STUDY PROTOCOL

A Randomized, Double-Blind, Vehicle-controlled, Parallel, Phase II Study to Evaluate Efficacy and Safety of CSTC1 in Patient with Diabetic Foot Ulcers

Project Number:	CSTC1-01
Investigational Product:	CSTC1
Sponsor:	Charsire Biotechnology Corp.

LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
[redacted]	[redacted]
CABG	Coronary Artery Bypass Graft
CMH test	Cochran-Mantel-Haenszel test
CRA	Clinical Research Associate
CRF	Case Report Form
[redacted]	[redacted]
CSTC1	CHARSIRE Trauma Complex
DFU	Diabetic Foot Ulcer
EC	Ethics Committee
EDC	Electronic Data Capture
GCP	Good Clinical Practice
[redacted]	[redacted]
[redacted]	[redacted]
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ITT	Intent-to-treat
IQR	Inter-quartile Range
[redacted]	[redacted]
LEA	Lower Extremity Amputations
NOAEL	No-observed-adverse-effect-level
[redacted]	[redacted]
PI	Principle Investigator
[redacted]	[redacted]
PP	Per-Protocol
PTCA	Percutaneous Transluminal Coronary Angioplasty
RBC	Red Blood Cell
SAE	Serious Adverse Event
SOP	Standard Operation Procedure
[redacted]	[redacted]
WBC	White Blood Cell

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PROTOCOL SUMMARY

Full Title	A Randomized, Double-Blind, Vehicle-controlled, Parallel, Phase II Study to Evaluate Efficacy and Safety of CSTC1 in Patient with Diabetic Foot Ulcers
Short Title	CSTC1 for Diabetic Foot Ulcers Phase II Study
Project No	CSCT1-01
Study Phase	Phase II
Sponsor	Charsire Biotechnology Corp.
Objectives	To evaluate the efficacy and safety of CSTC1 in patient with diabetic foot ulcers
Study Design	Randomized, double-blind, vehicle-controlled, multiple center, parallel study
Study Population	Subject aged at least 20 years old in Taiwan diagnosed with diabetic ulcer (which is selected by investigator and defined as the target ulcer) on the foot that not healing for at least 4 weeks
Number of Subjects	The sample size is determined to be 80 versus 20 subjects (4:1 ratio) for Treatment versus Control groups, 100 subjects in total. To ensure the completion of 100 evaluable subjects, around 125 subjects will be recruited.
Study Product, Dose, Route, Regimen	CSTC1 [redacted] topical application, 2 times daily
Duration of Administration	12 weeks of treatment or confirmed complete ulcer closure, whichever comes first, and up to 12 weeks of post-treatment follow-up for subject with confirmed complete ulcer closure after at most 12 weeks of treatment; Subjects failed to achieve complete ulcer closure will be arranged for 4 weeks of safety follow-up
Subject Assignment	Subjects who meet all eligible requirements for entry into the study will be randomized into one of the treatment group or vehicle control group in 4:1 ratio as shown below: <u>Treatment Group:</u> CSTC1 <u>Control Group:</u> Matched vehicle
Subjects Inclusion Criteria	A subject is eligible for the study if all of the following apply: 1. With either gender aged at least 20 years old 2. With at least one diabetic foot ulcer (including ulcers on the lower legs) and not healing for at least 4 weeks. The largest diabetic foot ulcer will be selected as target ulcer. If two or more ulcers have the largest size, the one with worst grade will be selected. If two or more ulcers have the largest size and grade, the one with longest duration will be selected.

	3. The target ulcer is classified as grade 1 to 2 ulcer according to modified Wagner system and with ulcer size of 1cm^2 to 50 cm^2 4. The target ulcer should be confirmed without active infection 5. Subject should be free of any necrosis or infection in soft and bone tissue; 6. Subject has signed the written informed consent form
Subjects Exclusion Criteria	Any subject meeting any of the exclusion criteria will be excluded from study participation. 1. With active osteomyelitis 2. With target ulcer size decreased by at least 30% after at least 2 weeks of standard-of-care-only period or any other recorded regular therapy before Randomization visit 3. With poor nutritional status (albumin < 2g/dl), poor diabetic control (HbA1c > 12%), anemia (hemoglobin < 8 g/dL), a leukocyte counts < 2,000/mm^3, abnormal liver function (AST, ALT>3 x upper limit of normal range) 4. Requiring treatment with systemic corticosteroids, immunosuppressive or chemotherapeutic agents within 28 days prior to Screening visit 5. Presence of necrosis, purulence or sinus tracts that cannot be removed by debridement 6. Receiving revascularization surgery or endovascular therapy performed <8 weeks before entry in the study 7. With known or suspected hypersensitivity to any ingredients of study product and vehicle 8. With coronary heart disease with myocardial infarction, coronary artery bypass graft (CABG), or percutaneous transluminal coronary angioplasty (PTCA) within 3 months prior to study 9. Pregnant or lactating or premenopausal with childbearing potential but not taking reliable contraceptive method(s) during the study period 10. Enrollment in any investigational drug trial within 4 weeks before entering this study 11. With any uncontrolled illness judged by the investigator that entering the trial may be detrimental to the subject
Primary Endpoint	**Efficacy**: The incidence of complete ulcer closure during up to 12 weeks of treatment period *Complete ulcer closure is defined as 100% skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits 2 weeks apart. Subjects with complete ulcer closure at week-12 visit and confirmed at week-14 visit will be considered as success.*

Secondary Endpoints	**Efficacy**: 1. The ulcer closure time *Defined as the time to complete ulcer closure* 2. The complete ulcer closure rate *The proportion of subjects with complete ulcer closure at each post-treatment visit* 3. Percentage change in ulcer size for each post-treatment visit *Calculated as (Ulcer size at post treatment visit - Ulcer size at baseline)/(Ulcer size at baseline)* **Safety**: 1. Adverse event incidence 2. Change in physical examination results 3. Net change from baseline in laboratory test results (include hemoglobin, hematocrit, RBC, platelet, WBC with differential counts, HbA1c, fasting glucose, AST, ALT, creatinine and BUN) 4. Net change from baseline in vital signs
Statistical Analysis	**Analysis population** Three data sets will be introduced for statistical analysis. *Intent-to-treat (ITT) population:* – All randomized subjects who have received at least one dose study medication. *Per-protocol (PP) population:* – A subset of ITT population – Fulfill all inclusion and exclusion criteria – Dosed with at least 8 weeks study drug or with early confirmed complete ulcer closure AND with at least 70% treatment compliance – Did not receive any prohibited treatment during treatment period – With any post-treatment assessment relevant to efficacy endpoint up to end of dosing period Efficacy endpoints will be analyzed on ITT and PP population. Demographics, baseline characteristics, and safety endpoints will be analyzed on ITT population. Conclusion of efficacy will be made according to the result of ITT population analysis. **Analysis** Demographics and baseline characteristics will be summarized for each group by using descriptive statistics.

The efficacy endpoints will compare the CSTC1 group with vehicle group. Incidence of ulcer closure and ulcer closure rate at endpoint will be analyzed with Cochran-Mantel-Haenszel test (CMH test). Ulcer closure time will be estimated by using Kaplan-Meier methods and compared between groups by using log-rank test. Percentage change in ulcer size will be analyzed by using Analysis of variance (ANOVA) model with treatment group and center as factors. Further efficacy analyses with ankle brachial index (ABI) (<0.7 or not) as stratification may be performed if necessary.

For safety analyses, adverse events will be reported by treatment groups and by physiological systems as appropriate. Incidence of adverse events between treatments will be analyzed by CMH test. 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.

Additionally, descriptive statistics will be provided for all of the endpoints. Frequency table will be provided for categorical data, while mean, standard deviation, maximum, minimum, median, inter-quartile range (IQR), and 95% two-sided confidence interval will be calculated for continuous measurements. In addition, endpoints of time to event will be presented by using the life table and Kaplan-Meier plot. The Kaplan-Meier estimates will also be provided for these time-to-event endpoints.

All treatment group comparisons will be conducted with significance level of 0.05.

SCHEDULE OF ASSESSMENTS

	Screening[1]	Initial[2] Phase	Randomization[3] (Baseline)	Dosing/Observation								Follow-up			
Visit No.	1	2	3	4	5	6	7	8	9	10	11[11]	12	13	14	15
Week relative to Baseline visit	-3~-2	-2~0	0	1	2	3	4	6	8	10	12	14	16	20	24
Visit Window (days)	NA	±4	NA	±4	±4	±4	±4	±4	±4	±4	±4	±4	±4	±4	±4
Informed consent signed and given	X														
Screening number assignment	X														
Randomization			X												
Demographic data & medical history	X														
Inclusion and exclusion criteria	X		X												
Pregnancy test for applicable subjects	X										X				
Ulcer Evaluation[4]	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examinations	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Ankle Brachial Index[5]	X														
Laboratory test: hemoglobin, hematocrit, RBC, platelet, WBC with differential counts, HbA1c, fasting glucose, AST, ALT, creatinine and BUN, albumin	X[6]		X[7]								X[8]				
Record concomitant medication(s)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Standard of Care		X	X	X	X	X	X	X	X	X	X				
Record adverse events (AE)[9]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Dispense trial medication			X	X	X	X	X	X	X	X					
Returned medication count				X	X	X	X	X	X	X	X				
Diary Dispensing			X												
Diary Return				X	X	X	X	X	X	X	X				
Complete exit form													X		X
Dismiss subject[10]													X		X

Footnote:

1: Could be on the same day as the first day of Initial Phase or randomization visit if subject's Initial Phase can be waived

2: Can be waived if subject's diabetic foot ulcer(s) has been treated for at least 2 weeks, and is still eligible to enter this study

3: Could be on the same day as the last day of Initial Phase

4,10: Subjects with confirmed complete ulcer closure up will be arranged for ulcer evaluation for follow-up period; Subjects not with complete ulcer closure will take 4 weeks of safety evaluation

5: The two blood pressures for calculating ABI, ankle and brachium, should be measured on the same side (left to left, right to right) and should be on the same side of target diabetic foot ulcer. However, the blood pressures of brachium may not be available for some patients. For such case, investigator is suggested trying to measure pressure of brachium of another side.
6: Test results within 14 days before Screening visit are acceptable; HbA1c test result within 28 days can be accepted. Albumin test will only be done at Screening and Randomization visits
7: Test results within 28 days before Randomization visit are acceptable
8: Test results within time window are allowed
9: AE should be recorded since informed consent is signed
11: For subject who has been confirmed complete closure before week-12 visit should be arranged for visit 11 assessment.

1. BACKGROUND AND RATIONALE

1.1 General Introduction

Diabetic foot ulcers (DFUs) are a common problem with clinically serious sequelae. It has been reported that annually, about 1% to 4% of those with diabetes, which is believed that more than 23 million people in the United States have, develop a foot ulcer; 10% to 15% of those with diabetes will have at least one foot ulcer during their lifetime. DFUs place patients at the risk of lower extremity amputations (LEAs), which is an extremely complicated. In the U.S., around 80,000 LEAs annually are conducted on diabetics.[1,2] In 2005, the overall rate of hospital discharge for new LEA was about 4.3 per 1,000 diabetic people.[3] As a result, DFUs are wildly acknowledged a source of major distress and morbidity for diabetic patient and also an enormous drain on health-care resources. It then becomes an important issue to have an effective treatment to alleviate the ulcer worsening and even to achieve complete ulcer closure for DFU patients.

Wound healing process is a complex series of events that comprised of inflammatory, proliferative (including granulation, contraction, epithelialization) and remodeling phases. Excessive inflammatory stimuli may be deleterious and result in adverse consequence to wound healing. Antioxidants, on the other hand, significantly limit the delayed sequelae of tissue damage and stimulate wound healing process. As a result, many substances that intend to enhance the wound healing emphasis on their anti-inflammatory and antioxidant effects.[4,5]

[redacted]

[redacted]

[redacted]

1.2 Rationale and Justification for the Study

1.2.1 Rationale for the Study Purpose

[redacted]

1.2.2 Rationale for Doses Selected

[redacted]

1.2.3 Rationale for Study Population

[redacted]

1.2.4 Rationale for Study Design

[redacted]

2. OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objectives

The primary objective of this study is to evaluate the efficacy of CSTC1 in patient with diabetic foot ulcers.

2.1.2 Secondary Objectives

The secondary objective of this study is to evaluate the safety of CSTC1 in patient with diabetic foot ulcers.

The study purpose is for registration. The study results may be used for developing new drug application dossier.

2.2 Endpoints

2.2.1 Primary endpoints

Incidence of complete ulcer closure during up to 12 weeks of treatment period

Complete ulcer closure is defined as 100% skin re-epithelialization without drainage or dressing requirements confirmed at two consecutive study visits 2 weeks apart.

2.2.2 Secondary efficacy endpoints

1. The ulcer closure time

   Defined as the time to complete ulcer closure

2. The complete ulcer closure rate

   The proportion of subjects with complete ulcer closure at each post-treatment visit

3. Percentage change in ulcer size for each post-treatment visit

   Calculated as (Ulcer size at post treatment visit - Ulcer size at baseline)/(Ulcer size at baseline)

2.2.3 Safety endpoints

1. Adverse event incidence
2. Change in physical examinations
3. Net change from baseline in laboratory test results (include hemoglobin, hematocrit, RBC, platelet, WBC with differential counts, HbA1c, fasting glucose, AST, ALT, creatinine and BUN)
4. Net change from baseline in vital signs

3. STUDY POPULATION

3.1 Subjects Enrollment

The sample size is determined to be 80 versus 20 subjects (4:1 ratio) for Treatment versus Control groups, 100 subjects in total. To ensure the completion of 100 evaluable (= per-protocol) subjects, around 125 subjects will be recruited. All of the subjects will be recruited in Taiwan. Any subject fulfills all of the recruitment criteria will be screening and/or randomized into the study.

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

1. If a patient consents to participate and meets the eligibility criteria but a delay occurs in starting to participate in due to certain change in situation such 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 such that patient's medications have now stabilized

In these situations, if randomization has not occurred;

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 both the CRF and the site master list
4. The patient needs to sign a new ICF as part of the screening procedure.

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

3.2 Inclusion Criteria

A subject is eligible for the study if all of the following apply:

1. With either gender aged at least 20 years old
2. With at least one diabetic foot ulcer (including ulcers on the lower legs) on the foot and not healing for at least 4 weeks. The largest diabetic foot ulcer will be selected as target ulcer. If two or more ulcers have the largest size, the one with worst grade will be selected. If two or more ulcers have the largest size and grade, the one with longest duration will be selected.
3. The target ulcer is classified as grade 1 to 2 ulcer according to modified Wagner system and with ulcer size of 1cm^2 to 50 cm^2
4. The target ulcer should be confirmed without active infection.
5. Subject should be free of any necrosis or infection in soft and bone tissue;
6. Subject has signed the written informed consent form

3.3 Exclusion Criteria

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

1. With active osteomyelitis

2. With target ulcer size decreased by at least 30% after at least 2 weeks of standard-of-care-only period or any other recorded regular therapy before Randomization visit
3. With poor nutritional status (albumin < 2g/dl), poor diabetic control (HbA1c > 12%), anemia (hemoglobin< 8 g/dL), a leukocyte counts < 2,000/mm^3, abnormal liver function (AST, ALT>3 x upper limit of normal range)
4. Requiring treatment with corticosteroids, immunosuppressive or chemotherapeutic agents within 28 days prior to Screening visit
5. Presence of necrosis, purulence or sinus tracts that cannot be removed by debridement
6. Receiving revascularization surgery or endovascular therapy performed <8 weeks before entry in the study
7. With known or suspected hypersensitivity to any ingredients of study product and vehicle
8. With coronary heart disease with myocardial infarction, coronary artery bypass graft (CABG), or percutaneous transluminal coronary angioplasty (PTCA) within 3 months prior to study
9. Pregnant or lactating or premenopausal with childbearing potential but not taking reliable contraceptive method(s) during the study period
10. Enrollment in any investigational drug trial within 4 weeks before entering this study
11. With any uncontrolled illness judged by the investigator that entering the trial may be detrimental to the subject

3.4 Withdrawal Criteria

[redacted]

3.5 Subject Replacement

[redacted]

3.6 Concomitant Medications

[redacted]

4. STUDY DESIGN

This study is designed as a randomized, double-blind, vehicle-controlled, multiple center and parallel trial to evaluate the efficacy and safety of CSTC1 in patients with diabetic foot ulcer.

The potential subjects with diabetic foot ulcer at Screening visit will be arranged to enter an initial phase for 2 weeks in which subjects receive only standard of care, unless subject has receive any other regular therapy for at least 2 weeks. Subjects whose study ulcer size decreases by at least 30% during this initial standard-of-care phase or previous regular therapy period will be excluded from the study at investigator's discretion. The remaining eligible subjects will be randomly assigned to receive either one of topical application of CSTC1 dose or CSTC1 matched vehicle, 2 times daily.

The treatment duration for each subject is 12 weeks or up to confirmed complete ulcer closure, whichever comes first. That is, subjects will receive treatment for at most 12 weeks, which consists of 8 visits located at weeks 1, 2, 3, 4, 6, 8, 10, and 12. Subjects confirmed complete ulcer closure during treatment period will be arranged for a 12 week post-treatment follow-up. Subjects failed to achieve complete ulcer closure at week-12 visit will be arranged for 4 weeks of safety follow-up. Subjects happening to attain complete ulcer closure at week-12 visit will not receive further treatment but needs to be confirmed at week-14 visit. If confirmation of complete ulcer closure is reached at week-14 visit, the subject will continue the post-treatment follow-up visit until week-24 visit. Otherwise, the subject will be arranged for safety follow-up until week-16 visit.

After the treatment period, the post-treatment follow-up period for subjects confirmed complete ulcer closure will consist of 4 visits at weeks 2, 4, 8, 12 after confirmation of complete ulcer closure. The safety follow-up period for subjects failed to achieve complete ulcer closure at week-12 visit will consist of 2 visits at weeks 14 and 16.

4.1 Treatment Assignment

Eligible subjects will be assigned randomization number in sequential order and each of the randomization will determine the allocation of one of the two treatment groups in 4:1 ratio as shown below:

1. CSTC1 two times daily
2. CSTC1 matched vehicle, two times daily

Treatments should be continued for a total of 12 weeks or until confirmation of complete ulcer closure, whichever comes first. After treatment period, additional 12 weeks of follow-up period is arranged for subjects confirmed complete ulcer closure to help distinguish actual ulcer healing from transient ulcer coverage, determine if the investigational product affects the strength of ulcer closure relative to standard care, and monitor for adverse effects on surrounding tissue

4.2 Randomization and Blinding

Permuted block randomization method will be applied to generate randomization codes. Each randomization number will be assigned to individual subject according to the time-sequence for screened subject becoming eligible.

Both CSTC1 and its matched vehicle will be prepared to be identical in all aspects to achieve the double-blind purposes. All subjects will take proper amount of CSTC1 or CSTC1 matched vehicle per application to ulcer area.

5. TRIAL MATERIALS

5.1 Trial Product (s)

Topical formulation of CSTC1 is a vapor fraction from seeds of Glycine max (L.) Merr. and composition thereof. The marker ingredients (impurity) of CSTC1 are Raffinose pentahydrate, Stachyose hydrate, Daidzin, and Genistin. The comparator used in this study is CSTC1 matched vehicle. Both investigational products will be applied daily twice a day.

Soybean was extracted using two methods, vapor fraction (C1) and ethanol extraction followed by concentration and drying (A1). 0.3% of A1, shown to reach the most active in wound healing in animal model when combined with the water extracted composition to form the drug substance. The final drug product, CSTC1, contains 0.3% of A1 and 85.95% water extracted composition in a cream base with a total of 30g per tube shown as below:

Code Name	CSTC1
Component	2148.75g C1 and 7.5g A1; cream base 343.75 g
Amount per tube	30g/tube

*Cream base: Stearic acid, Stearyl alcohol, Tween 80, Borneol, Potassium hydroxide, Benzyl alcohol

The placebo was made with a cream base and coloring agents with the ingredients and amount as follows:

The active and vehicle cream is manufactured and packed with 30 g in weight in a LDPE tube by Charsire Biotechnology Co., Ltd.

5.2 Storage and Drug Accountability

Adequate supplies of either CSTC1 or CSTC1 matched vehicle will be packaged for one visit dispense. The total number of tubes of investigational product planned for each subject will be 100 and each subject is expected to use at most 1 tube per day. Each subject will be dispensed for up to 8 visits at visits 3 to 10. At each visit of visits 3 to 9, investigational product should be supplied to the subject for use based on the number of tubes of unused investigational product the subject possesses and to the extent of date that the subject may return with remaining eligibly for next visit. Therefore, the number of tubes supplied at each visit will be calculated as (date of subject expected to return for next visit – date of current visit + 4 days of time window - number of tubes the subject possesses).

The trial medication for both treatment groups will be packaged in an identical way. Drug accountability will be recorded for the weight of test drug given to the subjects and returned to site by the subjects. Subject will be dispensed diary cards to record the times in each day they apply the study medications to the treated area.

All the products should be stored at room temperature (under 30° C) and be managed by the hospital pharmacies.

5.3 Dose Regimen

[redacted]

[redacted]

5.4 Treatment Compliance

[redacted]

6. STUDY MEASUREMENTS

6.1 Ulcer assessment

[redacted]

6.2 Physical Examination and Vital Signs

Physical examination conducted in this study will include the following items: general appearance, skin, eyes, ears, nose, throat, head and neck, heart, joints, chest and lungs, abdomen, lymph nodes, musculoskeletal, nervous system, and others

Vital signs measurement will consist of blood pressures, pulse rate, respiratory rate, and body temperature. Respiratory rate, 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.

Physical examination and vital signs will be performed at all visits throughout this study except initial phase visit.

6.3 Laboratory Examination

Laboratory examination to be measured in this study will consist of

- Hematology: hemoglobin, hematocrit, RBC, platelet, WBC with differential counts

- Biochemistry: AST, ALT, fasting glucose, HbA1c, serum creatinine, blood urea nitrogen (BUN), albumin

Laboratory examination will be conducted at Screening visit, Randomization (baseline) visit, and week 12 visit. Albumin will only be test at Screening and Randomization visits for judging subject eligibility. Laboratory test results within 14 days before Screening visit and 28 days before Randomization visit are acceptable; HbA1c test result within 28 days can be accepted.

6.4 Standard-of-Care Procedures

[redacted]

7. STUDY SCHEDULE

This study is designed as a randomized, double-blind, parallel, vehicle-controlled trial. The potential subjects with diabetic foot ulcer may be arranged to enter an initial phase for 2 weeks in which subjects receive only standard of care before randomization. The subjects confirmed eligible at the randomization visit will be randomly assigned to receive either one of CSTC1 or its matched vehicle treatment, two times daily. The subjects in either treatment group will be given 12 weeks of treatment and arranged a 4 week safety follow-up (if not achieving complete ulcer closure at week 12 visit) or be given up to confirmed complete ulcer closure and arranged 12-week post-treatment follow-up (if achieving confirmed complete ulcer closure before or at week-12 visit).

The schedule of assessment is tabulated in front of the protocol main text. 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.

7.1 Screening visit

(-3 to -2 weeks relative to Randomization (baseline) visit or within 1week randomization visit if initial phase is not required)

1. Explain the nature of the study and have subject to read and sign an Informed Consent Form
2. Assign subject screening number
3. Obtain Demographic, including:
   - Gender
   - Date of Birth
   - Height
   - Weight
4. Screen subject for inclusion/exclusion criteria
5. Medical history, including:
   - History of diabetic mellitus
     - Date of onset of diabetic mellitus
     - Date of onset of first and current episode of diabetic ulcer foot
     - Therapy for current episode of diabetic ulcer foot
   - General medical history: All medical histories within one year before screening visit are to be recorded, using the body system categories outlined below. For each history, the specific medical terminology for the disease/disorder/condition, the date of diagnosis, and the history status (resolved or ongoing) will be documented.
6. Perform pregnancy test for female with childbearing potential (Note: not with childbearing potential means post-menopausal (defined as at least one year without any menstruation) or documented status of post hysterectomy)
7. ABI measurement
8. Vital signs
9. Perform physical examinations

10. Obtain blood samples for hematologic and biochemical tests (Test results within 14 days before screening visit are acceptable; HbA1c test results can be within 28 days.):
11. Ulcer evaluation
12. Record concomitant medication(s)
13. Record adverse event(s)

7.2 Initial Phase visit (only for applicable subjects)

(-2 to 0 weeks relative to Randomization (baseline) visit)

1. Dispense instruction and material for Standard-of-Care
2. Record of Adverse Events
3. Record changes on concomitant medication(s)

7.3 Randomization visit

(Day 0)

1. Re-confirm eligibility of subject
2. Ulcer evaluation
3. Vital signs
4. Perform physical examinations
5. Obtain blood samples for hematologic and biochemical tests (The tests can be waived if the tests have been performed within past 28 days)
6. Assign randomization number to eligible subject
7. Record changes on concomitant medication(s)
8. Dispense material of standard-of-care
9. Record adverse event(s)
10. Dispense trial medication
11. Dispense diary to patient

7.4 Evaluation visits during treatment period in visits 4 to 11

(Weeks 1, 2, 3, 4, 6. 8, 10, 12 with time window ±4 days)

Subjects confirmed complete ulcer closure before week-12 visit will be arranged for post-treatment follow-up.

1. Ulcer evaluation
2. Vital signs
3. Perform physical examinations
4. Obtain blood samples for hematologic and biochemical tests (will only be performed at week 12 visit or when subject complete the treatment period)
5. Record changes on concomitant medication(s)
6. Dispense material of standard-of-care

7. Record adverse event(s)
8. Collect unused treatment medication(s) (which is dispensed at prior visit)
9. Dispense trial medication (excluding week 12 visit)
10. Collect and check diary (which recorded from previous visit)
11. At week-12 visit only, perform pregnancy test for female with childbearing potential

7.5 Follow-up visits

The follow-up visits will be performed at 2, 4, and 8 weeks after for subjects achieving confirmed complete ulcer closure during treatment period and at 2 weeks after treatment period (week 14 or visit 12) only for those not.

(The visit day will be week 14 for visit 12, week 16 for visit 13, and week 20 for visit 14, each with time window±4.)

1. Ulcer evaluation
2. Vital signs
3. Perform physical examinations
4. Record changes on concomitant medication(s)
5. Record adverse event(s)

7.6 Final visit

The final visit will be performed at 12 weeks ± 4 days after confirmation of complete ulcer closure for subjects achieved and at visit 13 (week 16 ± 4 days) for those not.

1. Ulcer evaluation
2. Vital signs
3. Perform physical examinations
4. Record changes on concomitant medication(s)
5. Record adverse event(s)
6. Complete an exit form in the case report form and dismiss the subject

8. ADVERSE EVENTS

8.1 Definitions

- *Adverse Event (AE):*

  An AE is any untoward medical occurrence in a patient or clinical investigation participant administered a study medication and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a study medication, whether or not related to the study medication. Laboratory abnormalities should not be recorded as AEs unless determined to be clinically significant by the Investigator.

- *Expected AE:*

  Expected AE are defined as any event, the specificity or severity of which is consistent with the current investigator brochure or other technique documents.

- *Unexpected AE:*

  Unexpected AE is defined as any event, the specificity or severity of which is not consistent with the current investigator brochure or other technique documents. "Unexpected", as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

- *Serious Adverse Event (SAE):*

  Serious Adverse Event (SAE): A Serious Adverse Event is defined as an AE meeting one of the following conditions:

  - Death during the period of protocol defined surveillance
  - Life Threatening Event (defined as a participant at immediate risk of death at the time of the event)
  - An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
  - Results in congenital anomaly or birth defect
  - Results in a persistent or significant disability/incapacity
  - Based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.2 AE/SAE Intensity and Relationship Assignment

- AE/SAE Intensity

  The investigator must rate the intensity for all AEs that occur during the study using the grades provided below:

  - Grade 1 (Mild): events require minimal or no treatment and do not interfere with the subject's daily activities.

- Grade 2 (Moderate): events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Grade 3 (Severe): events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Grade 4 (Life threatening): Any adverse drug experience that places the subject or participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.
- Grade 5 (Death)

- AE/SAE Relationship Assessment

The investigator will be asked to assess all AEs with respect to their causal relationship to the study drug according to the following classification:

- 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 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 "possible" soon after discovery, it can be flagged as requiring more information and later be upgraded to probable or certain as appropriate.
- Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to study agent/intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) 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 clinician Expected Events Related to Disease Process: Provide explicit definitions of the type(s), grade(s), and duration(s) of adverse event(s) that will be considered disease related.

8.3 Collecting, Recording and Reporting of Adverse Events

8.3.1 Collecting and Recording of AE

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 starting from signing ICF 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. The study subject will be followed until the event resolves or stabilizes at a level acceptable to the investigator. For patients receiving hemodialysis, taking regular arteriovenous fistula will not be considered as an AE.

8.3.2 Reporting of AE

Serious, alarming and/or unusual adverse events must be reported to the Sponsor/CRO contact within 24 hours of the investigator's knowledge of the event. Any event requiring hospitalization (or prolongation of hospitalization) that occurs during the course of a subject’s participation in a clinical study must be reported as a serious adverse event, except hospitalizations for the following:

- Social reasons in absence of an adverse event
- Surgery or procedure planned before entry into the study (must be documented in the CRF

The investigator is responsible to communicate details of medical emergencies in trial subjects to the Ethics Committee. Sponsor is responsible to inform the events to the regulatory authorities.

Fatal or life-threatening, unexpected ADRs should be notified to Taiwan National ADR Reporting Center by sponsor/CRO as soon as possible, but no later than 7 calendar days, after first acknowledged by the investigator, and a complete report should be followed 8 additional calendar days. This report 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 ADRs that are not fatal or life-threatening must be filed as soon as possible but no later than 15 calendar days after first acknowledged by the investigator that a case qualifies.

9. DATA ANALYSIS

9.1 Data Quality Assurance

The sponsor will provide paper CRFs or electronic CRFs (via Electronic Data Capture (EDC) system) for the recording and collection of subject data and for the collection of data. All CRFs will be completed as soon as possible after the subject's visit. Corrections to data on the CRFs will be documented or traced. The investigator will review CRFs to indicate that, to his/her knowledge, they are complete and accurate. If further changes are made after this, the investigator will need to again sign the investigator signature page or approve electronically. Designated source documents will be signed and dated by the appropriate study personnel.

9.2 Clinical Data Management

The investigator must agree to complete and maintain source documents and CRFs for each subject participating in the study.

The sponsor/CRO will be responsible for the processing and quality control of the data. Data management will be carried out as described in the sponsor/CRO's standard operating procedures (SOPs) for clinical studies.

The handling of data, including data quality control, will comply with regulatory guidelines and the sponsor/CRO's SOPs as well as provisions of the study-specific Data Management Plan.

10. SAMPLE SIZE AND STATISTICAL METHODS

10.1 Determination of Sample Size

[redacted]

10.2 Statistical and Analytical Plans

10.2.1 Analysis Population

Three data sets will be introduced for statistical analysis.

Intent-to-treat (ITT) population:

– All randomized subjects who have received at least one dose study medication.

Per-protocol (PP) population:

– A subset of ITT population

– Fulfill all inclusion and exclusion criteria

– Dosed with at least 8 weeks study drug or with early confirmed complete ulcer closure AND with at least 70% treatment compliance

– Did not receive any prohibited treatment during treatment period

– With any post-treatment assessment relevant to efficacy endpoint up to end of dosing period

Efficacy endpoints will be analyzed on ITT and PP population. Demographics, baseline characteristics, and safety endpoints will be analyzed on ITT population. The conclusion of efficacy will be made according to the results of ITT population analysis.

10.2.2 Efficacy Analyses

- **Primary efficacy endpoint**

[redacted]

[redacted]

- **Secondary efficacy endpoints**

[redacted]

10.2.3 Safety Analyses

[redacted]

10.2.4 Demography and baseline characteristics

[redacted]

10.2.5 Interim Analyses

[redacted]

11. ETHICAL CONSIDERATIONS

11.1 Informed Consent

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participants and their families.

Consent forms will be IRB approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participants should have sufficient opportunity to discuss the study and process the information in the consent process prior to agreeing to participate. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants 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.

11.2 IRB review

This protocol and the associated informed consent documents must be submitted to the IRB for review and approval. The study will not be initiated until the IRB provides written approval of the aforementioned documents and until approval documents have been obtained by the Principal Investigator and sponsor or CRO.

No changes from the final approved protocol will be initiated without the IRB/EC's prior written approval or favorable opinion of a written amendment, except when necessary to eliminate immediate hazards to the study participants or when the change involves only logistics or administration. Any significant deviation from the protocol when no approved amendment exists will be regarded as a protocol violation, and will be addressed as such during the reporting of the study.

11.3 Confidentiality of Data and Patient Records

Include procedures for maintaining participant confidentiality, any special data security requirements, and record retention per the sponsor's requirements.

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all 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 participants in this study. The clinical study site will permit access to such records.

The information provided by sponsor in this protocol and the associated Clinical Investigator's Brochure and the data generated by this clinical study are to be considered as confidential property of sponsor.

12. PUBLICATIONS

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.

13. RETENTION OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation should be retained by the PI in a secure storage facility. The records should be accessible for inspection and copying by authorized authorities.

All study documentation at the clinical site and sponsor records will be archived in accordance with ICH GCP, applicable regulations, the sponsor's quality standards and SOPs. Study records should not be destroyed without prior written agreement between sponsor or CRO and the study investigator.

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