RETROSPECTIVE STUDY PROTOCOL

A Retrospective Record-review Study in Patients Who Had Participated in the ON101CLCT02 Diabetic Foot Ulcer Trial

NCT#: NCT05135130

Principal Investigator:	Hsin-Han Chen, M.D.
Development phase:	NA
Sponsor:	Oneness Biotech Co., Ltd.
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AMENDMENT HISTORY

Amendment No.	Version No.	Version Date	Authors of changes	Summary of Revisions
1	V1.1	03-Sep-2021	Sponsor	1. Remove the questionnaire part (original 3.4.2) in the research method 2. Remove the outcomes related to non-medical cost and QoL 3. Remove the subgroups related to the patients' current statuses.

INVESTIGATOR SIGNATURE PAGE

The study will be carried out in accordance with the protocol, the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), and in accordance with local legal and regulatory requirements. All personnel involved in the conduct of this study have completed human subject protection training.

Principal Investigator	**Signature:**	**Date:**
_____	_____	_____
Name, Title		

SPONSOR SIGNATURE PAGE

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Sponsor's representative:	**Signature:**	**Date:**
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TABLE OF CONTENT

LIST OF ABBREVIATIONS

CRF	Case Report Form
CRO	Contract Research Organization
DFU	Diabetic Foot Ulcer
DM	Diabetes Mellitus
EMR	Electronic Medical Records
HRQoL	Health-Related Quality of Life
ICF	Informed Consent Form
ICD-9-CM	International Classification of diseases, 9th Revision with Clinical Modification
ICD-10-CM	International Classification of diseases, 10th Revision with Clinical Modification
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDF	International Diabetes Federation
IRB	Institutional Review Board
QoL	Quality of Life
UK	United Kingdoms
USA	United States of America

PROTOCOL SYNOPSIS

I. Protocol title:

A retrospective record-review study in patients who had participated in the ON101CLCT02 diabetic foot ulcer trial

II. Objectives:

To understand the life and financial burden of diabetic foot ulcers (DFUs) on patients, this study is proposed to retrospectively collect data on disease status and medical utilization in DFU patients through medical record review.

III. Study procedures:

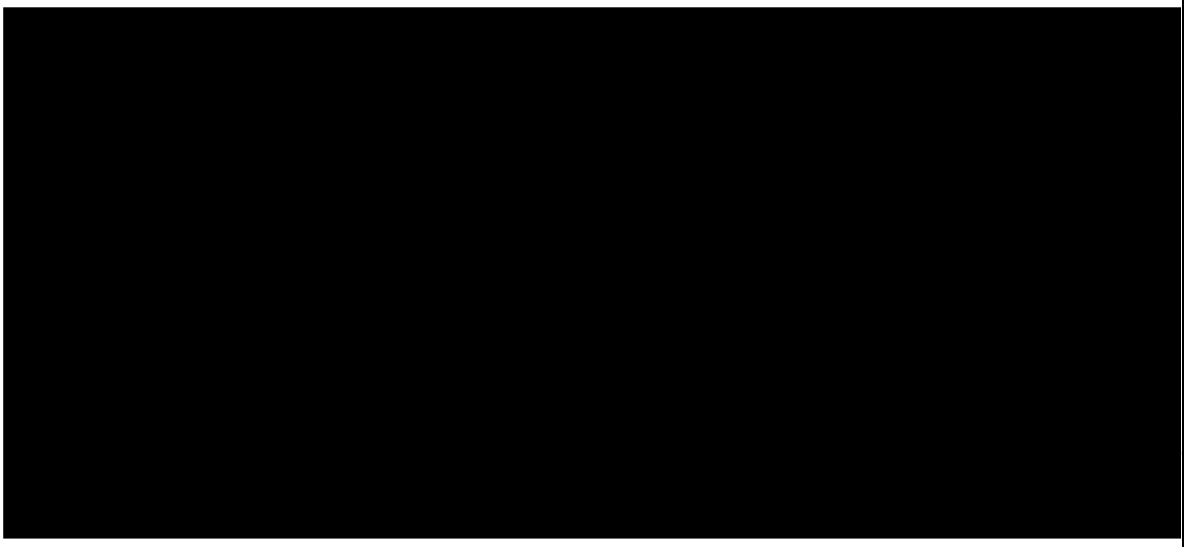
- **Study population:** The ON101CLCT02 trial enrolled 236 subjects and was conducted in Taiwan, China, and USA. The target population of this study consists of the Taiwanese DFU patients who had completed or early withdrawn from the ON101CLCT02 trial (188 of them). No additional inclusion or exclusion criteria will be applied.
- **Study Sites:** Seven (7) out of the 11 sites participated in the ON101CLCT02 trial, including Tri-Service General Hospital, China Medical University Hospital, Linkou Chang Gung Memorial Hospital, Kaohsiung Chang Gung Memorial Hospital, Chi Mei Medical Center, MacKay Memorial Hospital, National Taiwan University Hospital
- **Study duration:**
  - **Review of medical records:** Up to two years after the patient had completed or early withdrawn from the ON101CLCT02 trial

IV. Data collection:

The following data will be collected from the medical records:

1. Weight and measurement date
2. Blood pressure and measurement date
3. Ankle-brachial index and measurement date
4. Biochemical results (HbA1c, BUN, WBC, CRP, Creatinine) and measurement date
5. DM complications (neuropathy, peripheral arterial disease, retinopathy, kidney disease)
6. Undergoing dialysis or not, if so, hemodialysis or peritoneal dialysis
7. For the healed target DFU in ON101CLCT02 trial, whether any recurrence, if so, the date
8. Survival status and date of death, if applicable
9. The number of DFU wounds and the size, severity, duration, location, etiology, etc of each wound

10. Medical utilization

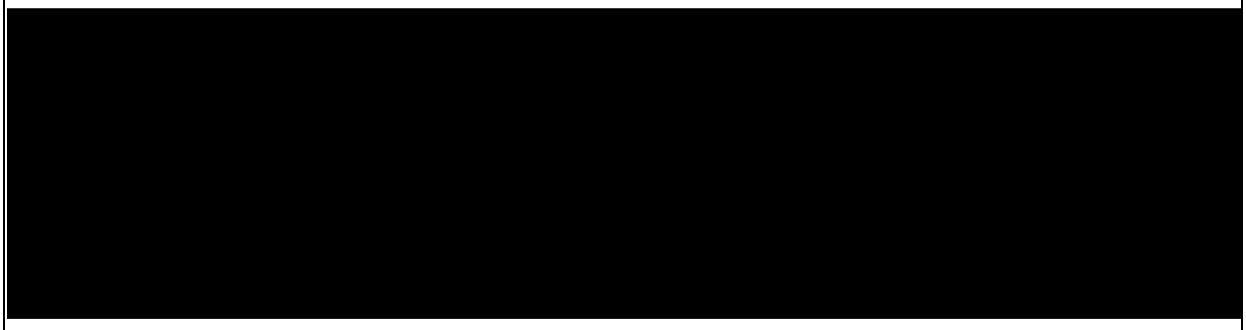


11. Whether infection occurred, if so, number of times and date
12. Whether there is gangrene, if so, number of times and date
13. Whether there is amputation, if so, major or minor, the location, success or failure, date
14. Whether there is any plastic or reconstructive surgery, such as skin grafts, flap surgery, etc., if so, the frequency, success or failure, and date

V. Statistics:

Continuous variables will be presented in mean, median, standard deviation, range (maximum, minimum), and 95% confidence interval and will be compared with T-test. Categorical variables will be presented in frequency and proportion. The Fisher's exact test will be used to compare the differences between the categorical variables. Statistically significant is defined by two-tailed, $p<0.05$. The correlation of continuous variables will be analyzed by linear regression.

The outcome measures include:



Subgroup analyses include:

1. The target ulcer has healed when completing or withdrew from the trial ON101CLCT02
2. Baseline HbA1c >7%

The medical cost of illness of DFUs may be assessed for the above subgroups.

1. INTRODUCTION

1.1. Background Information

According to current statistics from International Diabetes Federation (IDF), diabetes mellitus (DM) is on the rise all over the world. In 2017, it was predicted that 451 million people (aged 18–99 years) globally had diabetes. The number is predicted to rise to 693 million by 2045 [1]. Diabetic foot ulcer (DFU) is a severe complication of DM, of which the lifetime incidence has been estimated to be 19 to 34% among DM patients [2]. The global prevalence of DFU was reported to be 6.3% [3], and the number in Taiwan between 2001 and 2015 was 0.5 to 0.8% [4].

DFU can result in a heavy medical burden due to infections, as well as a reduction in quality of life (QoL) and an increase in mortality. More than half of DFUs are infected [5], and the amputation rate is approximately 2% among all DFU cases [6]. The health-related quality of life (HRQoL) was low in DFU patients, and the physical HRQoL was especially affected after amputation [7,8]. A patient with a DFU has a 2.5-fold higher risk of death at 5 years than a DM patient who does not have a foot ulcer [9].

The intensive medical care of DFU leads to drastic financial costs. In the USA, the annual healthcare cost per patient due to DFUs was about $15,000, and excess work-loss costs of about $3,000 were observed for private insured patients [10]. The medical expenditure of diabetes-related ulceration and amputation was about £900 million per year in the UK, accounting for almost 1% of the National Health Service budget [11]. In China, the average cost per DFU patient was about ¥42,000 in 2020 and has increased about 2.7-fold in the past 6 years [12]. The mean annual medical cost for DFU treatment in Taiwan per patient were estimated to be around $4,700 USD [13]. Overall, DFU causes noticeable expenses on medical care around the globe as well as in Taiwan.

1.2. Study Rationale

Chen *et al.* has estimated the total cost of DFU with over 800,000 patients, and Tai *et al.* has analyzed the medical utilization of DFU, the percentage of debridement and amputation among DFU patients in Taiwan [4,13]. However, the information on DFU severity, number of wounds, duration, and lab data was inaccessible due to the lack of medical records in the previous studies. With available clinical data, a detailed analysis of medical expenditure on DFU can be conducted and further explore the correlations between the risk factors and medical costs.

The identification of cost-effective and cost-saving treatments that can assist minimize the DFU healthcare burden has been ongoing [14]. The efficacy of ON101 (Fespixon cream®) has been proven, but its impact on medical expenditure is yet to be determined. Therefore, to understand the preliminary result on the cost of illness after introducing ON101 as a DFU treatment, a retrospective study based on medical records is proposed to analyze whether there are differences between the costs of illness in DFU patients who used ON101 or Aquacel® in the ON101CLCT02 trial.

2. RESEARCH OBJECTIVES

The purpose of the study is to evaluate the medical cost of illness for DFUs on the patients who had used ON101 or Aquacel® in the ON101CLCT02 trial.

3. RESEARCH METHODS

3.1. Study Design

This is a retrospective, multicenter, chart-review study. The study is designed to review the electronic medical records (EMR) of the patients who participated in the ON101CLCT02 trial. The EMR to be reviewed includes outpatient, inpatient, and emergency visits due to DFUs.

Data from the date of the DFU patient completed or early withdrew from the ON101CLCT02 trial up to two years after his/her completion or early withdrawal from the ON101CLCT02 trial are to be reviewed and collected.

Additional phone interview will be conducted if some protocol specified information is missing in the patient's EMR.

3.2. Study Population and Sample Size

The ON101CLCT02 trial enrolled 236 subjects and was conducted in Taiwan, China, and USA. The target population of this study consists of the Taiwanese DFU patients who had completed or early withdrawn from the ON101CLCT02 trial (188 of them).

No additional inclusion or exclusion criteria will be applied.

3.3. Study Sites

There were 11 sites that had participated in the ON101CLCT02 trial. This study will be conducted at 7 out of the 11 sites due to feasibility.

List of sites:

1. Chang Gung Medical Foundation Linkou Chang Gung Memorial Hospital
2. Chang Gung Medical Foundation Kaohsiung Chang Gung Memorial Hospital
3. China Medical University Hospital
4. Chi Mei Medical Center
5. MacKay Memorial Hospital
6. National Taiwan University Hospital
7. Tri-Service General Hospital

3.4. Data Collection

The following data will be collected from the EMR. Only the latest available data within the predefined data collection period, i.e., up to two years after the subject had completed or early withdrawn from the ON101CLCT02 trial will be recorded. The date of measurement or visit will be recorded too.

Category	**Variable(s)**
Demographic	Weight (unit: kg)
Blood Pressure	Systolic/Diastolic blood pressure (unit: mmHg) Ankle-brachial index (Left/Right; value)
Biochemistry	HbA1c (Glycated Hemoglobin A1c) (unit: %) BUN (Blood Urea Nitrogen) (unit: mg/dL) WBC (White Blood Cell) (unit: $10^3/mm^3$) CRP (C-Reactive protein) (unit: mg/dL) Creatinine (unit: mg/dL)
Comorbidities	DM complications (Yes; specify the diagnosis/No) Neuropathy/Peripheral vascular disease/Retinopathy/Kidney disease/Cardiovascular disease Dialysis (Yes; specify the interval/No) Hemodialysis/Peritoneal dialysis
DFU Characteristics	Number of DFUs Each DFU:

	Location (Left/Right; Plantar/Non-plantar) Etiology (Neuropathy/Neuro-ischemia/Peripheral vascular disease/Other) Duration (month) Severity (Wagner grade 0-5) Area (mm^2) Target ulcer of ON101CLCT02 trial (Yes/No)
Other	Survival (Yes/No) Date of death Status of the healed target ulcer of ON101CLCT02 trial Recurrence (Yes/No) and date of recurrence

The following data will be collected from the EMR. All the available data within the predefined data collection period, i.e., up to two years after the subject had completed or early withdrawn from the ON101CLCT02 trial will be recorded. The date of measurement or visit will be recorded too.

Category	Variable(s)
Management for Comorbidities	DM complications (Yes; specify the diagnosis /No) Neuropathy/Peripheral vascular disease/Retinopathy/ Kidney disease/Cardiovascular disease Treatment or management received (e.g., medication use, therapy, surgery, etc.) for any of the above comorbidities and the date
Medical Utilization	[redacted]

	[redacted]
DFU Disease Status and Treatment	

3.5. Outcome Measures

[redacted]

3.6. Subgroup analysis

The medical cost of illness due to DFUs may be assessed for the following subgroups:

1. The target ulcer has healed when completing or withdrew from the trial ON101CLCT02
2. Baseline HbA1c >7%

4. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

4.1 Analysis Plan

The baseline demographic and clinical characteristics will be described using descriptive statistics. Continuous variables will be summarized using number of values (n), mean, standard deviation (SD), median, range (minimum and maximum) and 95% confidence interval (CI). Frequencies and percentages will be reported for all categorical data.

Statistical analysis will be performed using T-test or Fisher's exact. Statistically significant is defined by two-tailed, $p < 0.05$. The correlation of continuous variables will be analyzed by linear regression, if applicable.

The effectiveness of treatment of ON101 and Aquacel® will be estimated with the clinical outcomes using cost-effectiveness or cost-benefit analyses. The total cost for DFU and the incremental cost-effectiveness ratio will be calculated.

4.2 Missing Data

No imputation strategies will be implemented for missing data.

5. DATA HANDLING AND RECORD KEEPING

5.1 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the Investigators.

Oneness Biotech Co., Ltd. or its designee(s) will be responsible of data management and quality

review, analysis and report the study data.

5.2 Data Handling Conventions

Data will be extracted from the EMRs of the 11 study sites listed in Section 3.3. Data will be collected according to the following procedures by the Investigators or designees:

1. Identify the subject who had completed or early withdrawn from ON101CLCT02 trial
2. Enter clinical data from EMR to the CRF.
3. Conduct phone interview with the subject if some information is missing in the EMR. Enter the information provided to the CRF.
4. Verification of data by second personnel
5. Remove patient personal identifiers (e.g., names, identification numbers, and medical record numbers) and send the de-identified data to the designated personnel for data management, validation, and analyses

5.3 Data Capture Methods

Data will be written to CRFs designed by Bestat Pharmaservices Corp. CRFs will be used for recording all data from each subject in this study. CRFs must be typewritten or printed legibly using black ballpoint pen or completed electronically.

Data documented in the CRFs will be entered into the study database powered by Microsoft® Excel® by the Sponsor or designated personnel. Queries regarding the unclear data will be reviewed and be sent to the investigators for clarification. A data clarification form will be applied for the queries if necessary. Designated site staffs are required to reply to the queries and make any necessary changes to the data. The queries will be resolved, and corresponding updates will be made to the database if necessary. Once all queries have been resolved, the database will be locked. The locked data will be exported to generate the subject listings, tabulations, or statistical analyses.

5.4 Study Records Retention

Study documents should be retained for a minimum of 2 years after the completion of the study. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor, if

applicable. It is the responsibility of the Sponsor to inform the Investigators when these documents no longer need to be retained.

6. QUALITY CONTROL AND QUALITY ASSURANCE

All aspects of data collection, management or analysis will be conducted according to the study protocol. Programming for this project will be conducted by a primary statistician and validated by a separate analyst. For all data processing steps, the validation analyst will review the program along with input and output data sets.

7. PROTECTION OF HUMAN SUBJECTS

7.1. Ethical Approval and Subject Consent

The protocol and other study documents will be submitted to the IRB of each study site for review and approval. Approval of the protocol must be obtained before any data collection is initiated. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study.

Patient's consent is to be obtained prior to any procedures if deemed necessary by the IRB. Only an IRB-approved consent form is to be used.

7.2. Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating Investigators, their staff, and the Sponsor and their agents. Patient-identified code will be used to improve patient privacy. The Investigators must assure that patients' privacy will be maintained and that their identities are protected from unauthorized parties.

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 monitors or other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigators, including but not limited to medical records (office, clinic, or hospital) and pharmacy records for the patients in this study. The study sites will permit access to such records.

7.3. Protocol Amendments

Any change of this protocol that affect study objectives, study design, study procedures, patient population, or significant administrative procedures will require a formal amendment to the protocol. Any proposed protocol amendments must be sent in written form to the applicable IRB. Prior to implementation, an amendment must be approved by the Sponsor, the Investigator, and the applicable IRB.

The same applies to the informed consent form.

8. PUBLICATION POLICY

Following completion of the study, the Investigators are expected to publish the results of this research in a scientific journal. Member journals of The International Committee of Medical Journal Editors (ICMJE) have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is Sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For grants and cooperative agreements, it is the responsibility of Oneness Biotech Co., Ltd. to register the trial in an acceptable registry.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from registering trials in a public registry such as ClinicalTrials.gov.

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