 (61.4) [‡]	<i>n</i> = 28 42.8 (45.0) [¶]	<i>n</i> = 24 47 (87)	<i>n</i> = 28 46 (84)	0.80
PAR related to ulcer area at baseline (ITT ulcer population)					
≥ 1 cm ²	<i>n</i> = 32 59.4 (45.5)	<i>n</i> = 32 29.3 (42.2)	<i>n</i> = 32 82.0 (77.6)	<i>n</i> = 32 29.2 (55.4)	0.007
< 1 cm ²	<i>n</i> = 43 58.2 (68.1)	<i>n</i> = 41 43.0 (101.1)	<i>n</i> = 43 100 (70.4)	<i>n</i> = 41 92.1 (87.7)	0.52

SD, standard deviation; IQR, interquartile range; ITT, intention-to-treat; PP, per protocol.

**p*-values were obtained by Mann-Whitney test adjusted for multiplicity of statistical testing (no * means not adjusted for multiplicity of testing).

[†]Duration of ulcer data was missing for 1 control ulcer.

[‡]There is a significant improvement in healing between those treated with EDX110 with ulcers of ≤ 6 months duration compared with those treated with EDX110 with ulcers of > 6 months duration (*p* = 0.015; Mann-Whitney).

[¶]There is no significant difference in healing of ulcers of ≤ 6 months duration treated in the control group compared with healing of those ulcers of > 6 months duration treated in the control group (*p* = 0.42; Mann-Whitney).

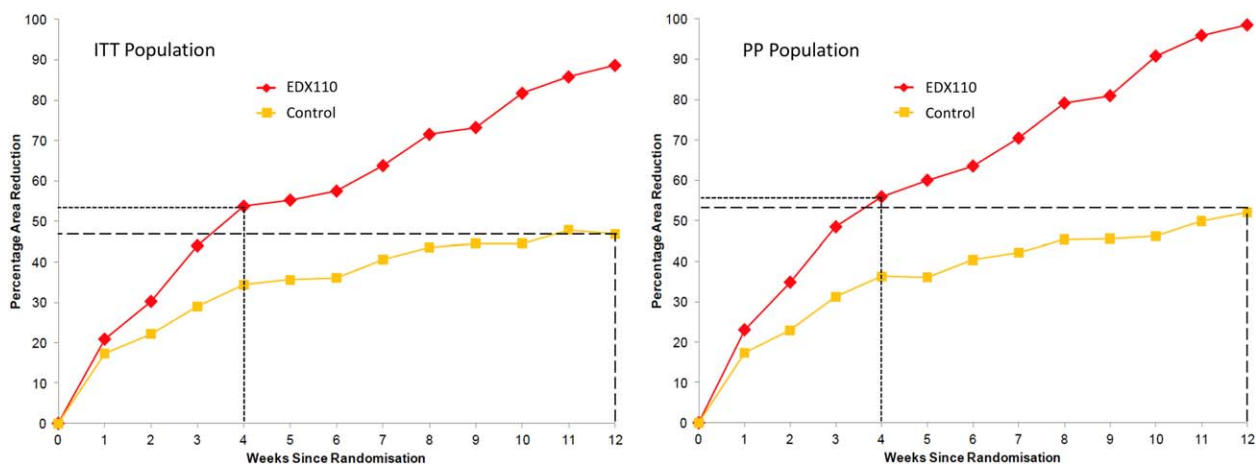


Figure 3. The progression of median PAR over the 12-week treatment for the ITT population and PP population indicating there is no significant difference between the PAR achieved by EDX110 in 4 weeks compared to the PAR achieved by SOC in 12 weeks. ITT, intention-to-treat; PP, per protocol. [Color figure can be viewed at wileyonlinelibrary.com]

No clinically meaningful difference was seen in offloading between the 2 arms; 58% of patients received offloading in each group and this corresponds with the plantar location of 63% of ulcers in the EDX group and 60% in the control group.

DISCUSSION

This multicenter, prospective, observer blinded, randomized controlled trial has shown that treatment of diabetic foot ulcers with EDX110 was safe and well tolerated. Furthermore, the PAR at 12 weeks was significantly improved in the EDX110 group compared with the control group. The percentage of DFUs demonstrating >50% PAR at 4 weeks additionally showed a significant difference in favor of EDX110. The rate of complete healing improved significantly in 12 weeks with EDX110 in the PP population and also in the ITT population, if the strictest definition of ITT had been followed. The ulcers treated with EDX110 were associated with a significantly smaller number of SAEs which were related or possibly related to the study ulcer compared to those treated with SOC dressings. ($p = 0.049$)

Thus, there may be a significant clinical benefit gained using EDX110 in a therapy area where recent overviews concluded that currently there is no robust evidence that any advanced dressing type is more effective than basic wound contact dressings.^{5,6,25}

In the Eurodiale prospective study on determinants of outcome in diabetic foot disease, the combination of infection with ischemia had a major negative impact on healing rates of DFUs.¹⁰ In this present real-life study, which allowed entry to participants with clinically infected ulcers and/or an ABPI as low as 0.5, the greater reduction in ulcer area may be attributed to the ability of EDX110 to generate a sustained release of NO that overcomes the issue of infection and ischemia. In many chronic ulcers, and especially in patients with diabetes, the generation of NO is lost or impaired.²⁶ Previous development of a functional NO wound treatment has been complicated by the very short half-life of the gas. EDX110 has overcome this issue with a sustained release of NO. This prolonged action of NO may be a major factor in the improved healing rate and makes EDX110 applicable to any chronic ulcer situation where ischemia and infection are factors in delayed healing. In the infected ulcers treated with EDX110, there was a doubling of complete healing compared to those treated with standard of care dressings. However, the number of infected ulcers was small and the number that healed approached, but did not reach, significance. Nevertheless, there are strong *in vitro* data to support the antimicrobial activity of NO as the skin's "natural antibiotic,"¹⁶ and EDX110 appears to be a useful treatment for infected ulcers.

The beneficial effect of EDX110 was particularly useful in the subgroup of ulcers ≥ 1 cm² at baseline and in the subgroup of ulcers of ≤ 6 months duration relative to the control group. Furthermore, within the EDX110 group itself, there was also a significant improvement in PAR of ulcers of ≤ 6 months duration compared to that of ulcers of > 6 months duration. This was not seen in the control group. Modern diabetic foot care encourages early presentation to specialist care, in keeping with advice and

findings from the UK National Diabetic Foot Audit.²⁷ Such a policy would facilitate the optimum healing effect of EDX110 when applied to ulcers of short duration.

This study may be seen to have certain limitations. Reduction in ulcer size was used as the primary outcome as opposed to the commonly utilized outcome of complete healing. Reduction in ulcer size was used for two scientifically legitimate reasons. Firstly, complete healing measured by the investigator is both subjective and nonblind, opening the outcome to bias. The use of an accurate, quantitative and reproducible measuring system allowed a nonsubjective, nonbiased measure of percentage reduction in ulcer size to be the primary outcome. Secondly, PAR is a clinically relevant outcome as it reflects the "healing" effect of standard of care dressings or EDX110 on the whole group and accounts for ulcers that are improving, static or, importantly, increasing in size, as well as those that heal. Therefore, an observer blinded outcome was the strongest alternative to remove bias and subjectivity. Also, the ProNOx1 study included all-comers and, therefore, contained a small number of participants with multiple ulcers which may be perceived as a limitation in that the inclusion of these participants may detract from the purity of the statistical analysis. However, a mixed model analysis, assessing the impact of variables, including the presence of multiple ulcers, showed that the only factor that significantly affected outcome was treatment arm and a direct comparison of the results showed that the inclusion of multiple ulcers had negligible effect.

A further possible limitation of the study was the choice of a nonstandardized dressing protocol in the control group with inevitable lack of blinding. There is no evidence that any specific dressing is superior in clinical practice and thus, investigators were allowed their optimal choice dependent on the ulcer condition at each visit. This was arguably a much tougher comparator for EDX110. True blinding would have required the use of a placebo dressing, which would not have offered optimal treatment to the control group. At best this would have been judged an unfair advantage for EDX110, at worst it would be unethical treatment of the participants. A further limitation with respect to dressings was that the mean number of dressing changes was greater in the EDX110 group. While this might be perceived as an advantage for EDX110, there is no evidence to suggest more frequent dressing changes improve healing, or it would be standard practice to change all dressings more frequently.

In the past decade, there has been growing concern that the results from many RCTs cannot be generalized to the wound care population.⁹ However, in the present trial, a "real world" wound care population was studied, including participants with moderate ischemia and ulcers complicated by infection, and EDX110 still met its primary endpoint and showed substantial promise. It was well tolerated, with significantly smaller numbers of SAEs which were related or possibly related to the study ulcer compared to those treated with SOC dressings. It also significantly improved the reduction of ulcer area compared with SOC dressings, notably in those with a duration of less than six months.

The trial was registered at ClinicalTrials.gov, number NCT01982565.

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AUTHOR CONTRIBUTIONS

MEE, AJMB, MJC, AJ, ATT, JES were responsible for study conception and protocol development.

MEE, HJB, PJC, CND, RD, BK, GL, SMR, TES, MJY, JES were involved in data acquisition and management.

MJC was responsible for data analysis.

MEE, MJC, AJMB were responsible for data interpretation and drafting the manuscript.

All authors reviewed and contributed to revisions of the manuscript.

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