STUDY PROTOCOL

A Pilot Phase I/II Study to Evaluate the Safety and Efficacy of TWB-103 in Treating Lower Limb Ulcers on Patients with Diabetes Mellitus

Protocol Number:	**18-FDF-C002**
Investigational product:	**TWB-103**
Sponsor:	**Transwell Biotech Co., Ltd.**
Version Number:	**v3.0, March 25, 2019**

Confidentiality Statement

This protocol is provided for the purpose of conducting a clinical research study. The information contained in this document is confidential and, except to the extent necessary to obtain informed consent or IEC/IRB approval, cannot be disclosed unless required by governmental regulation. Persons to whom any portion of the contents of this document is disclosed must be informed that the information is confidential and may not be further disclosed by them.

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Sponsor Approval(s):

Bin-Ru She

Chief Technical Officer

Transwell Biotech Co., Ltd.

_____ _____

Signature Date

STATEMENT OF COMPLIANCE

The signature below constitutes the approval of this protocol and the attachments and assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and ICH guidelines. No deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

Principal Investigator:

Title:

Affiliation:

Address:

Phone:

Email:

Signature: _____ **Date:** _____(dd-mmm-yyyy)

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: A Pilot Phase I/II Study to Evaluate the Safety and Efficacy of TWB-103 in Treating Lower Limb Ulcers on Patients with Diabetes Mellitus

Study Description: This study is designed in a Phase I/II, single-arm manner. This study plans to enroll 10 subjects (for 8 evaluable, per-protocol subjects) with diabetic lower limb ulcers for treating with up to 12 weekly applications of TWB-103 and evaluating the safety and efficacy of TWB-103. TWB-103 will be applied to the target ulcer wound once a week by the investigator, starting from Day 1, until wound closure is confirmed or up to 12 weekly applications. An ulcer wound is considered healed only after closure is confirmed at the visit 2 weeks later. Therefore, a wound closure should be observed at three consecutive visits (i.e. a duration of 2 weeks) for an ulcer to be deemed as healed. Following the confirmation of wound closure or completion of 12-week treatment period, whichever comes first, subjects will start a 24-week follow-up period.

The potential subjects with diabetic lower limb ulcer will be arranged to receive standard of care only for at least 2 weeks during screening period. Any subject whose study ulcer size decreases by at least 30% after this standard of care will be excluded from the study.

If the study ulcer wound is not closed after TWB-103 treatment, standard of care will be applied during the following 24 weeks of follow-up. If the study ulcer wound is healed after TWB-103 treatment, appropriate standard of care should still be applied for protection.

The first 3 subjects will be recruited sequentially with a 3-week staggering in the initiation of study treatment. Patients who experience ≥ Grade 1 allergic reaction at least possibly related to the investigational product (section 8.4.3.2), has > Grade 2 skin infection in the ulcer wound, or the treatment for the ulcer wound infection requires subject's hospitalization, intravenous infusion or muscular injection of antibiotics, must not receive any further TWB-103 treatment. Additionally, if major surgery debridement is required, the subject should stop TWB-103 treatment. The study will be stopped when ≥ 3 subjects received TWB-103 have developed grade 3 or above AEs according to CTCAE 5.0, independent of attribution. If the cause of AE can be identified and resolved, the study may be resumed.

Objectives: **Primary Objective:** To assess the safety profile of TWB-103 administered to subjects with diabetic lower limb ulcers

Secondary Objective: To explore the efficacy of TWB-103 administered to subjects with diabetic lower limb ulcers

Endpoints: **Primary Endpoint:** Incidence of adverse events (AEs) and serious adverse events (SAEs). Pre-existing abnormalities are considered as AE or SAE only when the conditions escalate. The following events should be discussed

specifically:
1. Study wound and its periphery: Infection, pain, pruritus/irritation, skin dysfunction/blister, osteomyelitis, cellulitis, edema/swelling and unexpected surgery
2. Non-study area and systemic: Infection, pain, pruritus/irritation, skin dysfunction/blister, osteomyelitis, cellulitis, edema/swelling, unexpected surgery, accidental injury, abnormal lab test and general disorders

Secondary Endpoints:

1. The percentage of subjects with confirmed study wound closure at each week up to 12 weeks.
   * Wound closure is defined as "full epithelialization of the wound with the absence of drainage or dressing requirement and without sign of abscess under the epithelium". An ulcer is considered healed only after wound closure is re-confirmed at the visit 2 weeks later. Therefore, a wound closure should be observed at three consecutive visits (i.e. a duration of 2 weeks) for an ulcer to be deemed as healed.
2. Time (days) to confirmed wound closure for those subjects whose wounds are healed during treatment period and during the study period.
3. Reduction of individual wound area for up to 12 weeks.
4. Reduction of wound area at each week up to 12 weeks.
5. General granulation rate and quality of granulation tissue.
6. Reduction of wound volume for individual subject measured by 3D camera and analysis software up to 12 weeks (only for those subjects who have received the 3D measurement).

Study Population: Ten subjects aged at least 20 years old (inclusive) with diabetic lower limb ulcers presented for at least 4 weeks are planned to enroll in Taiwan.

Inclusion Criteria In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Adults at least 20 years of age.
2. With diagnosed diabetic mellitus (DM), e.g. currently under DM medication treatment, or with HbA1c $\geqq$6.5% but $\leqq$ 12%, or with fasting plasma glucose $\geqq$126 mg/dL (7.0 mmol/L), or with plasma glucose $\geqq$ 200 mg/dL (11.1 mmol/L) in the two-hour 75-gram oral glucose tolerance test (OGTT).
3. With at least one cutaneous ulcer on or below malleoli, and not healing for at least 4 weeks (the $\geqq$2-week standard of care period can be counted as part of the 4-week ulcer history).
4. With ankle brachial index (ABI) $\geq$ 0.4 on the limb with the study ulcer. For subjects with 0.6 $>$ ABI $\geqq$0.4, the investigator will arrange for providing proper treatment to the subject, such as improving circulation by medication or surgical procedures, etc.
5. The study wound is not prone to infection (the wound can be maintained clean and well bandaged, there is no nearby infected lesion, and there is no history of recurrent infections).
6. The wound should allow complete sealing of the wound by Tegaderm™ film.
7. The study wound size is between 1~33 cm^2. The wound depth should be at

least full-thickness ulcer (penetrating skin). The depth of wound may reach ligament, joint capsule, fascia, or tendon. There should be no sign of osteomyelitis. The wound does not exceed Wagner Grade 2[7].

8. Under the standard care of Investigator for at least 2 weeks, the study wound has not shown significant healing. Significant healing is defined as the following: The area of healed tissue reaches at least 30% of the area of initially presented wound.
9. When the subject has more than one wound which met the inclusion criteria, only one wound is selected as the study wound. The other wounds will be treated by standard cares.
10. Except hyperglycemia, co-morbidities are under control and non-life threatening as determined by the Investigator based on medical history, physical examination, vital signs, or clinical laboratory tests, etc.
11. A negative pregnancy test at Screening. This applies to any female subject with childbearing potential.
12. Agrees to use acceptable contraceptive methods while on study (from signing informed consent form to the end of the study).
    Acceptable contraceptive methods include:
    a. Established use of oral, injected or implanted hormonal methods of contraception
    b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
    c. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps)
13. Able to follow the Investigator's instruction on wound care.
14. With signed informed consent form.

Exclusion Criteria

Any subject meeting any of the exclusion criteria will be excluded from study participation:

1. Being pregnant or nursing.
2. With autoimmune disease other than diabetes, e.g. lupus erythematosus, multiple sclerosis.
3. With current malignancy or hypo-immunity.
4. With history of recurrent cancer, metastatic cancer, cancer which has high probability of metastasis, or cancer on the limb where the study wound is located.
5. With any serum chemistry or hematology abnormalities below
    a. AST or ALT > 5 × ULN
    b. Serum albumin < 2.0 g/dL
    c. Serum creatinine concentration is > 2.5 mg/dL
    d. WBC is < 2,000/μL
    e. ANC is <1,000/μL
    f. Platelet count is <100,000/μL
6. With history of HIV infection
7. With history of alcoholism or drug abuse.
8. Received any cell-based product at the study wound.
9. Received an investigational drug, device or biological/bioactive treatment within 30 days prior to Screening Visit.
10. With any clinical condition or significant concurrent disease judged by the investigator to complicate the evaluation of the trial treatment.
11. With history of sensitivity to materials of bovine, porcine origin, or

human serum albumin.
12. With active infection or active osteomyelitis in the study wound.

Phase: I/II

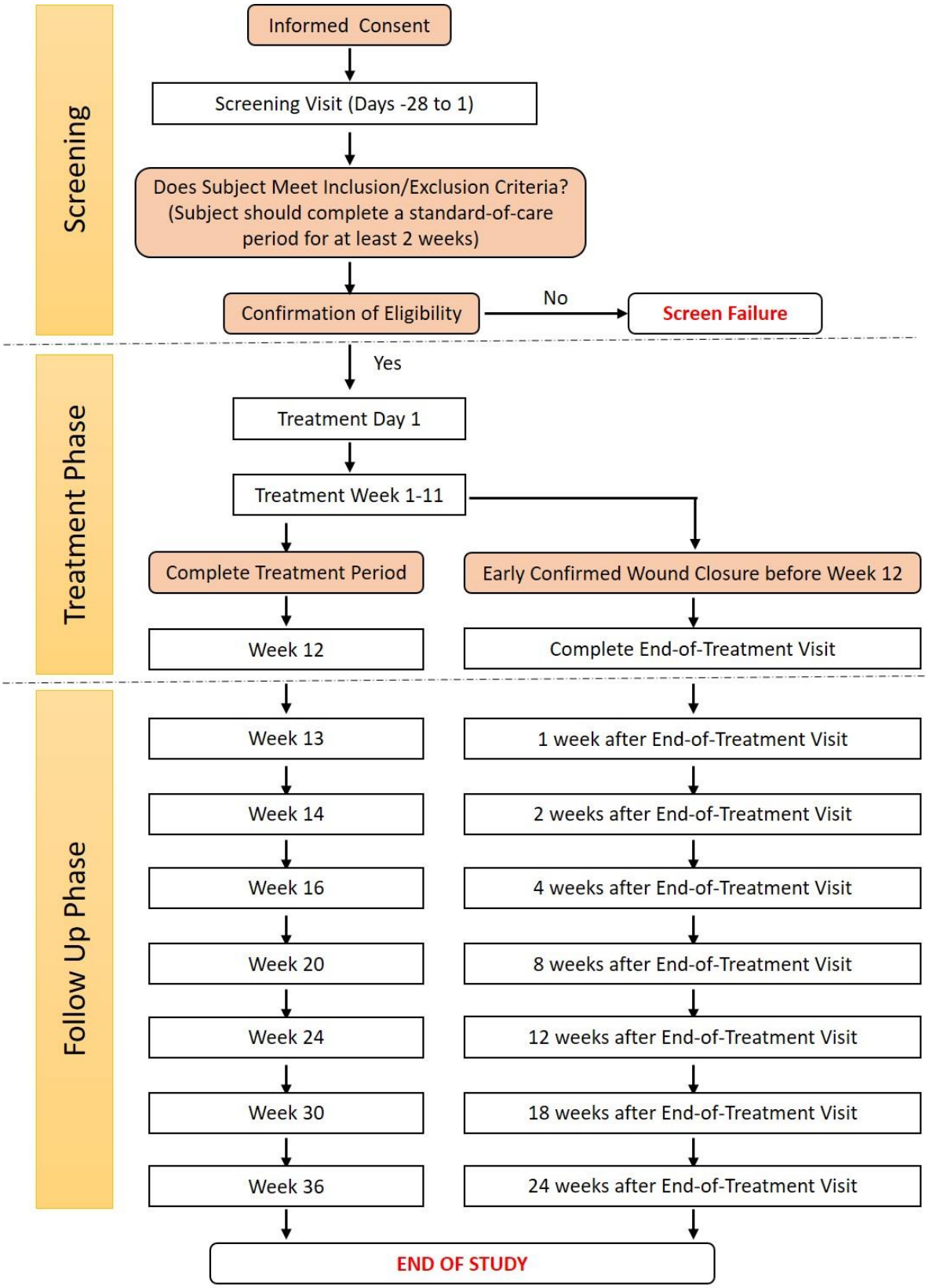
Study site: Multiple sites in Taiwan

Study Intervention: TWB-103 (mixture of TWB-102 Cell and TWB-103 Hydrogel, 6×10^5 cells/ml). TWB-102 Cell is live human fetal dermal fibroblast.
The dosage used in this study is 0.15 ml TWB-103/cm^2 of ulcer area.

Study Duration: Approximately 18 months

Participant Duration: Approximately 10 months

1.2 SCHEMA


Screening
Informed Consent
Screening Visit (Days -28 to 1)
Does Subject Meet Inclusion/Exclusion Criteria? (Subject should complete a standard-of-care period for at least 2 weeks)
Confirmation of Eligibility
No
Screen Failure
Yes
Treatment Phase
Treatment Day 1
Treatment Week 1-11
Complete Treatment Period
Early Confirmed Wound Closure before Week 12
Week 12
Complete End-of-Treatment Visit
Follow Up Phase
Week 13
1 week after End-of-Treatment Visit
Week 14
2 weeks after End-of-Treatment Visit
Week 16
4 weeks after End-of-Treatment Visit
Week 20
8 weeks after End-of-Treatment Visit
Week 24
12 weeks after End-of-Treatment Visit
Week 30
18 weeks after End-of-Treatment Visit
Week 36
24 weeks after End-of-Treatment Visit
END OF STUDY

1.3 SCHEDULE OF ACTIVITIES (SOA)

Visit	**Screening[1,2]**	**Treatment[3,4]**			**Follow-up[3,5]**	**Unscheduled[6]**
Visit number	**1**	**2**	**3~13**	**14**	**15~21**	**-**
Week(s) relative to Day 1 / **Procedures**	**Within 4 weeks prior to Day 1[9]**	**Day 1[9]**	**1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11**	**12 or End of Treatment**	**13, 14, 16, 20, 24, 30, 36 or 1, 2, 4, 8, 12, 18, 24 (after End-of-Treatment)[3]**	**-**
Target day	**-28 ~ 1**	**-**	**8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 (± 2)**	**85 (± 2)**	**92 (± 2), 99 (± 2), 113 (± 3), 141 (± 6), 169 (± 6), 211 (± 6), 253 (± 6) or 7 (± 2), 14 (± 2), 28 (± 3), 56 (± 6), 84 (± 6), 126 (± 6), 168 (± 6) (after End-of-Treatment)[3]**	**-**
Obtain signed informed consent	X					
Assign identification number	X					
Obtain demographic characteristics	X					
Medical history	X					
Medication history	X					
Eligibility[7]	X[7]	X[7,8]				
Physical exam	X	X[8]	X	X	X	X
Height and Weight	X					
Vital signs	X	X[8]	X	X	X	X
Pregnancy test (urine or serum) for applicable subjects	X[1]		X[10]	X[4,11]		(X)
Laboratory test	X[1]		X[10]	X[4,11]		(X)
PRA test: Class I HLA	X[1]			X[4,11]		(X)
Ankle Brachial Index	X[1]					
Ulcer evaluation[13]	X[2]	X[8]	X[8]	X	X	(X)
Standard of Care[12]	X[2]	X[8,14]	X[8,14]	X	X	(X)
TWB-103 application		X	X			
Adverse event	X----------X					
Concomitant medications	X----------X					

1. Procedures performed between signing ICF and Day 1 are acceptable. However, procedures should be completed before the TWB-103 application if performed on Day 1; in this case, the same procedures scheduled for Day 1 can be waived.
2. During this screening period, subjects will receive standard-of-care to the target ulcer wound for at least 2 weeks to observe the wound healing rate (inclusion criteria #8). The wound size before and after the standard-of-care treatment should be recoded. The choice of standard-of-care will be at investigator's discretion based on the subject's physical condition.
3. If any subject has confirmed wound closure before week 12, he/she will stop TWB-103 application and complete assessments of End-of-Treatment (Week 12). Those subjects who have confirmed wound closure before week 12 will have follow-up visits at 1 week, 2 weeks, 4 weeks, 8 weeks, 12 weeks, 18 weeks and 24 weeks after the End-of-Treatment Visit.
4. If the withdrawal is decided during TWB-103 treatment period, the withdrawn subject will be encouraged to complete the assessments of Week 12, within 1 week of the withdrawal decision, as End of Study (EOS) visit, before exiting from the study. Laboratory, pregnancy and PRA tests done within 1 week prior to EOS visit are acceptable.
5. If the withdrawal is decided during the follow-up period, the withdrawn subject will be encouraged to complete the assessments of Week 36, within 2 weeks of the withdrawal decision, as EOS visit, before exiting from the study.
6. Unscheduled visit will be arranged when investigator considers necessary. Actions (pregnancy test, laboratory test, PRA test, ulcer evaluation, standard of care) to be performed will be per investigator's decision.
7. Eligibility will be checked during screening and at Day 1 Visit. The eligibility re-check at Day 1 will be based on the results from Day 1 Visit (vital signs and physical exam) and other information collected during screening.
8. Procedures to be performed before TWB-103 application. If debridement is performed as part of the standard of care, debridement should be performed before TWB-103 application.
9. The baseline value of each assessment will be the value assessed closest and prior to the first dose of TWB-103.
10. Pregnancy test and laboratory test will be performed at Week 5 Visit only. Pregnancy test and laboratory test performed within 1 week prior to Week 5 Visit will be acceptable.
11. Laboratory, pregnancy and PRA tests done within 1 week prior to Week 12 or End-of-Treatment visit are acceptable.
12. Standard of care will be performed at the investigator's discretion.
13. Ulcer evaluation includes wound size, wound volume (optional), granulation rate and quality of granulation tissue, and grading. The wound grading will be performed at screening only. The ulcer evaluation before and after the standard-of-care treatment should be recorded during screening period (also see footnote 2).
14. Revascularization is prohibited during treatment period (Visit 2~Visit 13).

2 INTRODUCTION

2.1 STUDY RATIONALE

Diabetes Mellitus is a common illness worldwide. As of 2017, an estimated 425 million people had diabetes worldwide[1]. Diabetes can cause several complications and diabetic foot ulcer (DFU) is a major complication of diabetes mellitus. DFU is most common medical complication causing diabetics to get medical treatment. The global average prevalence of diabetic foot ulcer is 6.4% of diabetic patients, with variation between 3% in Oceania to 13% in North America[2]. Approximately 1% of people with diabetes suffer lower-limb amputation[1]. 85% of those amputations in diabetic patients have preceded foot ulceration[2]. In a survey conducted in 2011, around 1.3% of diabetic patients develop foot ulcer and 0.7% of diabetic patients have lower limb amputations[3]. As a result, DFU is wildly acknowledged a source of major distress and morbidity for diabetic subjects, and also an enormous drain on health-care resources. Seeking effective treatments for DFU has become an important issue for patients and healthcare practitioners.

Diabetic foot ulcers are most commonly caused by poor circulation, high blood sugar (hyperglycemia), nerve damage and irritated or wounded feet. Poor circulation and high blood sugar usually make ulcers more difficult to heal. Nerve damage can reduce the pain sensation, which increases risk of further wounding. Additionally, diabetic patients often have cracking or splinter due to dry skin, which will increase the chance of diabetic patients to develop foot ulcers.

Treatment for diabetic foot ulcer varies depending on the cause, the severity and condition of ulcer wound. A good wound cleansing and a moist wound environment, such as daily saline, are common self-care for diabetic foot ulcer. A debridement to remove necrotic or infected tissues can help ulcer healing. Management of wound infection, a serious complication of a foot ulcer, is also typical to prevent the deterioration of the ulcer wound. Evaluation and correction of peripheral arterial insufficiency will increase the circulation and improve the healing of ulcer. Moreover, pressure from walking can make an infection worse and an ulcer expand, particularly for overweight patients, and therefore stay off the feet can prevent pain and ulcers. Patients may benefit from using appropriate therapeutic footwear, such as diabetic shoes, compression wraps and shoe inserts. Surgical intervention may be also suggested by the doctors to alleviate pressure around the ulcer. Many topical over-the-counter treatments are available for foot ulcers, including dressings containing silver or silver sulphadiazine cream, polyhexamethylene biguanide (PHMB) gel or solutions, iodine (either povidone or cadexomer), or medical grade honey in ointment or gel form.

Although there are many standard treatments for diabetic foot ulcer, as described above, diabetic foot ulcer usually results from multiple pathological factors (e.g. vascular insufficiency, altered cellular activity, and a dysfunctional extracellular matrix), leading to a deficient wound bed, which often fails to respond to conventional therapy alone. A multidisciplinary approach is therefore in need in giving better treatment outcomes for diabetic foot ulcer. Cell therapies have shown as a promising solution for diabetic wound healing, owing to the ability of cells to secret an array of factors that facilitate the wound healing. Particularly, the safety records of clinical use of dermal fibroblasts in wound healing have been superior in the past two decades. Moreover, fibroblast-containing products, including Apligraf®, Demagraft®, have been approved for treating diabetic foot ulcers. TWB-103 which contains cultured fetal dermal fibroblasts is being developed to overcome the deficits associating with the current cell products such as high pricing, lack of donor tissue and short shelf life, *etc*.

2.2 BACKGROUND

Wound healing is a complexed process where the skin can repair from damage. This process includes the following phases: hemostasis, inflammation, proliferation, and maturation[4]. The hemostasis phase requires the activation of platelets which induces blood clotting at the wound site to stop bleeding. During inflammation phase, damaged and dead cells, bacteria and other pathogens or debris are cleaned out by white blood cells mediated phagocytosis. Following the inflammation phase, the new tissue starts to grow. New blood vessels are formed by vascular endothelial cells. Fibroblasts proliferate, migrate and produce extracellular matrix. Meanwhile, keratinocytes also proliferate, migrate and differentiate to form epidermis. During this granulation and epithelialization stage, fibroblasts and keratinocytes closely interact to grow new tissue. The last stage is the maturation and remodeling where the collagen will realign and unnecessary cells will be removed by apoptosis.

It is well-recognized that fibroblasts are critical for the wound healing. The use of fibroblasts as a therapeutic strategy is logical. The fibroblast-containing products, such as Apligraf® and Demagraft®, have been approved for treating diabetic foot ulcers.

Dermagraft® is a cryopreserved three-dimensional dermal substitute containing human neonatal dermal fibroblasts growing on a bioabsorbable mesh. The materials secreted from fibroblasts, including human dermal collagen, matrix proteins, growth factors, and cytokines help to restore the dermal bed of the diabetic foot ulcer and to improve the wound healing. In a randomized, controlled, and multicenter study, 314 patients with diabetic foot ulcer were randomized to either the Dermagraft® treatment group or control (conventional therapy)[5]. During the 12-week observation period, patients in the treatment group received up to 8 weekly application of Dermagraft®. Among those patients, 30.0% (39 of 130) of patients with Dermagraft® treatment, comparing to 18.3% (21 of 115) of control patients (P = 0.023), showed

complete wound healing by the end of week 12. Additionally, Dermagraft® group experienced significantly fewer ulcer-related adverse events.

Apligraf® is an allogeneic bio-engineered skin equivalent that contains an upper epidermal layer and a lower dermal layer. The epidermal layer is formed by cultured human foreskin-derived neonatal keratinocytes while the dermal layer is formed by cultured human foreskin-derived fibroblasts growing in bovine type I collagen matrix. Therefore, Apligraf® mimics the normal skin. In the study of Apligraf® for diabetic foot ulcer, 208 patients were randomly assigned to either with Apligraf® (112 patients) or saline-moistened gauze (96 patients, control group)[6]. Patients had up to 5 weekly application of Apligraft® and were followed for 12 weeks. The results showed that 63 (56%) Apligraf®-treated patients achieved complete wound healing compared with 36 (38%) in the control group (P = 0.0042) during the 12-week follow-up period. The median time to complete closure were 65 days and 90 days for Apligraf®-treated group and control group, respectively.

From the experience above, using fibroblasts to treat diabetic foot ulcers is feasible and also effective. TWB-103, developed by Transwell Biotech Co., Ltd., is a mixture of TWB-102 cell and TWB-103 hydrogel. TWB-102 cell contains live human fetal dermal fibroblasts (FE002-SK2 cells). In the current clinical trial for treating donor site wound, to each cm^2 of wound about 4 μL of TWB-102 cell product (which contains 30,000 FE002-SK2 cells) is applied. The hydrogel is used to dilute and stabilize cells and confine the cells within targeted wound area without disturbance to the therapeutic functions of the cells. The safety and efficacy of TWB-103 are tested in the following studies.

Non-Clinical Experience

Effect of the cellular components of TWB-103 on the migration of primary human keratinocytes

It is well established that TWB-102 cells stimulate keratinocyte migration in a co-culture system. Assay for such effect of TWB-102 cells is routinely conducted as a part of product release testing of TWB-102 cells.

Effect of the cellular components of TWB-103 on the proliferation of primary human keratinocytes

Three studies indicated that live TWB-102 cells, killed/broken TWB-102 cells and mitomycin C-treated FE002-SK2 cells were all capable of stimulating keratinocyte proliferation.

Semi-quantitative array profiling of TWB-102 cell

The cell extracts from three lots of TWB-102 cell products showed good consistency between lots. All three lots of cells contains moderate to high level of receptors for cell growth signals (Axl, gp130, EGFR, VEGF R1), adhesion proteins involving cell migration (ALCAM, ICAM-1, VCAM-1), growth factors

(bFGF, BDNF, TGF b1, HGF), modulators of growth factor (Follistatin, IGFBP-3, IGFBP-6, OPG), angiogenic factor (ANG-1), inflammatory factor (MIF), and factors which modulate degradation of tissue matrix (PAI-1, uPAR, TIMP-1, TIMP-2). Analysis of culture media detected growth factors that were present through the interaction of TWB-102 cells and keratinocytes.

Treating full-thickness wounds in nude rat study with TWB-103

A GLP study was carried out using TWB-103 product to treat excision wound on nude rats (n=100). On the back of each rat a full-thickness excision wound of 12 mm in diameter was created with a biopsy puncher. The rats were randomly divided into 3 treatment groups, each group had respectively received treatment of nothing (Group 1, sham control, n=40), 0.2 mL of hydrogel (Group 2, gel only, n=20), and 0.2 mL of hydrogel containing 120,000 TWB-102 cells (Group 3, cell+gel, n=40). After dosing, the wound was covered with a piece of Tegaderm™ and bandaged. All rats were dosed on days 0, 3, 6, and 9. Wound healing characterized by epithelialization was similar for all groups. There was no TWB-103-related abnormality on animals sacrificed on days 10, 21, 28 and 56. The evaluation on the migration and persistence of the grafted cells in TWB-103 product suggested that the product cells were effectively eliminated after wound healing and did not pose long term risk on treated animals. No human cells or DNA were detected in any tissue of any animal examined other than the wound site, suggesting the lack of cell migration from wound site to other tissues.

Treating full-thickness wounds in diabetic pigs with TWB-103

A porcine study was conducted to evaluate the safety and potential efficacy of TWB-103 for treating diabetic wounds. Pigs were injected with streptozotocin (STZ) for induction of hyperglycemia. Six wounds were created on the back of each of 4 pigs. The dimension of each wound was L 2cm x W 2cm x D 0.5 cm (down to fascia). The wounds were divided into 4 groups which respectively received treatment of A. Sham, B. 0.3 mL of TWB-103 hydrogel (no cell), C. TWB-103 hydrogel/46,900 cells/cm^2, and D. TWB-103 hydrogel/ 93,800 cells/cm^2. There were 6 wounds for each treatment group. The wounds were dosed on Day 0 (wound creation day), Days 3, 6, 9, and 14. Pigs were sacrificed on Day 34 when wounds were almost healed and tissues were cut off from the wounds for histological evaluation. The results showed that from Day 6 to Day 16 the healing rates of wounds receiving cell treatment were slightly faster than the sham group (but without statistical significance when analyzed by One-way ANOVA). No adverse effect was detected. Although the pigs in this study sustained hyperglycemia through the study period the wound healing appeared to generally follow normal wound healing course. The pathological phenomenon associating with chronic wounds was not apparent in this study.

Clinical Experience

TWB-103 (6×10^5 cells/ml) is currently under investigation for its safety and efficacy profile in subjects with split-thickness donor site wound. Subjects are recruited from Taiwan and Japan. Subjects can have up to 3 applications of TWB-103 (0.05 ml TWB-103 containing 30,000 cells /cm^2 of wound area) during a 14-day treatment period. TWB-103 is well-tolerant in all subjects up to now. No efficacy data is available currently due to blinding.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

The investigational product, TWB-103, is being proposed as an indication for managing diabetic lower limb ulcer in adult to aid the regeneration of functional skin. TWB-103 consists of one frozen vial of living allogeneic fibroblasts, and one vial of gelatin-based hydrogel. Given the extensive experience and knowledge on gelatin, it is generally recognized as safe by the FDA. The manufacturing process of TWB-103 has ensured to minimize the potential risk factors and completed extensive tests for those potential risk factors. However, as a cell product containing cultured human allogeneic cells and porcine gelatin, TWB-103 has the following potential risks which cannot be totally excluded: possible infection transmitted from the animal-derived cell culture reagents (bovine pathogens from fetal bovine serum; porcine pathogens from porcine trypsin), cells and the skin tissue donor (including Parvovirus B19) or acquired during the manufacturing process, immunological reactions leading to rejection to the ingredients (including gelatin) or to the residues carried from the manufacturing process (residual fetal bovine serum and porcine trypsin) by the recipient, inadequacy wound healing, cell distribution to sites distal to the wound beds and induction of systemic toxicity and tumorigenicity.

Because no antibiotic or other antimicrobial agent was used in any stage of cell production, it is expected that TWB-103 has no anti-infection activity. TWB-103 contains rich nutrients which may provide for microbial growth. However, with appropriate dressing, so far no incidence of wound infection had occurred in any animal studies or ongoing clinical trial.

Viral safety of the products had been evaluated and ensured at multiple levels which included testing of mother donor, examination of fetus, testing of all cell banks, testing of representative batch of product, and viral reduction plus testing of manufacturing materials from human and animal sources in compliance with international standards.

To address the tumorigenicity potential of the product, several studies had been conducted which included karyotyping to check chromosome stability of the initial donor cells and cells in final product, soft-agar assay to check the anchorage-dependence of cell growth, nude-mice study in which cells were

subcutaneously injected according to FDA guidance and nude rat study in which cells were topically applied to full-thickness wounds. No sign related to tumorigenicity was detected in any of these studies.

Regarding immunological responses toTWB-103, neither animal studies (using immune-compromised and normal immune animals) nor ongoing clinical study detected any concerning inflammatory or allergic responses.

TWB-103 is well-tolerated in all subjects up to now in the split-thickness donor site wound study. Besides, there was no report of immunological reaction, tumorigenicity or malignant transformation, or disease transmission resulting from the fetal dermal fibroblasts which were isolated and cultured similarly to FE002-SK2 in the compassionate clinical use in Switzerland for more than 15 years.

2.3.2 KNOWN POTENTIAL BENEFITS

Chronic wounds are characterized by the wound beds that are poorly vascularized, with decreased cell vitality and with imbalanced deposition/degradation of tissue matrix. As the result, granulation is often deterred and epithelialization impeded. Fibroblasts are integral part of granulation tissue. They produce both matrix components and enzymes for regeneration of the matrix. They also promote vascularization by recruiting endothelial cells and stimulating VEGF production. Ultimately, fibroblasts closely interact with keratinocytes in the epithelialization process. TWB-103 provides vital fibroblasts which may promote wound healing by modulating tissue matrix, enhancing vascularization and promoting keratinocyte proliferation and migration.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Because TWB-103 is an allogenic cell product, it may raise concerns of exposing subjects to the short-term risks such as infection, allergic reaction, as well as the long-term risks like tumorigenicity. However, chronic wounds which fail to respond to standard of care may put subjects at risks ranging from wound deterioration to amputation. Cell therapy may provide the bioactivity needed for initiating the healing process. The manufacturing of TWB-103 is stringently controlled to minimize the contamination of microbes and impurities. The product is well tolerated in the animal studies and ongoing clinical trial. Considering the proposed efficacy of TWB-103 in speeding wound healing, the benefit of TWB-103 is believed to outweigh the risks for subjects with diabetic lower limb ulcer.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To assess the safety profile of TWB-103 administered to subjects with diabetic lower limb ulcers	Incidence of adverse events (AEs) and serious adverse events (SAEs). Pre-existing abnormalities are considered as AE or SAE only when the conditions escalate. The following events should be discussed specifically: 1. Study wound and its periphery: Infection, pain, pruritus/irritation, skin dysfunction/blister, osteomyelitis, cellulitis, edema/swelling and unexpected surgery 2. Non-study area and systemic: Infection, pain, pruritus/irritation, skin dysfunction/blister, osteomyelitis, cellulitis, edema/swelling, unexpected surgery, accidental injury, abnormal lab test and general disorders	All adverse events will be recorded to carefully evaluate the safety of TWB-103.
Secondary		
To explore the efficacy of TWB-103 administered to subjects with diabetic lower limb ulcers	1. The percentage of subjects with confirmed target wound closure at each week up to 12 weeks. * Wound closure is defined as "full epithelialization of the wound with the absence of drainage or dressing requirement and without sign of abscess under the epithelium". An ulcer is considered healed only after wound closure is re-confirmed at the visit 2 weeks later. Therefore, a wound closure should be observed at three consecutive visits (i.e. a duration of 2 weeks) for an ulcer to be deemed as healed. 2. Time (days) to confirmed wound closure for those subjects whose wounds are healed during treatment period and during the study period. 3. Reduction of individual wound area for up to 12 weeks. 4. Reduction of wound area at each week up to 12 weeks. 5. General granulation rate and quality of granulation tissue. 6. Reduction of wound volume for individual subject measured by 3D camera and analysis software up to 12 weeks (only for those subjects who have received the 3D measurement).	The efficacy of TWB-103 for wound healing is observed in multiple dimensions, including percentage of subjects presented wound closure, time to wound closure, reduction of wound area/volume and the appearance of wound bed.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This study is to evaluate if applying TWB-103 on diabetic ulcer wound can speed up the wound healing in subjects with diabetic lower limb ulcer which has not shown healing for at least 4-week duration. This study is designed in a Phase I/II, single-arm manner. This study plans to enroll 10 subjects (including 8 evaluable per-protocol subjects) with diabetic lower limb ulcers for treating with up to 12 weekly applications of TWB-103 and evaluating the safety and efficacy.

Eligibility will be checked during screening and at Day 1 Visit. The potential subjects with diabetic lower limb ulcer will be arranged to receive standard of care only for at least 2 weeks during screening period. The standard of care will be performed at the investigator's discretion based on the condition of each subject. Any subject whose study ulcer size decreases by 30% or more after this standard of care will be excluded from the study.

TWB-103 is applied once a week by the investigator at the study site, starting from Day 1, until wound closure is confirmed or up to 12 weekly applications. An ulcer is considered healed only after closure is confirmed at the visit 2 weeks later. Therefore, a wound closure should be observed at three consecutive visits (i.e. a duration of 2 weeks) for an ulcer to be deemed as healed. For wound closure which is first observed at Week 11 or Week 12, and has a confirmation at Week 13 or Week 14, respectively, will be considered as a healed wound closure within 12 weeks. If any subject has confirmed wound closure before week 12, he/she will stop TWB-103 application and complete assessments of End-of-Treatment (Week 12).

Following the confirmation of wound closure or completion of 12-week treatment period, whichever comes first, subjects will start a 24-week follow-up period. Subjects will have follow-up visits at weeks 13, 14, 16, 20, 24, 30 and 36, if the 12-week treatment period is completed. Those subjects who have confirmed wound closure before week 12 will have follow-up visits at 1 week, 2 weeks, 4 weeks, 8 weeks, 12 weeks, 18 weeks and 24 weeks after the End-of-Treatment Visit.

If the study wound is not closed after TWB-103 treatment, standard of care will be applied during the following 24 weeks of follow-up. If the study wound is healed after TWB-103 treatment, appropriate standard of care should still be applied for protection.

The first 3 subjects will be recruited sequentially with a 3-week staggering in the initiation of study treatment. Any subject who experiences ≥ Grade 1 allergic reaction at least possibly related to the investigational product (section 8.4.3.2), has > Grade 2 skin infection in the ulcer wound, or the treatment for the ulcer wound infection requires subject's hospitalization, intravenous infusion or muscular injection

of antibiotics, must not receive any further TWB-103 treatment. Additionally, if major surgery debridement is required, the subject should stop TWB-103 treatment. The study will be stopped when ≥ 3 subjects received TWB-103 have developed grade 3 or above AEs according to CTCAE 5.0, independent of attribution. If the cause of AE can be identified and resolved, the study may be resumed.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This study is intended to explore the safety and efficacy of TWB-103 in subjects of diabetic lower limb ulcer. As a pilot study, in order to gain bounty clinical experience with few subjects, it is designed as a single-arm small scale study. Because of the nature of TWB-103, whose cell part is stored at -70 ~ -80℃, it will be applied to the wound only at the study site by the investigator. Based on the expectation that diabetic ulcers usually heal slowly even under advanced therapeutic regimen, subjects are scheduled to receive TWB-103 treatment once a week for up to 12 applications. A 24-week follow-up is also required to observe long-term safety and to further evaluate the wound healing status.

4.3 JUSTIFICATION FOR DOSE

The current cell density in each mL of prepared TWB-103 is 600,000 cells/mL. In the nude rate study, 0.2 mL of TWB-103 which contained 120,000 cells was applied to each excision wound of 12 mm in diameter (~106,200 cells/cm^2). This dosage was considered as safe because no significant abnormality was observed. In the hyperglycemia porcine wound study, full thickness excision wounds were treated with TWB-103 at 45,000 cells/cm^2 and 90,000 cells/cm^2 wound area. No adverse effect was detected. In the ongoing clinical trial donor site wounds have been treated with 0.05 mL of TWB-103 containing 30,000 cells/cm^2 wound area. Considering that donor site wounds have largely intact dermis in which substantial number of fibroblasts are present while the diabetic ulcers tend to be deficient of functional dermis, the dosage proposed to be used in this study will be 0.15 ml of TWB-103/cm^2 wound area, that will equal to 90,000 cells/cm^2 wound area. Furthermore, considering that there are probably 200,000~600,000 fibroblasts/cm^2 human skin, such dose is thought to be reasonable.

4.4 END OF STUDY DEFINITION

A subject is considered to have completed the study if he or she has completed the End-of-Treatment Visit (for withdrawal during treatment period) or Week 36 (or 24 weeks after the End-of-Treatment Visit) shown in the Schedule of Activities (SOA), Section 1.3. The study is considered completed when all subjects are no longer being examined or the last study visit or procedure shown in the SOA of all subjects of the study site have be performed.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Adults at least 20 years of age.
2. With diagnosed diabetic mellitus (DM), e.g. currently under DM medication treatment, or with HbA1c ≧6.5% but ≦ 12%, or with fasting plasma glucose ≧126 mg/dL (7.0 mmol/L), or with plasma glucose ≧ 200 mg/dL (11.1 mmol/L) in the two-hour 75-gram oral glucose tolerance test (OGTT).
3. With at least one cutaneous ulcer on or below malleoli, and not healing for at least 4 weeks (the ≧2-week standard of care period can be counted as part of the 4-week ulcer history).
4. With ankle brachial index (ABI) ≥ 0.4 on the limb with the study ulcer. For subjects with 0.6 > ABI ≧ 0.4, the investigator will arrange for providing proper treatment to the subject, such as improving circulation by medication or surgical procedures, etc.
5. The study wound is not prone to infection (the wound can be maintained clean and well bandaged, there is no nearby infected lesion, and there is no history of recurrent infections).
6. The wound should allow complete sealing of the wound by Tegaderm™ film.
7. The study wound size is between 1~33 cm^2. The wound depth should be at least full-thickness ulcer (penetrating skin). The depth of wound may reach ligament, joint capsule, fascia, or tendon. There should be no sign of osteomyelitis. The wound does not exceed Wagner Grade 2.
8. Under the standard care of Investigator for at least 2 weeks, the study wound has not shown significant healing. Significant healing is defined as the following: The area of healed tissue reaches at least 30% of the area of initially presented wound.
9. When the subject has more than one wound which met the inclusion criteria, only one wound is selected as the study wound. The other wounds will be treated by standard cares.
10. Except hyperglycemia, co-morbidities are under control and non-life threatening as determined by the Investigator based on medical history, physical examination, vital signs, or clinical laboratory tests, etc.
11. A negative pregnancy test at Screening. This applies to any female subject with childbearing potential.
12. Agrees to use acceptable contraceptive methods while on study (from signing informed consent form to the end of the study).

    Acceptable contraceptive methods include:

    a. Established use of oral, injected or implanted hormonal methods of contraception

    b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)

    c. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps)
13. Able to follow the Investigator's instruction on wound care.
14. With signed informed consent form.

5.2 EXCLUSION CRITERIA

Any subject meeting any of the exclusion criteria will be excluded from study participation:

1. Being pregnant or nursing.
2. With autoimmune disease other than diabetes, e.g. lupus erythematosus, multiple sclerosis.
3. With current malignancy or hypo-immunity.
4. With history of recurrent cancer, metastatic cancer, cancer which has high probability of metastasis, or cancer on the limb where the study wound is located.
5. With any serum chemistry or hematology abnormalities below
   a. AST or ALT > 5 × ULN
   b. Serum albumin < 2.0 g/dL
   c. Serum creatinine concentration is > 2.5 mg/dL
   d. WBC is < 2,000/μL
   e. ANC is <1,000/μL
   f. Platelet count is <100,000/μL
6. With history of HIV infection
7. With history of alcoholism or drug abuse.
8. Received any cell-based product at the study wound.
9. Received an investigational drug, device or biological/bioactive treatment within 30 days prior to Screening Visit.
10. With any clinical condition or significant concurrent disease judged by the investigator to complicate the evaluation of the trial treatment.
11. With history of sensitivity to materials of bovine, porcine origin, or human serum albumin.
12. With active infection or active osteomyelitis in the study wound.

5.3 SCREEN FAILURES

Screen failures are defined as subjects who consent to participate in this trial but do not meet all the criteria for participation in this trial. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects, to meet the regulatory authority requirement and to respond to queries from regulatory authorities and IRBs/IECs. Minimal information includes demography, screen failure details, and any serious adverse event.

Re-screening for subject's eligibility will be allowed if the subject is willing to participate in the study and one of the following conditions meets:

1. If a subject consents to participate and meets the eligibility criteria but a delay occurs in starting the participation and that some measurement relevant to eligibility became invalid owing to the delay.

2. If the cause of screen failure has now resolved or adequately treated with medication, and the subject condition has now stabilized.
3. If there is an alternative manner to indicate that the subject may be suitable.

In these situations,

1. A new CRF will be used
2. A new screening number will be assigned to the person
3. The person will be marked as having been re-screened on the source document
4. The old CRF will be completed as fully as possible and data on the old CRF is not copied to the new CRF.
5. The subject needs to sign a new ICF as part of the screening procedure.

It is not appropriate to re-screen a subject if he/she has previously failed to meet the eligibility criteria and no further changes or treatments have been able to indicate that the subject may be suitable.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

TWB-103 is a mixture of TWB-102 Cell and TWB-103 Hydrogel. TWB-102 Cell is a frozen vial containing 0.4 mL of $3x10^6$ human fetal dermal fibroblasts and TWB-103 Hydrogel is a refrigerated glass vial containing 6 mL of gelatin-based hydrogel. To prepare for clinical application, TWB-102 Cell vial will be warmed to thaw and TWB-103 Hydrogel will be warmed to liquefy. Then cells from one vial will be mixed with 4.6 mL of hydrogel in a syringe to result in a 5 mL mixture containing 6×10^5 cells/mL. If less than 1 mL of TWB-103 will be applied, the mixture will further be transferred to a 1 mL syringe before it starts to thicken.

6.1.2 DOSING AND ADMINISTRATION

Dosing

Each subject will receive TWB-103 weekly up to 12 applications or till confirmed wound closure, whichever comes first. TWB-103 is applied to the ulcer at appropriate amount based on the ulcer area. The dosage of TWB-103 used in this study is 0.15 ml TWB-103/cm^2 of ulcer area. Detailed protocol of preparing TWB-103 for clinical application will be provided by study sponsor.

Before Administration

(1) Before TWB-103 Treatment starts, the wound dressing and topical drugs used during screen period should be removed first. Attention should be paid to viscous substances such as gel or ointment and anti-microbial substances because they may interfere with the subsequent TWB-103 treatment by conferring physical barrier or cytotoxicity to cells in TWB-103. If such substance has been applied previously, the substance should be thoroughly washed off by flushing with sterile saline or wiping off with saline-saturated cotton swabs.

(2) Wound debridement may be performed based on Investigator's judgment. Epinephrine is allowed for reducing bleed.

(3) When bleeding stops, an OPSITE™ transparent film is placed on the wound and the wound area is traced on the film. The area of the wound is estimated using the grid pattern on the film. The investigator is allowed to perform additional wound measurement with a 3D camera combined with software which is capable of estimation of wound dimensions.

(4) Surgical removal of excessive granulation tissue and minor wound debridement are allowed during study period. However, such events should be recorded and photo images before and after the procedure should be taken.

Administration

(1) TWB-103 is pushed from the syringe to the wound. The wound is then covered by Tegaderm™ transparent film. If the wound is positioned in a way that TWB-103 might flow-off easily, a collagen wound dressing (provided by Sponsor) can be placed within the wound after TWB-103 administration and before covering the wound with the Tegaderm™ transparent film. It is appropriate that the collagen dressing loosely fills the wound after it is applied. Prewetting of the collagen dressing is not suggested since TWB-103 contains sufficient liquid.

(2) Then, gauze pad, additional dressings, fixation bandages or sleeves, etc. may be applied over the wound for further protection.

(3) Gauze pad and dressing on top of Tegaderm™ transparent film should be replaced as frequently as needed for good wound care.

Note: For consistency, only one type of collagen dressings may be used for each patient. "One type of dressings" means the dressings under the same medical device approval number by Taiwan Ministry of Health and Welfare which normally covers products with various dimensions. The suitable collagen dressings are those which have no obvious cytotoxicity and do not physically obstruct the subsequent weekly administration of TWB-103 (the bulk of the dressing can be gently removed with saline rinse). The collagen dressings are of bovine or porcine origin since only patients non-allergic to bovine or porcine materials are included in this study.

Self-Care by Patient between Hospital Visits

Between applications of TWB-103 in hospital, if the transparent film becomes loose or displaced, fluid strike-through occurs or the area adhered by the film becomes itchy, the following management is applied:

(1) The gauze, dressing and Tegaderm™ transparent film are removed.

(2) The wound is gently cleaned with gauze moisturized with normal saline, then pat-dried with gauze. No effort is required to remove the residual TWB-103 (or the collagen dressing, if it is present) on wound bed.

(3) The wound is applied with appropriate wound dressing following instruction of the investigator (see below). No additional TWB-103 is reapplied until next scheduled application.

(4) Investigator provides instruction for appropriate dressing and sufficient amount of dressing to be applied by the subject at home based on the following principles:

- The dressing maintains the wound at moist condition, e.g. using Mepilex® or Mepilex® Border for high exudates wounds and using Mepilex® Border Lite or Mepilex® Lite for low-medium exudate wounds.
- The dressing is retained on wound by ways that will not cause itchiness or irritation to the subject, e.g. by adhesives well tolerable to the subject, by elasticated bandages, such as Tubifast®.
- The dressing should not stick to the wound bed, to avoid damaging the newly formed tissue.
- The dressing should be able to be removed completely with easiness, to avoid interfering with subsequent TWB-103 treatment.

(5) In principle, dressings with anti-microbial activity should not be used for study wounds during TWB-103 treatment period unless approved by sponsor after consultation. Sponsor's approval will be based on the confirmation that the anti-microbial activity of the dressing is not toxic to human dermal fibroblast or keratinocyte. Likewise, the use of topical treatment for infection should also have sponsor's approval. However, systemic treatments (oral administration) to control infection (≤ Grade 2) are allowed.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The sponsor is responsible for supplying the investigator(s)/study site(s) with the investigational products (IP). The IP must not be used for the purposes other than this trial, and the clinical trial pharmacist or designated qualified personnel must record the quantity of IP supplied/returned by/to the sponsor to maintain clear drug inventory control. Acquisition of IP from pharmacy for clinical administration as well as the amount of IP applied to each wound should also be recorded.

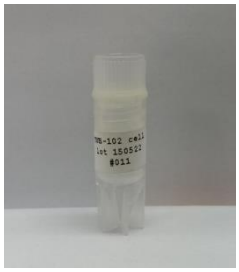
The investigator or designated qualified personnel must retain all unused and leftover IP until the study monitor has confirmed accountability data and the sponsor has given instruction for the final handle of the IP. Any discrepancy noted will be investigated, resolved, and documented prior to returning or destruction of unused study drug. Leftover or unused IP should be returned to the sponsor for destruction or be destroyed at the study site according to standard institutional procedures after drug accountability has been conducted by the sponsor or representative.

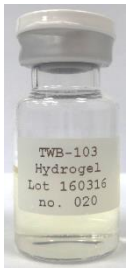
6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

TWB-103 used in this study will be prepared, packaged, and labeled under the responsibility of a qualified person at sponsor with Standard Operating Procedures (SOPs) of sponsor, Good Tissue Practice (GTP) guidelines, International Conference on Harmonization Good Clinical Practice (ICH GCP)

guidelines, and applicable local laws/regulations. TWB-102 Cell is supplied in a frozen plastic vial, with the secondary package of aluminum pouch. TWB-103 Hydrogel is supplied in a refrigerated glass vial, with the secondary package of aluminum pouch. The product will be labeled with descriptions of "Clinical trial use only" as well as other required information in the local language according to the regulatory requirements.

The Figure below shows the representative appearance of IP in primary and secondary packages.

Primary package of TWB-102 Cell	**TWB-102 Cell vial in aluminum pouch**
	

Primary package of TWB-103 hydrogel	**TWB-103 hydrogel vial in aluminum pouch**
	

6.2.3 PRODUCT STORAGE AND STABILITY

Once packaged and stored at -70 ~ -80ºC, TWB-102 Cell has at least 6 months of shelf-life. TWB-102 cell vials should be directly transferred from dry ice (shipping condition) to a freezer which has been validated for maintaining temperature not higher than -70ºC. If no suitable freezer is available, a well-insulated container loaded with dry ice can be used. In such case, the TWB-102 cell vial should be fully buried within dry ice until use.

Stored at 2~6ºC, the TWB-103 Hydrogel has 12 months of shelf life after manufacturing. TWB-103 hydrogel vials should be stored in a validated 4ºC refrigerator. The hydrogel is in gel-form at cold temperature and the vials can be stored in standing or lying position.

The two components of TWB-103 are stored separately but will be used together by the bedside.

6.2.4 PREPERATION

TWB-103 should be prepared by mixing a vial of TWB-102 cells (0.4 mL) with 4.6 mL of hydrogel in a 5 mL syringe and let the mixture cool to thicken enough for the convenience of applying it to wound. A water-bath set at 37ºC is required for the procedure. Briefly, a hydrogel vial is removed from the aluminum pouch and put in a water bath to be warmed to liquefy. When hydrogel is fully liquefied (taking about 20 min), a cell product in aluminum pouch is removed from the ultra cold storage (deep freezer or dry ice), then the cell vial is immediately removed from the pouch and put in the water bath for quick thaw. It usually takes about 80 seconds to thaw the cells. After thaw, the cell solution from one vial is drawn into a 5 mL-syringe through an 18-G needle, and then 4.6 mL of hydrogel is drawn into the same syringe. The hydrogel and cells are thoroughly mixed in the syringe. If less than one mL of TWB-103 will be applied to the wound, the mixture is transferred to 1 mL- syringe through a syringe connector. The syringe is then set aside at room temperature until it thickens and convenient to be applied onto the wound. If the wound is deep and there is no concern for the IP run-off from wound, the IP need not thicken before applied to the wound.

After the cell vial is thawed, the cells should be soon mixed with hydrogel. The maximum time between cell-thaw and mixing with hydrogel is 30 minutes. The prepared TWB-103 (i.e. mixture of thawed cells and hydrogel) is stable at room temperature (or up to 37ºC) for at least 2 hours, providing adequate flexibility of preparation time for the study staff.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

As this is an open-label study, no blinding will be used in this study.

6.4 STUDY INTERVENTION COMPLIANCE

TWB-103 will be administered at the study site at scheduled visits. Subject's compliance should not be a severe issue. However, the study monitor will assure the subject's compliance with the study protocol.

6.5 CONCOMITANT THERAPY/MEDICATION

Concomitant therapy is defined as any therapy including surgeries, vaccines, prescriptions, or over the counter medications. Subjects are allowed to receive routinely used medications or treatments for other indications which is judged by the investigator as not affecting the efficacy and safety assessments of this study. All therapies taken by the subject prior to the Screening Visit as well as throughout the study will be recorded on the appropriate page of the case report form (CRF)/electronic CRF (eCRF). Therapies will be categorized as follows:

- Major surgeries: will be recorded on a life time base
- Therapies for diabetic lower limb ulcer: will be recorded up to 2 months before Screening Visit
- All other medications: will be recorded up to 30 days before Screening Visit

This record will include the name of the therapy, frequency, unit dose, routes, dates of the drug is started and stopped (if medication/therapy is not ongoing), and the indication for the use of the drug.

6.6 PROHIBITED THERAPY/MEDICATION

Prohibited therapy or medication are defined as below:

- Any other investigational drug, device or biological/bioactive treatment during the study period.
- Any treatment/therapy applied to target wound area that may affect the efficacy evaluation of study medication at the investigator's discretion, such as revascularization, hyperbaric oxygen therapy, negative-pressure wound therapy, during TWB-103 treatment period.
- Dressings with anti-microbial activity should not be used for study wounds during TWB-103 treatment period unless approved by sponsor after consultation.
- Antibiotics administered via intravenous injection or intramuscular injection during TWB-103 treatment period.

6.7 STANDARD OF CARE FOR DIABETIC LOWER LIMB ULCERS

Standard care will be documented in CRF including the type of care (e.g., extent of debridement, use of concomitant medications), timing, and frequency. Parameters for consideration in choosing standard care procedures for diabetic lower limb ulcer include, but not limited, the following:

- Debridement: Removal of necrotic or infected tissue. During TWB-103 treatment period, if judged by the investigator as appropriate, lesser wound debridement is allowed. However, if major surgery is required, the subject should be withdrawn from this study.
- Off-loading: During study period, off-loading devices, such as clutches, wheel chair, off-loading

shoes, etc., may be applied as judged by Investigator.

- Revascularization or other treatment to improve circulation. However, revascularization is prohibited during treatment period (Visit 2~Visit 13).
- Maintenance of a moist wound environment.
- Management of wound infection: If the target wound has low grade infection (≤ Grade 2) during TWB-103 treatment, whether to stop TWB-103 treatment will be judged by the investigator and appropriate treatment to control the infection will be provided at the investigator's discretion. However, if wound is infected (> Grade 2) during TWB-103 treatment or the treatment for the ulcer wound infection requires subject's hospitalization, intravenous infusion or muscular injection of antibiotics, TWB-103 treatment must stop and be replaced by appropriate treatment to control the infection.
- Wound cleansing
- Good blood glucose control: Considering each subject's physical and physiological condition, "good blood glucose control" is defined as blood glucose level as good as possible with the help of physicians. Subjects will have appropriate medication and education to control the blood glucose level. The investigator will also monitor subjects' blood glucose level through the laboratory tests, including fasting glucose and HbA1c test results, collected during this study.

7 STUDY INTERVENTION DISCONTINUATION AND SUBJECT WITHDRAWAL

7.1 STUDY INTERVENTION DISCONTINUATION

If the target wound has low grade infection (≤ Grade 2) during TWB-103 treatment, TWB-103 treatment may continue or discontinue as judged by the investigator and appropriate treatment to control the infection will be provided at the investigator's discretion. However, any subject with > Grade 2 skin infection in the ulcer wound, or the treatment for the ulcer wound infection requires subject's hospitalization, intravenous infusion or muscular injection of antibiotics, must not receive any further TWB-103 treatment.

If allergy is judged as likely due to the investigational product, it is dangerous to continue using the investigational product. Therefore, any subject who experiences ≥ Grade 1 allergic reaction at least possibly related to the investigational product (section 8.4.3.2) must stop TWB-103 immediately.

During TWB-103 treatment period, if judged by the investigator as appropriate, lesser wound debridement is allowed. However, if major surgery is required, the subject should stop TWB-103 treatment.

7.2 SUBJECT WITHDRAWAL FROM THE STUDY

A subject may withdraw voluntarily or involuntarily from the trial due to any of the following conditions:

1. The subject voluntarily decides to withdraw her/his consent.
2. The subject's pregnancy is confirmed after enrollment till last dose of TWB-103.
3. The subject received or plans to receive any prohibited therapy as defined in section 6.6.
4. The subject dies or is lost to follow-up.
5. Any pathological event, clinical adverse event, laboratory abnormality, intercurrent illness, or any occurrence or change in the subject's status giving indication to the investigator that further participation in the study may not be the best interest of the subject.
6. The subject develops > Grade 2 skin infection in the ulcer wound, or the treatment for the ulcer wound infection requires subject's hospitalization, intravenous infusion or muscular injection of antibiotics.
7. The subject experiences ≥ Grade 1 allergic reaction at least possibly related to the investigational product (section 8.4.3.2).
8. The subject requires a major surgery debridement.

9. The study is terminated prematurely by the investigator, research institution, sponsor, IRB/IEC or regulatory authorities.

If the withdrawal is decided during TWB-103 treatment period, the withdrawn subject will be encouraged to complete the assessments of Week 12, within 1 week of the withdrawal decision, as End of Study (EOS) visit before exiting from the study. Laboratory, pregnancy and PRA tests done within 1 week prior to EOS visit are acceptable.

If the withdrawal is decided in the follow-up period, the withdrawn subject will be encouraged to complete the assessments of Week 36, within 2 weeks of the withdrawal decision, as EOS visit before exiting from the study.

The reason for withdrawal must be recorded. If a subject is withdrawn due to an AE, every effort will be made to follow the event until event resolves or stabilizes at a level acceptable to the investigator.

7.3 LOST TO FOLLOW-UP

A subject will be considered lost to follow-up if he or she fails to return for any scheduled visit and is unable to be contacted by the study site staff.
The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit within time window and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the subject. These contact attempts should be documented in the subject's medical record or study file.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

The baseline value of each assessment will be the value assessed closest and prior to the first dose of TWB-103.

8.1.1 ULCER EVALUATION-WOUND SIZE

The surface area of the ulcer will be estimated in the manner as follows. The ulcer along with an ulcer measuring ruler (OPSITE™ transparent film) will be photographed. An OPSITE™ transparent film will be placed on the wound and the wound area is traced on the film. The images will be processed and analyzed using the ImageJ software package (National Institutes of Health, http://rsb.info.nih.gov/ij/download.html). The ImageJ software read the computed tomographic data and calibrated the images automatically.

Wound closure was defined as “full epithelialization of the wound with the absence of drainage or dressing requirement and without sign of abscess under the epithelium”. An ulcer was considered healed only after closure was re-confirmed at the visit 2 weeks later. Therefore, a wound closure should be observed at three consecutive visits (i.e. a duration of 2 weeks) for an ulcer to be deemed as healed. For example, when the wound closure is first observed at Week 8 and subsequently at Week 9 and Week 10, the ulcer is “healed”. If the subject misses Week 9 or Week 10 visits or wound closure is not present at Weeks 9 and 10, this ulcer is not defined as “healed on week 8”.

8.1.2 ULCER EVALUATION-WOUND GRADING

The grading of ulcer will be performed at screening only as part of the eligibility check according to the Wagner Grading System[7], which is described below:

Grade 1: Superficial diabetic ulcer
Grade 2: Ulcer extension
1. Involves ligament, tendon, joint capsule or fascia
2. No abscess or osteomyelitis
Grade 3: Deep ulcer with abscess or osteomyelitis
Grade 4: Gangrene to portion of forefoot
Grade 5: Extensive gangrene of foot

8.1.3 ULCER EVALUATION-WOUND VOLUME (OPTIONAL)

The wound volume may be measured by using 3D camera and analysis software, which will be provided by the sponsor.

8.1.4 ULCER EVALUATION-GRANULATION RATE AND QUALITY OF GRANULATION TISSUE

Granulation rate and quality of granulation tissue will be recorded by comments from the investigator. Healthy granulation tissue is pink to red, moist, and shiny. Forming of healthy granulation tissue provides foundation for epithelialization and is an indicator of healing. Stalled granulation process often associates with prolonged inflammation, lack of vascularization, infection or other abnormalities. Hypergranulation may occur during wound healing, which may deter epithelialization. Should it occur, the excessive tissue may be trimmed off surgically before administration of TWB-103. Images before and after excision should be recorded.

8.2 SAFETY AND OTHER ASSESSMENTS

The baseline value of each assessment will be the value assessed closest and prior to the first dose of TWB-103.

8.2.1 INFORM CONSENT

The investigator must explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks involved, and any predicted discomforts. Each subject will be informed that participation in the study is voluntary and that he/she can withdraw their participation at any time.

All subjects must provide signed and dated informed consent prior to any study-related procedures. Only the Independent Ethics Committee (IEC) or Institutional Review Board (IRB) and the applicable regulatory authorities approved ICF can be used.

8.2.2 SCREENING, ELIGIBILITY, AND ENROLLMENT

Screening is defined as the process for identifying a candidate for the study and evaluating their eligibility to participate. Eligibility should be thoroughly checked by the investigators during screening and at Day 1 Visit before the application of TWB-103 based on the inclusion and exclusion criteria. Procedures for screening can be done between signing ICF and Day 1. However, procedures should be completed before

the TWB-103 application if performed on Day 1; in this case, the same procedures scheduled for Day 1 can be waived. The eligibility re-check at Day 1 Visit will be based on the results from Day 1 Visit (vital signs and physical exam) and other information collected during screening. A subject is recognized as enrolled into the study if this subject consented and is screened, with eligibility verified.

8.2.3 DEMOGRAPHICS

Demographic data, including the subject's birth year, race, and gender, will be recorded at the Screening Visit.

8.2.4 MEDICAL HISTORY

General medical history within 1 year before Screening Visit and malignancy on a life time basis should be recorded at Screening Visit. The history of diabetic lower limb ulcer for the lifetime base will be recorded in the "diabetic lower limb ulcer" part of the CRF, separately. All history recorded will include date of onset, diagnosis and current status.

8.2.5 LABORATORY, PREGNANCY AND PANEL REACTIVE ANTIBODY TESTS

Laboratory tests to be measured in this study will consist of the followings:

- Hematology: hemoglobin, hematocrit, RBC, platelet, and WBC with differential counts, ANC
- Biochemistry: total bilirubin, ALT, AST, fasting glucose, HbA1c, serum albumin, blood urea nitrogen (BUN), and serum creatinine

Laboratory and pregnancy tests performed within 1 week prior to Week 5 Visit, or within 1 week prior to Week 12 or End-of-Treatment visit, are acceptable.

Pregnancy tests can be done with serum or urine. Pregnancy tests can be waived if the female subject has menopausal for over 1 year, total hysterectomy, bilateral salpingo-oophorectomy or other clinical condition that completely eliminates childbearing potential, confirmed by the investigator.

For panel reactive antibody (PRA) test, all patients will be tested with class I human leukocyte antigen (HLA). PRA test performed within 1 week prior to Week 12 or End-of-Treatment visit, are acceptable.

When withdrawal occurs during TWB-103 treatment period, laboratory, pregnancy and PRA tests done within 1 week prior to EOS visit are acceptable.

8.2.6 PHYSICAL EXAMINATION, HEIGHT, AND WEIGHT

Height and weight will be collected at Screening Visit only.
Physical examination conducted in this study will include general appearance, skin, eyes, ears, nose, throat, head and neck, heart, chest and lungs, abdomen, extremities, lymph nodes, musculoskeletal, neurological, etc.

8.2.7 VITAL SIGNS

Vital signs measurement will consist of systolic/diastolic blood pressure, respiratory rate, pulse rate or heart rate, and body temperature. Respiratory rate, pulse/heart rate and blood pressure (systolic/diastolic) will be obtained after the subject has been at rest for at least 5 minutes in a sitting position.

8.2.8 ANKLE BRACHIAL INDEX

The ankle-brachial index (ABI) is the ratio of the blood pressure at the ankle to the blood pressure in the upper arm. The ABI is calculated by dividing the systolic blood pressure at the ankle by the systolic blood pressure in the arm.

8.2.9 STANDARD OF CARE

The choice of standard-of-care will be at investigator's discretion based on the physical condition of the subject. Subjects will receive standard of care for at least two weeks during screening as part of the eligibility check. If required, the investigator can provide standard of care to the subject during the TWB-103 treatment period. However, revascularization is prohibited during treatment period (Visit 2~Visit 13). The investigator will also provide standard of care to subjects in the follow-up period as a protection for subjects who have confirmed wound healing or as a treatment for those still have open wound. Refer to section 6.7 for details of standard of care.

8.2.10 ASSESSMENT OF ADVERSE EVENT

All AEs will be assessed for severity by the study investigator based on National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) 5.0. Further, the investigator has to judge if the AE is IP (TWB-103)-related. Refer to section 8.4 for details of AE definition and reporting.

8.3 STUDY SCHEDULE

The assessments or procedures to be performed at each study visit are listed below. The results of protocol specific assessments and procedures will be recorded in the source documents for all study

subjects and on the appropriate page of the CRF. Unscheduled visit will be arranged when investigator considered necessary.

8.3.1 SCREENING VISIT (VISIT 1)

The Screening Visit will be scheduled after signing ICF and within 4 weeks prior to Day 1 (Day -28 ~ Day 1). Procedures performed between signing ICF and Day 1 are acceptable. However, procedures should be completed before the TWB-103 application if performed on Day 1; in this case, the same procedures scheduled for Day 1 can be waived.

- Obtain signed informed consent
- Assign identifier number
- Demographics
- Medical history
- Medication history
- Physical examination
- Vital signs
- Pregnancy test (for applicable subjects only)
- Laboratory test
- PRA test
- Eligibility
- Ulcer evaluation (wound size, wound volume (optional), granulation rate and quality of granulation tissue, and grading; before and after standard of care for at least 2 weeks)
- Ankle Brachial Index
- Standard of care for at least 2 weeks
- Concomitant medication
- Adverse event

8.3.2 TREATMENT DAY 1 VISIT (VISIT 2)

- Physical examination
- Vital signs
- Standard of care (when standard of care is required, revascularization is prohibited during treatment period)
- Ulcer evaluation (wound size, wound volume (optional), granulation rate and quality of granulation tissue)
- Eligibility
- TWB-103 application

- Concomitant medication
- Adverse event

8.3.3 TREATMENT VISITS (VISIT 3~13)

Additional treatment visits will be performed weekly from week 1 ~ week 11.
(Day 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, all with a visiting window of ± 2 days)

- Physical examination
- Vital signs
- Pregnancy test (for applicable subjects only; only for Week 5, performed within 1 week before scheduled visit is acceptable)
- Laboratory tests (only for Week 5, performed within 1 week before scheduled visit is acceptable)
- Standard of care (when standard of care is required, revascularization is prohibited during treatment period)
- Ulcer evaluation (wound size, wound volume (optional), granulation rate and quality of granulation tissue)
- TWB-103 application
- Concomitant medication
- Adverse event

8.3.4 WEEK 12/END OF TREATMENT VISIT (VISIT 14)

(Day 85, with a visiting window of ± 2 days, or for early confirmed wound closure)
(If the withdrawal is decided during TWB-103 treatment period, the withdrawn subject will be encouraged to complete the assessments of Week 12, within 1 week of the withdrawal decision, as End of Study visit, before exiting from the study.)

- Physical examination
- Vital signs
- Pregnancy test (for applicable subjects only; performed within 1 week before scheduled visit is acceptable)
- Laboratory tests (performed within 1 week before scheduled visit is acceptable)
- PRA test (performed within 1 week before scheduled visit is acceptable)
- Standard of care (if necessary)
- Ulcer evaluation (wound size, wound volume (optional), granulation rate and quality of granulation tissue)
- Concomitant medication
- Adverse event

8.3.5 FOLLOW-UP VISITS (VISIT 15-21)

(Week 13, 14, 16, 20, 24, 30, 36 or 1 week, 2 weeks, 4 weeks, 8 weeks, 12 weeks, 18 weeks, 24 weeks after End-of-Treatment for early confirmed wound closure)
(Day 92 (± 2), 99 (± 2), 113 (± 3), 141 (± 6), 169 (± 6), 211 (± 6), 253 (± 6) or 7 (± 2), 14 (± 2), 28 (± 3), 56 (± 6), 84 (± 6), 126 (± 6), 168 (± 6) after End-of-Treatment for early confirmed wound closure)
(If the withdrawal is decided during the follow-up period, the withdrawn subject will be encouraged to complete the assessments of Week 36, within 2 weeks of the withdrawal decision, as End of Study visit, before exiting from the study.)

- Physical examination
- Vital signs
- Standard of care (if necessary)
- Ulcer evaluation (wound size, wound volume (optional), granulation rate and quality of granulation tissue)
- Concomitant medication
- Adverse event

8.3.6 UNSCHEDULED VISIT

Unscheduled visits will be arranged when investigators consider necessary.

- Physical examination
- Vital signs
- Concomitant medication
- AE/SAE reporting
- Other actions to be performed will be per investigator's decision

8.4 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.4.1 DEFINITION OF ADVERSE EVENTS (AE)

Based on ICH GCP, adverse event (AE) means any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product or intervention and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product/intervention, whether or not related to the medicinal (investigational) product/intervention.
Pre-existing abnormalities are considered as AE only when the conditions escalate.

Increment in wound size by 30% comparing to baseline should be reported as an AE irrespective of causality to the investigational products.

8.4.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes (based on ICH GCP):

- Death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

8.4.3 CLASSIFICATION OF AN ADVERSE EVENT

8.4.3.1 Severity of Event

All AEs will be assessed for severity by the study investigator using the following guidelines (based on NCI-CTCAE 5.0):

- Grade 1 (Mild): Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2 (Moderate): Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*
- Grade 3 (Severe): Hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**
- Grade 4 (Life threatening): Urgent intervention indicated
- Grade 5 (Death): Death

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

8.4.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study product assessed by the investigator who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study agent/intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study agent/intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- Probably Related: There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time sequence to administration of the study agent/intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- Possibly/Potentially Related: There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- Unlikely to be related: A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the investigational product) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

- Not related: The AE is completely independent of study agent/intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the investigator.

8.4.3.3 Expectedness

Sponsor and investigators will be responsible for determining whether an AE is expected or unexpected. An AE is considered "unexpected" if it is not listed in the investigator's brochure (IB), package insert, or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the protocol, as amended.

Expected adverse reactions are AEs that are known to occur for the study intervention being studied and should be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction. IB will be the source of the reference safety information used to determine the expectedness of the AE. Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study intervention.

8.4.4 ADVERSE EVENT REPORTING

AEs may be volunteered spontaneously by the study subject, discovered as a result of general questioning by the study staff, or determined by physical examination. All AEs will be recorded on the CRF. For all AEs, the investigator must pursue and obtain adequate information both to determine the outcome of the AE and to assess whether it meets the criteria for classification as a serious AE requiring immediate notification. Follow-up of the AE, even after the date of study drug discontinuation, is required if the AE persists.

8.4.5 SERIOUS ADVERSE EVENT REPORTING

The investigator will report to the sponsor/CRO contact any SAE within 24 hours of the investigator's knowledge of the event, regardless of the expectedness and causality to the study intervention. The reporting of the SAE to the regulatory authorities/IEC/IRB will comply with the local regulations and requirements.

Nevertheless, the following events will not be reported as a SAE:

- Hospitalization due to social reasons in absence of an adverse event
- Hospitalization due to surgery or procedure planned before entry into the study (must be documented in the CRF)

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the subject is stable. Other supporting documentation of the event may be requested by sponsor and should be provided as soon as possible.

The sponsor/CRO will be responsible for notifying the regulatory authorities of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after first acknowledged by the Sponsor. A complete report should be provided within 15 calendars by

sponsor's acknowledgement and must include an assessment of the importance and implication of the findings and/or previous experience on the same or similar medical products.
Serious, unexpected suspected adverse reactions that are not fatal or life-threatening must be filed as soon as possible but no later than 15 calendar days after first knowledge by the sponsor that a case qualifies.

8.4.6 PREGNANCY REPORTING

Any patient or patient's partner who becomes pregnant during the treatment period should be recorded and the information regarding this pregnancy should be collected. Any pregnancy occurs during the 30 days following the last dose of investigational product should also be reported and accompanied information should also be collected. When female patient or male patient's partner suspects a pregnancy during the treatment period or within 30 days of the last dose, a pregnancy test should be conducted. Once pregnancy is confirmed, the patient should stop the administration of investigation product immediately, if patient is still during the treatment period. The pregnancy should be reported to the sponsor using the pregnancy report form. The Obstetrician/Gynecologist of the female patient or male patient's partner should be notified the trial information. Because information of specific tests regarding this investigational product is not clear yet, whenever possible, a pregnancy should be followed to term or to any premature terminations reported. Additionally, the status of the mother and child should be reported to the sponsor after delivery. Although pregnancy occurring during the trial is not considered as an adverse event, any pregnancy complications should be recorded as AEs or SAEs.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Not applicable for this study.

9.2 SAMPLE SIZE DETERMINATION

It is planned to enroll 10 subjects to achieve 8 evaluable subjects.
A subject is considered evaluable if the subject fulfills the criteria of per-protocol population.

9.3 POPULATIONS FOR ANALYSES

The following populations will be introduced for statistical analysis.

Intend-to-treat (ITT) population:

- All enrolled subjects who have received at least one application of TWB-103
- With any post-treatment evaluation

Per-protocol (PP) population:

- A subset of ITT population
- Fulfill all inclusion and exclusion criteria
- Dosed with at least 9 applications of TWB-103, OR with early confirmed wound closure and at least 75% treatment application
- With efficacy endpoint measurement at wound assessment
- Not received any prohibited treatment during TWB-103 treatment period

Safety evaluations will be performed on ITT population. Analysis of efficacy endpoints will be performed on ITT and PP population. The main conclusion will be made on ITT population analysis results of the primary endpoint.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

There will be a separate formal statistical analysis plan (SAP) to be completed prior to database/data lock. The SAP generally includes additional statistical analysis detail (e.g., more detail of analysis populations, summary of statistical strategies). Any deviations from the planned analysis as described in the SAP will be justified and recorded in the final clinical study report.

The baseline value of each assessment will be the value assessed closest and prior to 1st application of TWB-103.

Descriptive statistics will be provided for all endpoints.

Continuous variables will be summarized by reporting the number of observations, mean, standard deviation, median, interquartile range (IQR = third quartile (Q3) - first quartile (Q1)), Q1, Q3, minimum and maximum.

Categorical variables will be summarized using frequency tables showing the number and percentage of subjects within a particular category.

Ordinal endpoints will be presented by transition tables.

Healing time from baseline to wound closure will be estimated by using Kaplan-Meier method.

The data changes from baseline value will be analyzed by one-sample t-test or Wilcoxon sign rank test. The baseline value of each assessment will be the value assessed closest and prior to 1st application of TWB-103. The Last Observation Carried Forward (LOCF) method will be applied for dealing missing data of the efficacy endpoints.
All comparisons will be conducted with significance level of 0.05, using 2-tailed tests where 95% confidence interval for difference and corresponding *p*-value will be presented.

9.4.2 ANALYSIS OF THE PRIMARY ENDPOINT

Adverse events observed during the study will be coded using Medical Dictionary for Regulatory Activities (MedDRA) and will be reported by System/Organ/Class (SOC) classified in MedDRA as appropriate.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINTS

For changes of wound area and (if data available) wound volume measured by 3D camera from the baseline values, data will be analyzed by using one-sample t-test or Wilcoxon sign rank test on ITT and PP population.

9.4.4 SAFETY ANALYSES

Adverse events will be coded by employing MedDRA. Each AE will be counted once only for a given subject. Severity, frequency, and relationship of AEs to study intervention will be presented by System Organ Class (SOC) and preferred term groupings. The information of AEs, such as start date, stop date,

severity, relationship, outcome, and duration, should be reported. Serious treatment-emergent AE should be presented either in a table or a listing.

Changes in physical examinations will be displayed for each individual system. Net changes from pre-treatment laboratory test results and vital signs will be analyzed by descriptive statistics.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Demographics and baseline characteristics will be will be summarized by using descriptive statistics.

9.4.6 PLANNED INTERIM ANALYSES

Interim analyses are not planned for this study.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

Consent forms describing in detail the study intervention, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention or administering study intervention. Different Informed Consent Forms (ICFs) subject to regulations and guidelines applicable to clinical studies of local countries and IRB/IEC requirements are submitted to regulatory authorities and IRBs/IECs, respectively, for approval.

In obtaining and documenting informed consent, the investigator must comply with regulations and guidelines applicable to clinical studies of local countries and IRB/IEC requirements and should adhere to International Conference on Harmonization Good Clinical Practice (ICH GCP). Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval for the protocol and the written informed consent form(s) and any other written information to be provided to the subjects. A separate screening consent will not be used for this study. The study consent must be signed prior to conducting study screening procedures.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the subject's study participation. Consent forms will be IRB/IEC-approved and the subject will be asked to read and review the document. The investigator will explain the study to the subject and answer any questions that may arise. A verbal explanation will be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as subjects. Subjects will have the opportunity to carefully review the written consent form and ask questions prior to signing. The subjects should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The subject will sign the informed consent document prior to any procedures being done specifically for the study. Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the signed informed consent document will be given to the subjects for their records. The informed consent process will be conducted and documented in the source document (including the date) and the ICF will be signed before the subject undergoes any study-specific procedures. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to subjects, investigator, the sponsor/CRO and regulatory authorities, whichever is applicable. If the study is prematurely terminated or suspended, the Principal Investigators (PIs) will promptly inform subjects, the IRB/IEC, and sponsor/ contract research organization (CRO), whichever is applicable and will provide the reason(s) for the termination or suspension. Subjects will be contacted, as applicable, and be informed of changes to study visit schedule.
Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects, i.e. ≥ 3 subjects received TWB-103 have developed grade 3 or above AEs according to CTCAE 5.0, independent of attribution. If the cause of AE can be identified and resolved, the study may be resumed.
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRBs/IECs and/or regulatory authorities.

10.1.3 CONFIDENTIALITY AND PRIVACY

Subject confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor/CRO. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidentiality. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB/IEC, or regulatory authorities may inspect documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB/IEC, policies of regulatory authorities, or sponsor requirements.

Subject data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the responsible CRO. This will not include the subject's contact or identifying information. Rather, individual subjects and their data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by responsible CRO staff will be secured and password protected.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the responsible CRO. After the study is completed, the archived data will be stored at CRO and transmitted to the sponsor. Future use of the archived data will need the permission of the sponsor. Data will not be removed if the subject is withdrawn.
The biological samples collected will be used only under the scope of this clinical study. Once the completion of analysis, the biological samples will be destroyed following the regulations and guidelines of each study site.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

The name and contact information of the key roles are provided in the following:

- Sponsor: Transwell Biotech Co., Ltd.
  No.12. YanFa 2nd Road, HsinChu Science Park, 30076, HsinChu, Taiwan
  TEL: +886-3-5670399

10.1.6 SAFETY OVERSIGHT

Safety oversight will be under the direction of the investigators and the sponsor.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of subject are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICH GCP, and with applicable regulatory requirement(s).

A separate plan will describe in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports. A monitoring plan usually focus on preventing or mitigating important and likely risks, identified by a risk assessment, to critical data and processes.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Quality control (QC) procedures will be implemented on the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the case report form (CRF)/electronic CRF (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs and concomitant medications) and clinical laboratory data will be entered into data capture system, a regulatory authorities compliant data capture system provided by the

responsible CRO. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be recorded/entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for a period complying with regulations and guidelines applicable to clinical studies of local countries after the last approval of a marketing application and until there are no pending or contemplated marketing applications or until a period complying with regulations and guidelines applicable to clinical studies of local countries have elapsed since the formal discontinuation of clinical development of the study intervention. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol or ICH GCP, requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations following the regulatory requirements of health authorities and IRB/IEC. All deviations must be sent to the reviewing IRB/IEC per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB/IEC requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

The data and information associated with this study may be used by sponsor now and in the future for the purposes of presentation, publication at discretion of sponsor or for submission to regulatory agencies. In addition, relative to the release of any proprietary information, sponsor reserves the right of prior review of any publication or presentation of data from this study.

In signing this protocol, the investigator agrees to the release of the data from this study and acknowledges the above publication policy.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

11 ABBREVIATIONS

ABI	Ankle Brachial Index
AE	Adverse Event
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
Bun	Blood Urea Nitrogen
CRF	Case Report Form
CRO	Contact Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DFU	Diabetic Foot Ulcer
DM	Diabetic Mellitus
DNA	Deoxyribonucleic Acid
EOS	End of Study
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GTP	Good Tissue Practice
HbA1c	Hemoglobin A1c
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IP	Investigational Product
IQR	Interquartile Range
IRB	Institutional Review Board
ITT	Intention-To-Treat
IUD	Intrauterine Device
IUS	Intrauterine System
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
NCI	National Cancer Institute
OGTT	Oral Glucose Tolerance Test
PHMB	Polyhexamethylene Biguanide
PI	Principal Investigator
PP	Per-protocol
PRA	Panel Reactive Antibody
QC	Quality Control
RBC	Red Blood Cell
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure

STZ	Streptozotocin
TFDA	Taiwan Food and Drug Administration
ULN	Upper Limit Normal
WBC	White Blood Cell

12 REFERENCES

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13 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
1.0	06 Jul 2018	Initial version	N/A
1.1	17 Aug 2018	Revise the guideline of standard of care, the use of antibiotics for infection (patient self-care, prohibited therapy/medication, the criteria of study intervention discontinuation and subject withdrawal), the duration of screening period, the storage of TWB-102 cell vials, and the future use of specimens. Add the upper limit of HbAlc as the inclusion criteria, and the test of panel reactive antibody.	Per the comments of TFDA
1.2	25 Sep 2018	Add the description to clarity the confirmation of wound healing. Add the recruiting rule (staggering) to the first 3 subjects. Add "good blood glucose control" to standard of care. Revise the stopping rule, definition of wound closure, inclusion criteria #10,	Per the comments of US FDA
1.3	12 Oct 2018	Revise the location and depth of ulcers. Add the lower limits for renal function, blood count and platelets count.	Per the comments of US FDA
2.0	10 Jan 2019	Revise the follow-up period, standard of care, and inclusion criteria.	Per the comments of Taiwan FDA and NTUH IRB
3.0	25 Mar 2019	Replace the collagen dressing product Kollagen resorb™ with similar products which are approved for marketing in Taiwan.	Per the comments of Taiwan FDA