Supplementary Online Content

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eMethods 1. Randomization Plan

eMethods 2. Procedure of Healing Judgment by Independent Evaluator

eTable 1. Institutional Review Board Approval Information of 21 Medical Centers

eTable 2. Efficacy and Safety Outcomes of the mITT Population

eTable 3. Hematology, Biochemistry, and Vital Sign Data at Visit 10 and Change From Baseline

eTable 4. Summary of Treatment-Emergent Adverse Events

eTable 5. Summary of Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term

eTable 6. Population Excluding Ulcers Reduced ≧10% During Screening

eFigure 1. ON101 Promotes Wound Healing Through Regulating M1 and M2 Macrophages

eFigure 2. Time to Healing in mITT

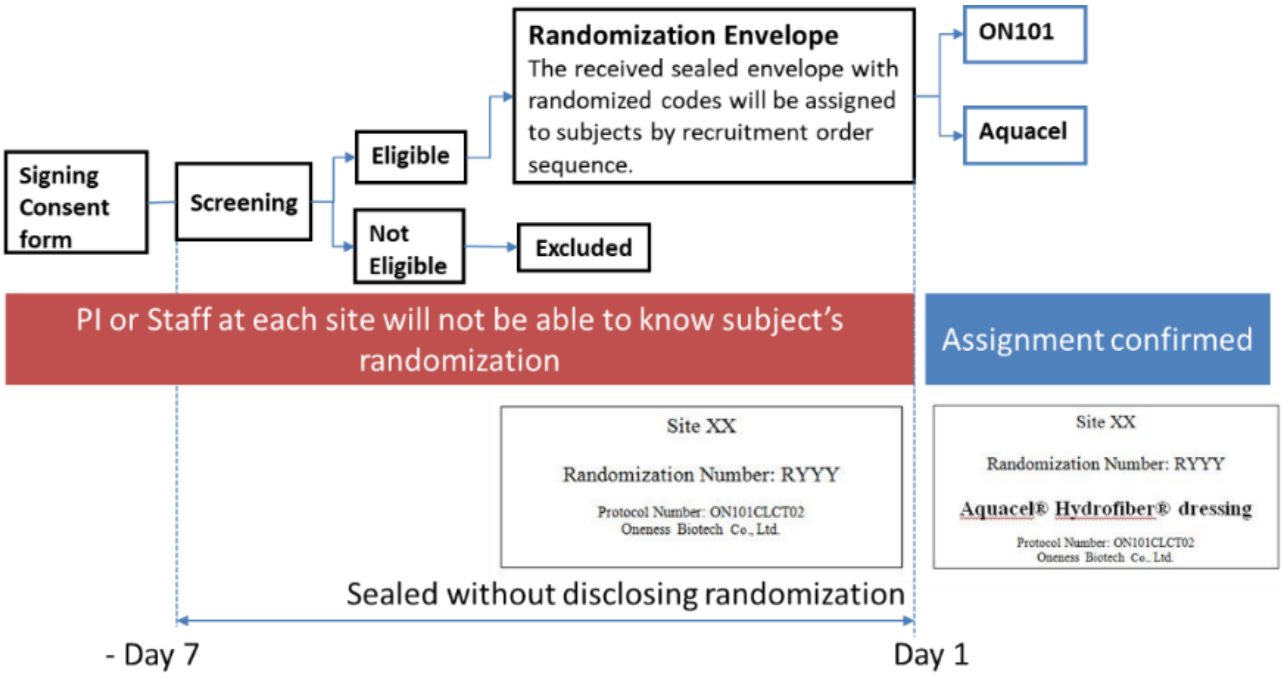
This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Randomization Plan

Subjects enrolled in this trial are to be randomized according to the following protocol.

1. Thirty random numbers are to be generated for each study site, with 15 generated for each of the two treatment groups.
2. Each randomization number has the format R001 to R030 that will be unique per site, but not unique across the study.
3. The package containing the sealed envelopes for each RCT study site will be mailed by post to a site representative before the site initiation visit.
4. Upon receipt of the sealed envelopes, the responsible site personnel will acknowledge delivery and confirm the envelopes were fully sealed upon receipt by signing a letter sent from an independent contract research organization which generates a randomization code.
5. The sealed randomization envelope will not disclose the group.
6. Each envelope will measure 2 1/2 inches by 4 1/4 inches, sealed with a label measuring 1 inch by 3 inches.
7. Once a subject is confirmed eligible, he/she will receive a sequential randomization number at baseline (Day 1) (i.e., the next eligible subject will always receive the lowest available randomization number).

- Flow Chart for Randomization:



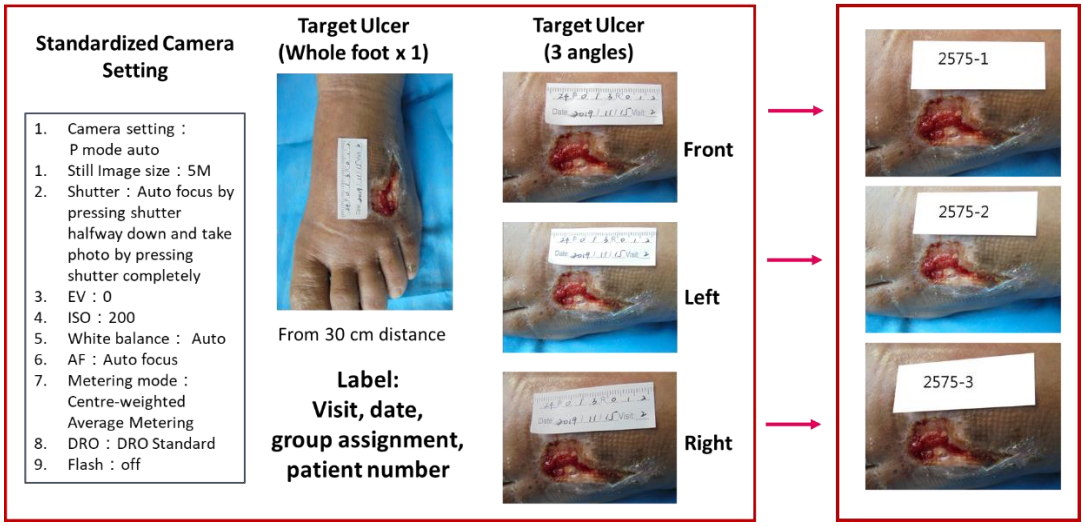
eMethods 2. Procedure of Healing Judgment **by Independent Evaluator**

1. Standardized Photographing Procedure

   Camera setting: mode, still image size, shutter timing, EV, ISO, white balance, AF, etc.

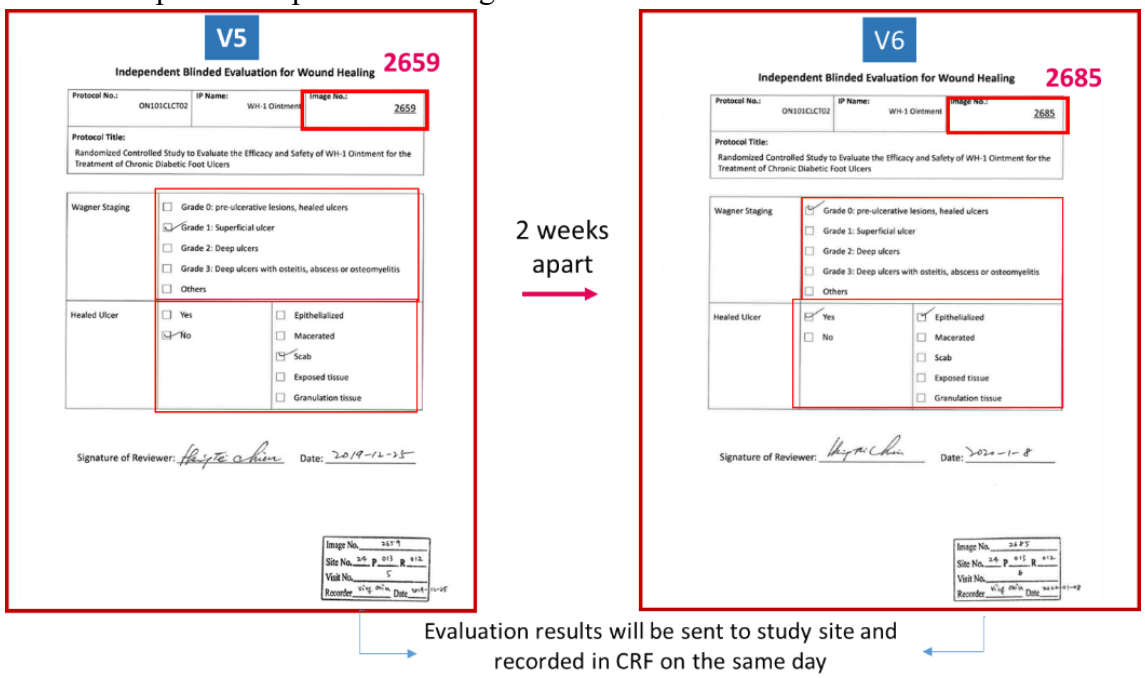
   Three images for the target ulcer (labeled with the patient no., site no., data of visit, and visit no.) will include one with the whole foot and three with a closer view from three angles.

2. Blinded Coding
   a. An independent staff member will assign codes to the images of each target ulcer by sequential order according to the time of receipt (new codes will replace the label on the target ulcer image so that the information of patient and site numbers, date of visit, and visit number will not be disclosed, to ensure blindness).
   b. A second independent staff member will verify the blinded coding in the encrypted database.
   c. New codes are entered into the Independent Blinded Evaluation for Wound Healing form for printing.
   d. A second independent staff member will deliver this evaluation form with digital images saved on a laptop to the independent blinded evaluator.
3. Target Ulcer Photographing and Sequential Coding by Blinded Procedure:
4. Examples of Images and Coding



5. Blinded Evaluation & Records - Blinded evaluation procedure
    a. The independent evaluator will judge the ulcer's healing status based on sequentially coded images and complete the evaluation form.
    b. A second independent staff member will retrieve the images and evaluation form. The independent evaluator will not keep any records.
    c. The second independent staff member will send the encrypted evaluation results to the study site.
    d. The staff of the study site will record the evaluation results by the independent evaluator in the case report form.
    e. Evaluation and recording need to be completed on the same day.
6. Examples of Blinded Evaluation

7. Example of one patient's coding number in different visits:



eTable 1. Institutional Review Board Approval Information of 21 Medical Centers

Site	Site Initial	Site Name	IRB#
01	CMUH	China Medical University Hospital, Taiwan	DMR100-IRB-224
02	NTUH	National Taiwan University Hospital, Taiwan	201209029MSA
03	MMH	Presbyterian Church in Taiwan Mackay Medical Foundation Mackay Memorial Hospital, Taiwan	12CT037A
04	TSGH	Tri-Services General Hospital, Taiwan	2-101-01-021
05	BTCGH	Buddhist Tzu Chi Medical Foundation Taipei Tzu Chi Hospital, Taiwan	01-FS05-061
06	CGMH-LK	Chang Gung Medical Foundation Linkou Change Gung Memorial Hospital, Taiwan	102-0020A
07	CMMC	Chi-Mei Medical Center, Taiwan	10207-006
08	CMUH-BG	China Medical University BeiGang Hospital, Taiwan	DMR100-IRB-224
09	MMH-TS	Presbyterian Church in Taiwan Mackay Medical Foundation Tamshui Mackay Memorial Hospital, Taiwan	12CT037A
10	CGMH-KH	Chang Gung Medical Foundation Kaohsiung Chang Gung Memorial Hospital, Taiwan	102-0020A
11	TPVGH	Taipei Veterans General Hospital, Taiwan	2016-04-006B
21	LPP	Limb Preservation Platform, Inc., US (LPP)	IRB tracking #20180770
22	RJH	Ruijin Hospital, Shanghai Jiaotong University School of Medicine, China (RJH)	(2018)伦审第(65)号
23	SDFH	The First Affiliated Hospital of Soochow University, China (SDFH)	(2017)伦审批第189 号
24	JSUH	Affiliated Hospital of Jiangsu University, China (JSUH)	(2018)伦审第(05)号
25	HNFH	The First Affiliated Hospital of Henan Science & Technology University, China (HNFH)	2018-007
26	STCMIH	Shanghai TCM-Interated Hospital, China (STCMIH)	2018-018-1
27	ZSFH	The First Affiliated Hospital, Sun Yat-sen University, China (ZSFH)	2018-058-01
28	NFH	Nanfang Hospital of Southern Medical University, China (NFH)	NFEC-2018-132
29	SYSH	Sun Yat-sen Memorial Hospital, Sun Yat-sen University, China (SYSH)	2019-YW-026
30	SDPH	Shandong Provincial Hospital, China (SDPH)	(2019)伦审第(38)号

eTable 2. Efficacy and Safety Outcomes of the mITT Population

	ON101 (n=118)	**Hydrocolloid dressing (n=112)**	**Significance**
Primary outcome (mITT)			
Percentage of complete healing (n) (%)	73 (61.9%)	38 (33.9%)	OR (95% CI) 3.15 (1.82-5.43); p<0.0001 †
Secondary outcomes (mITT)			
Healing time of 50th percentile of healed population, days (95% CI) ¶	98	NA	Hazard ratio (95% CI); p-value† 1.91 (1.29-2.83); p=0.001
Incidence of infection of the target ulcer	4 (3.39%)	7 (6.25%)	p=0.365*
Ulcer recurrence in the follow-up period#	15 (20.55%)	6 (15.79%)	p=0.617*

†Odds ratio and Hazard ratio with 95% CI and p-value using logistic regression model adjusted for treatment (fixed factor), baseline wound size in cm^2 and Wagner grade (covariates); ¶ Kaplan-Meier method; * Fisher's exact test.; # Ulcer recurrence was recorded once ulcer was completely healed and was observed during the follow-up period; NA: Not analysable, because till the end of treatment period the number of healed ulcers did not reach to 50% of total number in group.

eTable 3. Hematology, Biochemistry, and Vital Sign Data at Visit 10 and Change From Baseline*

Parameter	Status	ON101 Cream N = 122	Hydrofiber dressing N = 114	P-value
Hemoglobin (g/dL)	Mean (STD)	13.0(1.8)	12.6 (1.8)	0.148
	CFB, Mean (STD)	0.4 (1.2)	0.3 (1.3)	0.305
RBC (10^6/uL)	Mean (STD)	4.5 (0.7)	4.5 (0.8)	0.946
	CFB, Mean (STD)	0.1 (0.4)	0.1 (0.5)	0.930
Platelet (10^3/uL)	Mean (STD	242 (77.6)	259 (91.7)	0.126
	CFB, Mean (STD)	-14 (65.6)	2.5 (67.6)	0.048
WBC (10^3/uL)	Mean (STD	8.1 (3.0)	8.2 (2.5)	0.809
	CFB, Mean (STD)	0.4 (2.7)	0.7 (2.0)	0.402
Basophil (%)	Mean (STD	0.5 (0.4)	0.5 (0.4)	0.851
	CFB, Mean (STD)	-0.0 (0.5)	-0.0 (0.3)	0.943
Eosinophil (%)	Mean (STD	2.7 (1.7)	3.2 (2.3)	0.037
	CFB, Mean (STD)	-0.3 (2.2)	-0.5 (2.8)	0.196
Lymphocyte (%)	Mean (STD	27.6 (9.5)	28.6 (8.5)	0.425
	CFB, Mean (STD)	0.0 (7.9)	1.2 (7.5)	0.259
Monocyte (%)	Mean (STD	6.1 (1.8)	6.1 (1.8)	0.920
	CFB, Mean (STD)	-0.1 (1.8)	-0.3 (1.8)	0.451
Neutrophil (%)	Mean (STD	63.1 (10.3)	61.5 (9.8)	0.248
	CFB, Mean (STD)	0.4 (9.6)	-0.4 (8.3)	0.347
HbA1c (%)	Mean (STD	8.0 (1.8)	7.9 (1.6)	0.818
	CFB, Mean (STD)	-0.1 (1.6)	-0.2 (1.4)	0.580
Fasting glucose(mg/dL)	Mean (STD	159 (59.5)	156 (65.3)	0.768
	CFB, Mean (STD)	0.8 (64.9)	5.6 (75.4)	0.674
AST (IU/L)	Mean (STD	20.4 (9.4)	20.6 (7.6)	0.864
	CFB, Mean (STD)	-0.2 (9.6)	-0.2 (8.1)	0.907
ALT (IU/L)	Mean (STD	19.2 (10.3)	19.7 (10.8)	0.710
	CFB, Mean (STD)	-1.4(12.1)	0.9(10.0)	0.274
Albumin (g/dL)	Mean (STD	4.0(0.5)	4.1(0.5)	0.269
	CFB, Mean (STD)	0.0(0.4)	0.1(0.4)	0.225
Creatinine(mg/dL)	Mean (STD	1.1(0.5)	1.1(0.5)	0.815
	CFB, Mean (STD)	0.1(0.3)	0.1(0.2)	0.959
BUN (mg/dL)	Mean (STD	22.4(13.8)	20.9(8.8)	0.38
	CFB, Mean (STD)	1.9(12.5)	0.9(6.7)	0.392
Body weight (kg)	Mean (STD	74.0(16.7)	76.1(18.1)	0.360
	CFB, Mean (STD)	0.7(3.1)	0.3(3.1)	0.430
Systolic blood pressure (mmHg)	Mean (STD	134(18.4)	132(16.8)	0.302
	CFB, Mean (STD)	-0.6(19.3)	-1.1(18.5)	0.636
Diastolic blood pressure (mmHg)	Mean (STD	78.6(12.9)	76.2(10.4)	0.132
	CFB, Mean (STD)	-1.0(13.0)	-1.2(11.8)	0.313

V10, visit 10, end of treatment· *CFB, change from baseline, means the changed value at Visit 10 (end of treatment) from baseline (screening visit). P value was conducted by ANOVA

eTable 4. Summary of Treatment-Emergent Adverse Events*

System Organ Class	Preferred Term	ON101 Cream N = 122	Hydrocolloid dressing N = 114
		n (%) E	n (%) E
Patients with any AE		93 (76·2%) 368	90 (78·9%) 387
Blood and lymphatic system disorders	Total	2 (1·6%) 3	4 (3·5%) 4
	Anaemia	2 (1·6%) 3	3 (2·6%) 3
Eye disorders	Total	6 (4·9%) 8	8 (7·0%) 14
	Cataract	1 (0·8%) 1	3 (2·6%) 3
	Diabetic eye disease	3 (2·5%) 3	2 (1·8%) 2
Gastrointestinal disorders	Total	10 (8·2%) 11	4 (3·5%) 7
	Constipation	5 (4·1%) 5	0 (0·0%) 0
General disorders and administration site conditions	Total	9 (7·4%) 9	13 (11·4%) 14
	Peripheral Swelling	1 (0·8%) 1	4 (3·5%) 4
	Pyrexia	2 (1·6%) 2	3 (2·6%) 3
Infections and infestations	Total	32 (26·2%) 43	33 (28·9%) 44
	Abscess limb	4 (3·3%) 4	2 (1·8%) 2
	Cellulitis	8 (6·6%) 8	5 (4·4%) 5
	Infected skin ulcer	2 (1·6%) 2	3 (2·6%) 3
	Nasopharyngitis	3 (2·5%) 3	5 (4·4%) 5
	Upper respiratory tract infection	6 (4·9%) 7	7 (6·1%) 7
	Wound infection	0 (0·0%) 0	3 (2·6%) 3
Injury, poisoning and procedural complications	Total	11 (9·0%) 11	15 (13·2%) 17
	Wound	3 (2·5%) 3	2 (1·8%) 2
	Wound complication	1 (0·8%) 1	5 (4·4%) 6
Investigations	Total	3 (2·5%) 4	7 (6·1%) 11
	Glomerular filtration rate decrease	0 (0·0%) 0	4 (3·5%) 5
Metabolism and nutrition disorders	Total	13 (10·7%) 15	13 (11·4%) 13
	Gout	3 (2·5%) 4	3 (2·6%) 3
	Hyperlipidaemia	3 (2·5%) 3	2 (1·8%) 2
Renal and urinary disorders	Total	7 (5·7%) 9	10 (8·8%) 13
	Chronic kidney disease	1 (0·8%) 1	3 (2·6%) 3
	Diabetic nephropathy	2 (1·6%) 2	5 (4·4%) 5

System Organ Class	Preferred Term	ON101 Cream N = 122	Hydrocolloid dressing N = 114
		n (%) E	n (%) E
Respiratory, thoracic and mediastinal disorders	Total	3 (2·5%) 5	3 (2·6%) 6
Skin and subcutaneous tissue disorders	Total	29 (23·8%) 40	29 (25·4%) 44
	Blister	3 (2·5%) 3	2 (1·8%) 2
	Decubitus ulcer	3 (2·5%) 3	0 (0·0%) 0
	Dermatitis contact	1 (0·8%) 1	3 (2·6%) 3
	Diabetic foot	5 (4·1%) 5	5 (4·4%) 9
	Eczema	3 (2·5%) 3	4 (3·5%) 4
	Erythema	2 (1·6%) 2	3 (2·6%) 3
	Pruritus	3 (2·5%) 3	1 (0·9%) 1
	Skin ulcer	14 (11·5%) 16	12 (10·5%) 13
Vascular disorders	Total	8 (6·6%) 9	8 (7·0%) 9
	Hypertension	3 (2·5%) 3	6 (5·3%) 6
n: number of patients (percentage of patients), number of events,			

* All causes occurring in ≥ 2% in either group

eTable 5. Summary of Related Treatment-Emergent Adverse Events by System Organ Class and Preferred Term

	Preferred Term	ON101 Cream N = 122	Hydrocolloid dressing N = 114
		n (%) Events	n (%) Events
Any related TEAE		7 (5·7%) 11	5 (4·4%) 5
General disorders and administration site conditions	Peripheral swelling	1 (0·8%) 1	0 (0·0%) 0
	Pyrexia	0 (0·0%) 0	1 (0·9%) 1
Infections and infestations	Cellulitis	0 (0·0%) 0	1 (0·9%) 1
	Osteomyelitis	0 (0·0%) 0	1 (0·9%) 1
	Staphylococcal infection	1 (0·8%) 1	0 (0%) 0
Injury, poisoning and procedural complications	Wound complication	1 (0·8%) 1	0 (0·0%) 0
Investigations	Weight increased	1 (0·8%) 1	0 (0·0%) 0
Metabolism and nutrition disorders	Hyperuricaemia	2 (1·6%) 2	0 (0·0%) 0
Neoplasms benign, malignant and unspecified	Skin papilloma	0 (0·0%) 0	1 (0·9%) 1
Skin and subcutaneous tissue disorders	Dermatitis contact	1 (0·8%) 1	0 (0·0%) 0
	Diabetic foot infection	0 (0·0%) 0	1 (0·9%) 1
	Eczema	2 (1·6%) 2	0 (0·0%) 0
	Erythema	1 (0·8%) 1	0 (0·0%) 0
	Rash	1 (0·8%) 1	0 (0·0%) 0

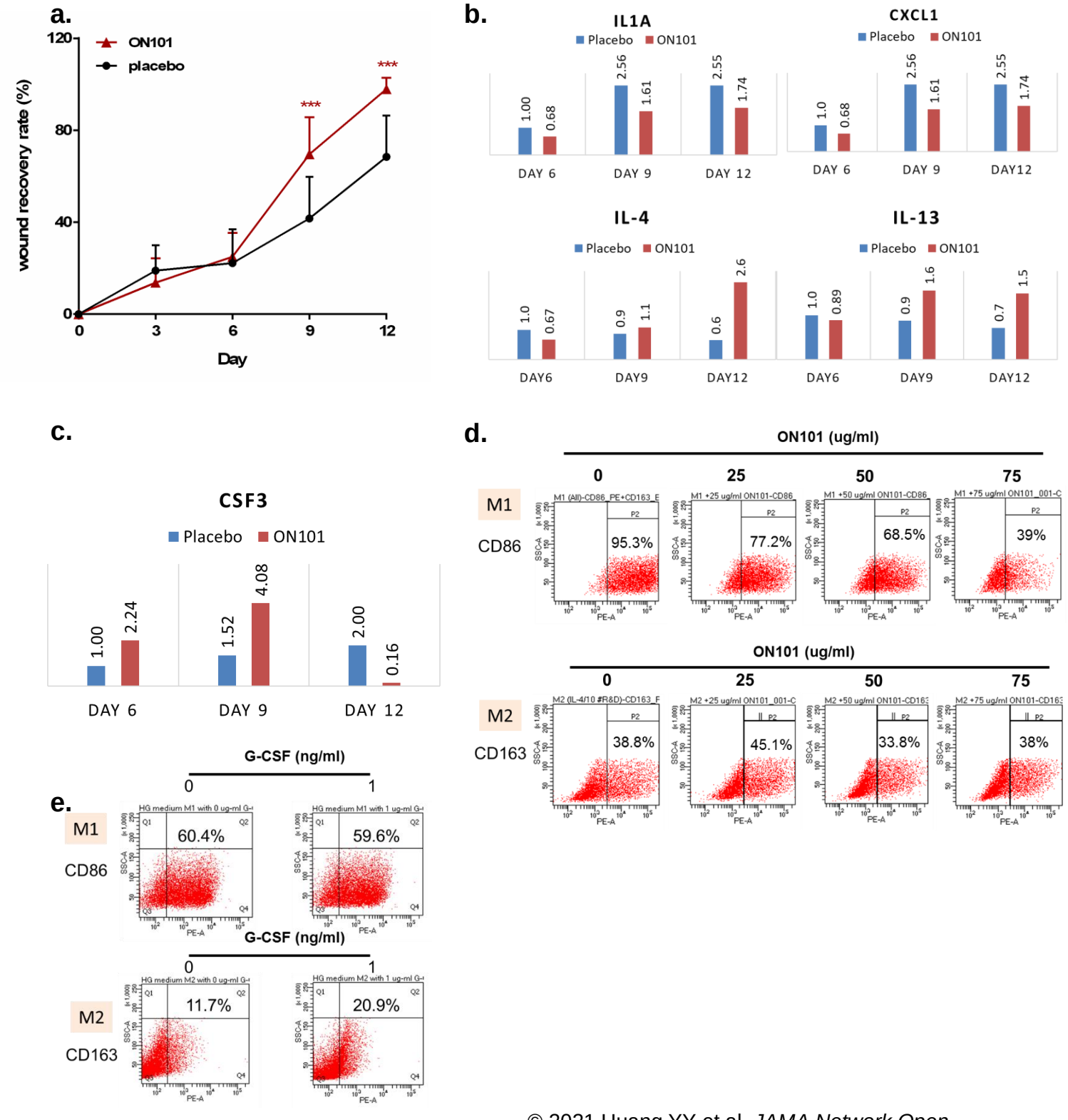
SOC, system organ class; PT, preferred term

eTable 6. Population Excluding Ulcers Reduced≧10% During Screening

Population excluding ulcers reduced >=10% during screening	ON101 Cream	Hydrocolloid dressing	Odds ratio*	P-value**
N	64	66		
Complete healing	32(50.0%)	18(27.3%)	2.44(1.16,5.15)	0.0077
Non-complete healing	32(50.0%)	48(72.7%)	0.0190	

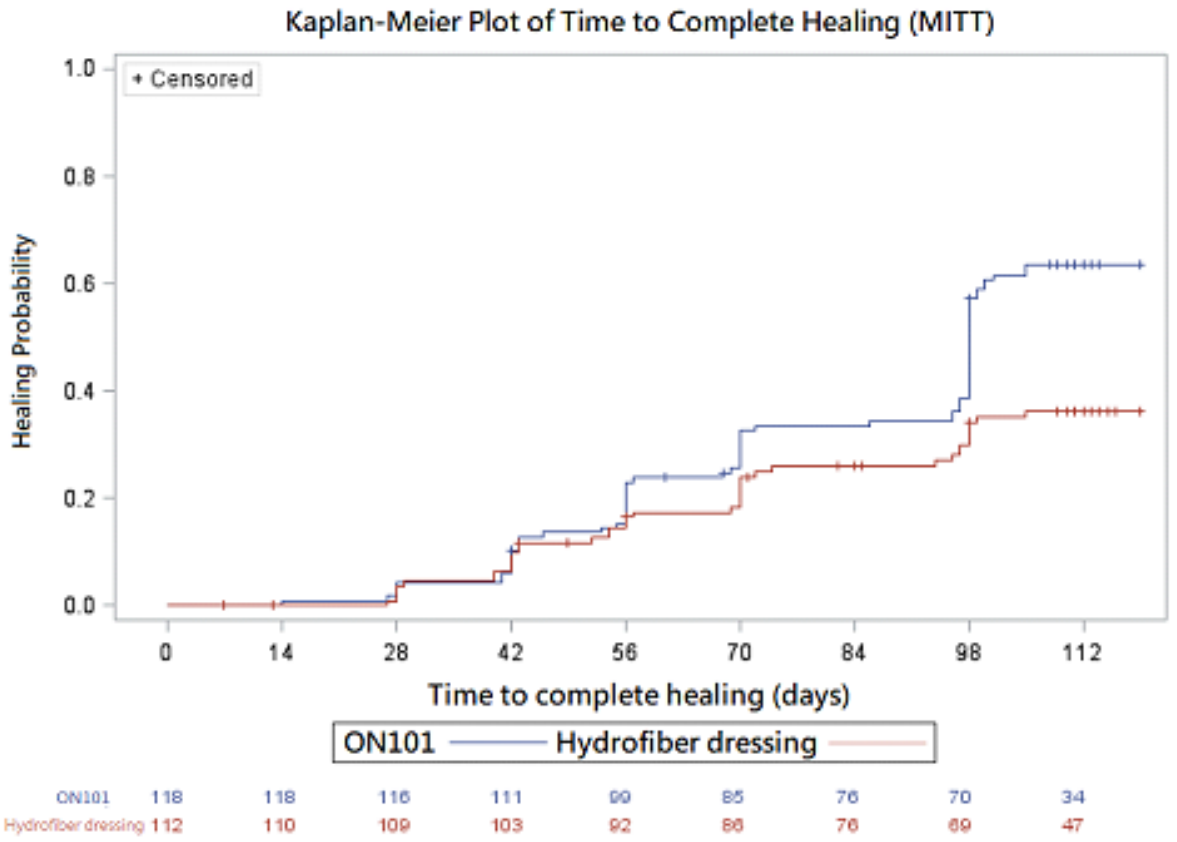
*: Odds ratio with 95% CI and p-value using logistic regression model adjusted by treatment (fixed factor), baseline wound size in cm^2 and Wagner grade (covariates).

**: Chi-square test



eFigure 1. ON101 Promotes Wound Healing Through Regulating M1 and M2 Macrophages

(a) - (c) A 6 mm- diameters wound was created on the back of Db/db mice (n=4 in each group/time point). Mice were topically applied either ON101 cream or placebo cream from day 3 after wounds were created twice daily until the harvest time. Wound size was measured and skin from wound edges were harvest at day 3, 6, 9, and 12. Total RNA was extracted for analysis gene expression by quantitative RT-PCR. (a) The averaged wound recovery rate was calculated by comparing the wound size to Day 0. *** $p<0.0005$. (b) and (c) Gene expression of indicated genes by Q-PCR panel analysis. The value of Placebo group in Day6 was used as normalization control. (b)M1-associated cytokines (IL-1a and CXCL1) were down-regulated in ON101 treatment group, while M2-associated cytokines (IL-4 and IL-13) were up-regulated in ON101 treatment group comparing to placebo group. (c) CSF3 (G-CSF symbol in human) expression was up-regulated upon ON101 treatment at Day 6 and Day 9. (d) and (e) were THP-1 cell lines polarization experiments which showed the dose-dependent inhibition of M1 marker (CD86) upon ON101 treatment (e) and GCSF could enhance the proportion of M2 (CD163) by using flow cytometry analysis.



eFigure 2. Time to Healing in mITT
The survival curve indicates the incidence of ulcers healed at each visit. Complete healing was defined as epithelization without drainage observed at 2 continuous visits. The mITT (modified intention-to-treat) cohort randomly assigned to the Aquacel group (n=112) or ON101 group (n=118) was used for analysis by Kaplan-Meier Analysis.