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STUDY PROTOCOL

A Multi-national, Multi-center, Prospective, Randomized, Double Blinded, Placebo-controlled Trial to Evaluate the Efficacy of HyperBox Cyclical Topical Wound Oxygen Therapy (TWO_2) in the Treatment of Chronic Diabetic Foot Ulcers

Device	HyperBox Cyclical Topical Wound Oxygen Therapy (TWO_2) System
Indication	Chronic, diabetic foot ulcers
Sponsor	AOTI Ltd. Qualtech House Parkmore Business Park West Galway, Ireland
Principal Investigator	Robert Frykberg, DPM MPH Chief of Podiatry & Residency Director Phoenix VA Healthcare System 650 E. Indian School Road Phoenix, AZ 85012, USA

Version 013.0 (September 24, 2016), Amendment 8.

PROTOCOL APPROVAL

Signature Page

By signing the protocol, we the undersigned confirm our agreement with the contents of the protocol and our commitment to comply with the procedures contained in the study manual, with the conditions and principles of Good Clinical Practice in accordance with the International Conference on Harmonisation (ICH) E6: “Good Clinical Practice: Consolidated Guideline,” 62 Federal Register 25,692 (1997) where and as adopted by the FDA and with all requirements of the relevant Regulatory Authorities and IRB/Ethics Committees.

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Investigator Signature Page

By signing the protocol, we the undersigned confirm our agreement with the contents of the protocol and study manual and our commitment to comply with the procedures contained within, with the conditions and principles of Good Clinical Practice in accordance with the International Conference on Harmonisation (ICH) E6: “Good Clinical Practice: Consolidated Guideline,” 62 Federal Register 25,692 (1997) where and as adopted by the FDA and with all requirements of the relevant Regulatory Authorities and IRB/Ethics Committees.

Principal Investigator

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PRINT NAME	**SIGNATURE**	**DATE**

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INSTITUTION NAME

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Contents

1. SYNOPSIS

Title	A Multi-national, Multi-center, Prospective, Randomized, Double Blinded, Placebo-controlled Trial to Evaluate the Efficacy of HyperBox Cyclical Topical Wound Oxygen Therapy (TWO_2) in the Treatment of Chronic Diabetic Foot Ulcers
Objectives	To evaluate the efficacy, safety and economic benefits of Cyclical Topical Wound Oxygen (TWO_2) Therapy in the treatment of chronic diabetic foot ulcers
Device	HyperBox Cyclical Topical Wound Oxygen Therapy (TWO_2) System applied 5 times weekly for 90 minutes for up to 12 weeks
Comparator treatment	Identical control device without cycling pressure of oxygen
Trial design	Double-blinded, multi-center, prospective, randomized, clinical trial
Setting	All subjects will be treated in a home care environment (home or equivalent facility)
Primary endpoint	Incidence of complete wound closure within 12 weeks
Secondary endpoints	• Time to complete wound closure • Change in wound size over time • Incidence of recurrence • Incidence of amputation • Incidence of adverse events and adverse device effects • Quality of Life – Pain, Mood and Function • Economic Analysis (EQ-5D, dressing and related medical history)
Type of subjects	• Foot ulcer below or at the malleoli graded as Grade 1 or 2 as assessed via the University of Texas Classification • Adequate perfusion with ABI > 0.7 **AND** TcpO2 > 30mmHg **OR** skin perfusion > 30mmHg **OR** Toe pressure > 30mmHg **OR** Duplex with biphasic waveforms below the knee • Ulcer size ≥ 1 cm^2 and < 20cm^2 after debridement • Ulcer duration ≥ 4 weeks but ≤ 1 year • Wound size reduction in a 2-week run-in period of ≤ 30%
Number of centers	Up to 30 centers in the US, Canada and Europe
Number of subjects	Conservative sample size estimation suggested a total sample size of 220 subjects. The trial will be using a group sequential design (GSD) allowing for interim analyses to be performed after one third of subjects complete 12 weeks and two thirds of subjects complete 12 weeks and potentially stopping the trial if stopping criteria are met.
Length of Study	It is estimated that subject recruitment of 73 subjects will take about 6 months.

2. INTRODUCTION

2.1. Background and Rationale

Foot disorders, such as ulceration, infection and gangrene are a major source of morbidity and a leading cause of hospitalization for persons with diabetes.[1] Ulceration, infection, gangrene, and amputation are significant complications of the diabetic foot ulcer and are estimated to cost billions of dollars each year.[2]

Foot disorders are also the leading causes of hospitalization in patients with diabetes mellitus.[2] Diabetic peripheral wounds are a major risk factor for lower extremity amputation.[3] Approximately 40-70% of all lower extremity amputations are performed in patients with diabetes, and some 100,000 non-traumatic lower-limb amputations were performed among U.S. diabetics in 2008 alone.[4] Even superficial diabetic wounds are often difficult to treat and show high rates of complications.[5]

Oxygen (O_2) is essential to wound healing. Local tissue hypoxia, caused by disrupted or compromised vasculature, is one key factor that limits wound healing.[6,7] It is well established that O_2 is vital in the synthesis of collagen, enhancement of fibroblasts, angiogenesis and leukocyte function.[8-10] O_2 also has key functions in energy metabolism[11,12] and in the inhibition of microbial growth.[13]

Clinical use of O_2 to promote wound healing began in the 1960's with the administration of systemic full body Hyperbaric Oxygen Therapy (HBO) to treat wounds.[13] Today HBO is usually administered in single or multi place chambers utilizing pressures of 2,500 mb and higher. HBO is reimbursed by the Center for Medicare and Medicaid Services in the US to treat certain wounds, including Diabetic Foot Ulcers (DFU's). A Cochrane review by Kranke et al. demonstrated that in people with foot ulcers due to diabetes, HBO significantly reduced the risk of major amputation and may improve the chance of healing at one year.[14] The availability of HBO facilities, contraindications, the need to transfer the patients to the HBO facilities and the risks of undesired systemic side effects, limits the widespread use of HBO to treat diabetic ulcers on a global basis.

In an effort to address some of these drawbacks, the principle of topical pressurized oxygen administration or topical wound oxygen therapy (TWO_2) was introduced in the late 60's.[15] The approach of topically oxygenating the wound is quite different from HBO. TWO_2 does not involve pressures anywhere near as high as in HBO. Additionally, TWO_2 is portable and can be administered in varied care sites, including in the patient's home. There have been a number of studies, including smaller Randomized Control Trials (RCTs) and Case Series etc., published demonstrating very positive outcomes with TWO_2.[15-22]

A previous study with the cycling TWO_2 device in a prospective, controlled clinical trial conducted in Canada examined the clinical efficacy of the TWO_2 device used in this proposed RCT. In outpatients with severe diabetic foot ulcers referred for care to a community wound care clinic, 14 out of 17 ulcers (82.4%) in the treatment group and 5 of 11 ulcers (45.5%) in the control group healed within 90 days. The median of wound healing in the oxygen group was 56 vs. 93 days in the control group ($p = 0.04$).

The concept of topical oxygen is still relatively unknown to the medical community as a whole and a larger well conducted randomized clinical trial is clearly needed to establish topical therapy as an important treatment in the management of diabetic foot ulcers.

This trial will evaluate the efficacy and potential economic benefits of TWO_2 in the treatment of chronic diabetic foot ulcers. Pre-trial and real-time events captured for each subject will be

used to provide data for health economic analyses.

2.2. Medical Device

The medical device being investigated in this trial is the HyperBox Cyclical Topical Wound Oxygen therapy (TWO_2) system manufactured by AOTI Ltd., Galway, Ireland.

The TWO_2 System operates by applying cyclical oxygen pressure directly to the wound site within a sealed and humidified environment. This provides a greater tissue oxygen diffusion gradient and increased tissue oxygenation, which enhances antimicrobial actions, stimulates angiogenesis and maximizes collagen production. The cyclical nature of the pressure also creates a sequential compression effect which helps reduce peripheral edema and stimulates wound site perfusion.

The TWO_2 system is intended as adjunctive therapy to standard wound care for acute or chronic wounds. It can be placed over a wound on the lower leg and when connected to a medical grade oxygen supply (Wall supply, Oxygen Concentrator or LOX system) which provides oxygen directly to the wound site with a pressure cycling between 5 to 50mbar at a frequency of approximately 2 cycles per minute. An integrated humidifier aids in keeping the wound moist during treatments.

The devices have US Food and Drug Administration (FDA) 510(k) clearance, Health Canada clearance and CE-Mark approval for the following indications:

Treatment of acute and chronic wounds on the lower extremity, such as;
Diabetic ulcers, Venous stasis ulcers, Post-surgical wounds, Gangrenous lesions, Decubitus/pressure ulcers, Amputations/infected stumps, Skin grafts, Burns, Frostbite.

2.3. Risk-Benefit Assessment

The HyperBox Cyclical Topical Wound Oxygen Therapy System is a non-invasive device that has been assessed to provide Non Significant Risk (NSR) per FDA guidelines. The device offers the same therapy as the predicate system, which has been used in the marketplace for over 20 years with no recorded adverse events. Based on this assessment the potential benefit of the therapy is expected to exceed any potential risks envisioned.

2.4. Compliance Statement

This trial will be conducted in compliance with the approved study protocol and ISO 14155:2011 (E) Clinical investigation of medical devices for human subjects — Good clinical practice as reflected in Good Clinical Practice in accordance with the International Conference on Harmonisation (ICH) E6: “Good Clinical Practice: Consolidated Guideline,” 62 Federal Register 25,692 (1997) where and as adopted by the FDA, and applicable regulatory requirements. Investigators should conduct the trial in compliance with these conditions, agreed to with AOTI, Ltd. and approved by Regulatory Authorities and IRB/Ethics Committees. Signature of the protocol by the investigator(s) and AOTI, Ltd. confirms this agreement.

3. OVERALL STUDY DESIGN AND PLAN

3.1. Overview

This is a multi-center trial, to be conducted at up to 30 sites in the United States, Canada, and Europe.

Inclusion criteria for participation in the trial include the presence of documented diabetes (Type I or II) with non-healing, full-thickness, University of Texas Classification of Diabetic Foot Ulcers Grade 1 and 2 diabetic foot ulcers of at least 4 weeks duration, but not greater than 1 year, measuring 1cm^2 or greater and less than or equal to 20 cm^2.

Subjects will be asked to sign a written informed consent form, approved by an IRB/Ethics Committee, prior to participation in the trial at the screening study visit. Each subject will be assigned a Subject Identification Number, which will be used for identification purposes in order to ensure subject confidentiality. All subject data held on the study Sponsor's database will be anonymised and identifiable only by Subject Identification Number.

Those who continue to meet all of the eligibility criteria after the 2 week run-in period will be enrolled into the trial and randomized to one of two treatment arms: TWO_2 therapy or, Control therapy.

In this trial a maximum of 220 subjects will be recruited. A Group Sequential Designs (GSD) with interims analyses after one third and two third recruited subjects has been chosen that will allow the trial to be stopped if TWO_2 therapy is obviously better than control therapy.

Subjects will be recruited as outpatients in participating wound care centers and clinics. At the screening visit the informed consent document must be signed, the wound will be measured and the subject will receive a standardized off-loading device for weight bearing wounds. The subject will from then on, be treated with predefined dressings used in the total treatment phase of the study.

A diary card will be given to every subject. Subjects will be instructed on keeping the simple diary card to record the use of the TWO2 or control device, dressing changes and to collect data for health economic analysis. A study ID card will also function as an alert to other providers of participation in a clinical study (example Appendix I).

After a run-in period of 2 weeks the subject will be randomized into the study if the wound-size reduction is less than 30% of the initial measurement and all other inclusion and exclusion criteria are met.

All subjects will receive 90 minutes of either TWO_2 therapy or control therapy at their home or nursing care facility 5 times a week for the treatment phase of up to 12 weeks. To secure double blinding no TWO_2 treatment will be done at the study centers. Delivery, installation and training on use of the TWO2 device or control device may differ according to geographical location and shall be performed by trained accredited home health service providers utilizing nurses or other registered healthcare providers, or by trained home/durable medical equipment (DME) providers and an expert on the TWO2 device or control device. The subject, or in some cases caregiver or healthcare professional (such as a home health nurse or aide), applying the TWO_2 therapy or control therapy will be adequately trained and assessed for competency on the use of the device, which will be reviewed monthly. Dressing changes at home (or equivalent) may be performed either by the subject, caregiver or healthcare professional such as a home health nurse or aide. Both the TWO_2 therapy and control therapy groups will visit the study center once per week for on

treatment study visits. Dressings will be pre-specified advanced moist wound therapy (AMWT) dressings, which will be changed at a minimum of once weekly according to the PI/Sub Investigator's (SubI) clinical judgment. Subjects will receive standardized off-loading for a weight bearing wounds or an open heel boot for posterior heel wounds. A Charcot Restraint Orthotic Walker boot (CROW boot) may be used where there is a need to protect a Charcot Foot deformity. Debridement will be performed as needed according to the PI/SubI clinical judgment.

The treatment phase of the study will be 12 weeks. At every on treatment study visit at the clinic, foot ulcers are photographed. The initial (baseline) measurement after debridement will serve as the standard. Digital images will be sent to a blinded central assessor to be assessed and measured.

Once wounds are initially considered closed, that visit will serve as the first of two confirmatory visit. All subjects will continue treatment for an additional 2 weeks, to confirm wound closure.[23] During this period, subjects will continue to receive TWO2 therapy or Control therapy, use and change dressings, and use the off-loading device, where it is required, per the protocol. For subjects whose wounds close at week 11 or later the treatment phase will continue to the second confirmatory visit.

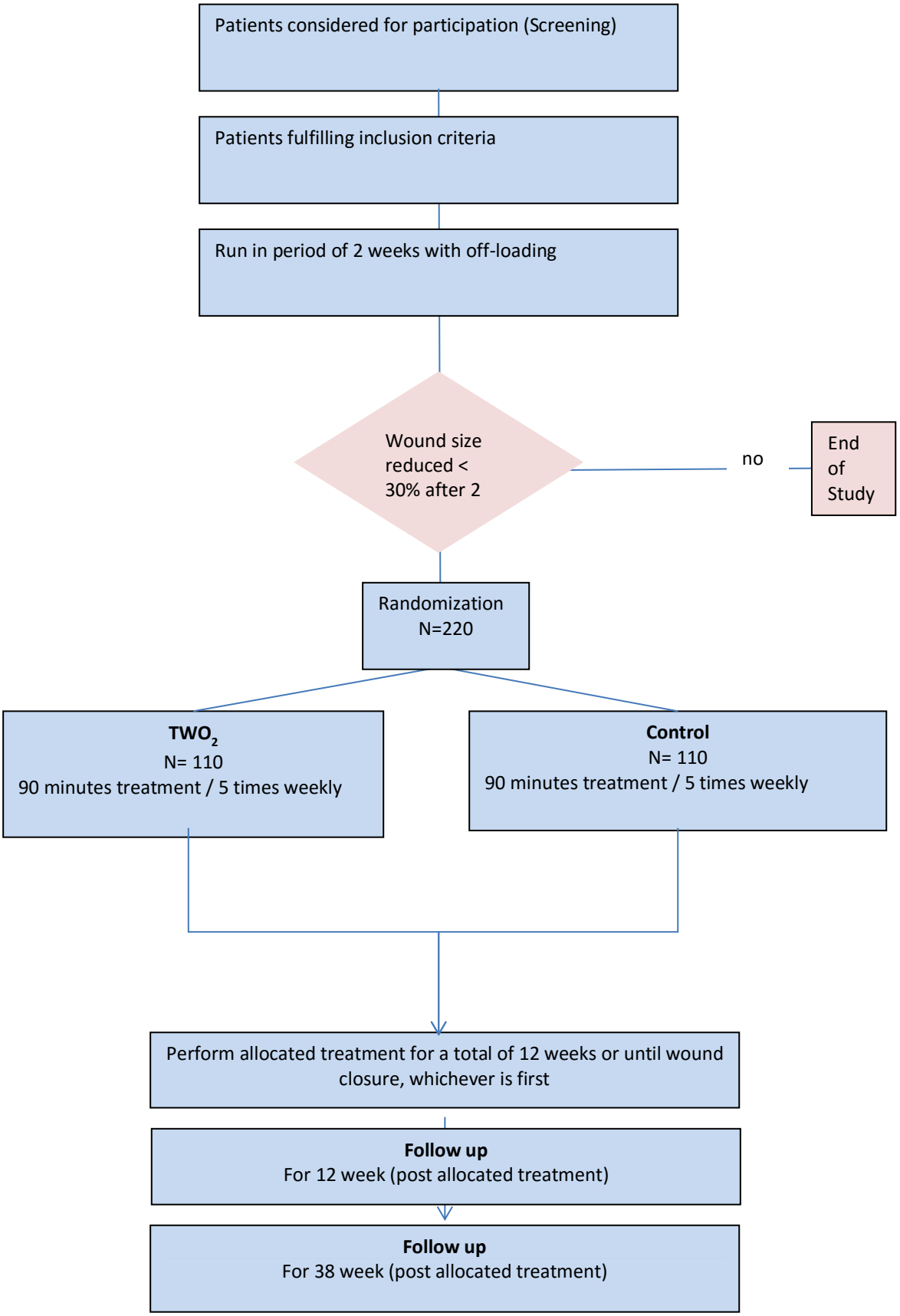
All subjects whose wounds are confirmed closed or who finished the treatment phase of 12 weeks – whichever is sooner - will enter into the post-treatment follow-up phase of 12 and 38 weeks.

Subjects who are withdrawn by the PI/SubI from the study treatment phase will be asked to return for the final week 12 on treatment visit and follow-up phase at 12 and 38 weeks.

The maximum duration for participation in the trial is 54 weeks. During the follow-up phase, subjects will receive standard care according to the clinician's recommendation and will be asked not to participate in another wound care trial in this period.

The start of the trial is scheduled for July 2014.

3.2. Flowchart of Subject Flow



3.3. Endpoints

3.3.1. Primary Study Endpoints

The primary endpoint is the:

Incidence of complete wound closure within 12 weeks.

Closure is defined as 100% skin re-epithelialization without dressing requirements which is confirmed by 2 consecutive study visits 2 weeks apart.

3.3.2. Secondary Study Endpoints

1. Time to complete wound closure
2. Change in wound size over time
3. Incidence of reoccurrence
4. Incidence of amputation
5. Incidence of adverse events
6. Quality of Life – Pain, Mood and Function
7. Health Economic Analysis

3.3.3. Safety Endpoints

Incidence, duration and severity of adverse events and device effects will be documented in every case. This includes:

- Incidence of severe infections needing hospitalization
- Malfunctioning of the device
- Worsening of the wound
- Death

3.3.4. Justification for Trial Design

The choice of using the "Incidence of complete wound closure" as the primary endpoint is in accordance with the recommendation of the FDA document "*Guidance for Industry, Chronic Cutaneous Ulcer and Burn Wounds, Developing Products for Treatment*".[23]

The choice of the parallel group design is the most appropriate for a wound healing study.

The treatment duration of 12 to 16 weeks is a standard in randomized clinical trials in wound care.[24-29] Two recent studies with the TWO_2 system in venous and diabetic ulcers showed that the majority of ulcers treated with topical oxygen show wound closure after 12 weeks.[21,22]

A double blind trial is the gold standard in trial design.

Although there are a number of published reports reporting positive results on topical oxygen treatment and diabetic foot ulcers there is no comparative trial so far. In order to overcome the uncertainty surrounding the expected benefit, the trial will be undertaken using group sequential design (GSD) allowing for interim analysis to be performed at intervals during the trial recruitment.

For the purposes of standardizing to reduce treatment variability the choice of dressings for this trial will be specifically limited.

All "active" dressings will be excluded from the trial. These include: antimicrobial dressings containing silver, iodine or Polyhexamethylene Biguanide (PHMB), dressings containing enzymes, and growth factors (Regranex® or dermal skin substitutes like Apligraf® or Dermagraft®).

Adjunctive treatments such as Hyperbaric oxygen therapy (HBO), Vacuum assisted closure (VAC), Heat therapy, Laser Therapy, Mechanical constant tension, Larval/maggot therapy (Biodebridement), Artificial Skin and Electrical stimulation for Tissue Repair (ESTR) are not allowed in this study.

No local antimicrobial treatments are allowed in the study. Systemic antibiotic treatment of infection will be prescribed according to local standards.

Histological studies in pigs treated with topical oxygen showed signs of improved angiogenesis and tissue oxygenation. Immunohistochemical analyses revealed a stronger presence of Vascular Endothelial Growth Factor (VEGF) in the tissue from oxygen treated wounds in pigs compared to the non-treated controls.[17] As no studies have investigated these effects in humans, punch biopsies from 20 randomized subjects may be planned at selected centers and taken at baseline and after 4 weeks.

Recent RCTs with patients with diabetic foot ulcers from Blume et al., 2008 investigating Negative Pressure Wound Therapy versus advanced Moist Wound Therapy and the paper from Löndahl, 2010 where Hyperbaric Oxygen Therapy was evaluated as an adjunctive therapy did not exclude infected ulcers. As oxygen is critical to fight infection in chronic wounds[33-35] and the TWO_2 device has been shown to eliminate infections and multi-resistant staphylococcus aureus (MRSA) [21,22,36], in this trial wound infections will not be excluded. However, any subject with wound infection must be under treatment at the time of randomization.

4. SELECTION OF TRIAL POPULATION

Healing chronic ulcers will have a positive impact on patient well-being and quality of life. In this study we aim to examine the efficacy of Topical Wound Oxygen (TWO_2) therapy with AMWT in the healing of diabetic foot ulcers and compare the outcome to that of AMWT and Control therapy in a double blind study.

In the event that a subject being considered for study enrollment has multiple diabetic foot ulcers, the ulcer to be considered as the index ulcer will be the largest one, which still meets all other inclusion criteria (Index ulcer).

4.1. Inclusion Criteria

A subject must meet all of the following criteria to be eligible for enrollment in the trial.

Ulcer characteristics

- Subject has a documented diagnosis of Diabetes mellitus Type 1 or 2
- Foot ulcer at or below ankle with duration of more than 4 weeks but no longer than 1 year
- If the index ulcer is a post amputation wound date of surgery must be > 30 days
- 2 week run in period with less than 30% wound size reduction
- University of Texas Grade 1A, 1B, 1C, 1D, 2A, 2B, 2C, or 2D (Appendix I)
- Ulcer is ≥1cm^2 and ≤ 20cm^2 after debridement at start of run-in period
- If more than one ulcer is present on the foot, only the largest is considered in the study (Index ulcer)
- Index ulcer must be ≥ 1cm away from any other ulcers present on the foot

Vascular status

- Adequate perfusion with ABI > 0.7 **And**
  TcpO2 > 30mmHg **OR** skin perfusion > 30mmHg **OR** Toe pressure > 30mmHg **OR** Duplex with biphasic waveforms below the knee
- No planned revascularization procedure or vascular surgery within the last/next 30 days

Device and study related

- Males and Females 18-89 years
- Subject and/or caregiver are willing and able to comply with all specified care and visit requirements
- Subject has a reasonable expectation of completing the study; according to the Investigator's clinical judgment

4.2. Exclusion Criteria:

Ulcer characteristics

- Evidence of gangrene on any part of affected limb
- Documented evidence of osteomyelitis on any part of affected limb
- Index ulcer has exposed bone
- Index ulcer exhibits signs of severe clinical infection that requires hospitalization or immediate surgical intervention
- Active Charcot foot on the study limb

Device and study related

- Subject participated in another investigational device, drug or biological trial within last 30 days
- Uncontrolled diabetes: HbA1c > 12 %
- Renal dialysis or serum creatinine > 2.5 mg/dl
- Known immune insufficiency
- Chronic steroid use or immunosuppressive agents within the last three (3) months or is anticipated to require them during the course of the study
- Active treatment for malignancy (not specific to study limb)
- Patient has a Deep Vein Thrombosis within the last 30 days
- Subject has received growth factor therapy (e.g., autologous platelet-rich plasma gel, becaplermin, bilayered cell therapy, dermal substitute, extracellular matrix) within the screening period
- Subject may not be pregnant at the time of treatment

4.3. Removal of Subjects from Therapy or Assessment

Subjects may discontinue study treatment and withdraw from the study at any time. The reasons for withdrawal may include the subject withdrawing their consent to participate in the study, or Investigator may decide to withdraw the subject from the study because of Adverse Events and Device Effects (ADE) or subject non-compliance.

Where subjects are removed from study treatment (TWO2 therapy or Control therapy) for other than non-compliance and continue to provide consent to participate, may continue with study visits to asses for primary and secondary endpoints. This may include:

- 12 week final treatment visit
- 12 week follow up
- 38 week follow up

If treatment is terminated and no follow up visits are anticipated, the final evaluation will be performed as completely as possible. In addition, any comments (spontaneous or elicited) or complaints made by the subject and the reason for termination will be recorded in the CRF and the subject's medical records.

5. RANDOMIZATION

The Sponsor will act as the central randomization center. After successful assessment of inclusion/ exclusion criteria at the 2 week run in visit, the subject will be randomized to one of two treatment arms: TWO_2 group or, Control group. Study sites will notify the Sponsor when a subject has met the criteria for randomization. In accordance with local requirements, the device service provider will be notified by the study site , who will provide the contact details of the subject for device delivery, set-up and training.

Randomization will then be centrally performed by the Sponsor by utilizing randomized permuted blocks of number using the ralloc function in Strata 11.0 Subjects will be randomized to the next available treatment group. The local Device Service Provider will be notified of the assigned device, by the Randomisation Centre, to be delivered directly to the successfully randomized subject. The Central Reading Centre, Investigators and Trial monitors will not be aware of the randomization group assigned.

5.1. Identification numbers

Each subject who has signed a written IRB/Ethics Committee approved Informed Consent Form will receive a unique subject ID number. The number will have 6 digits. The first two numbers will be the country code, the next two digits will define the center and the last two digits will be consecutive screened subjects in this center.

After all baseline medical data is recorded and the wound is documented at the 2 week baseline/randomization visit, the subject's number will become the subject identification number (Subject ID) for the remainder for the study. The investigator will not know to which treatment arm the subject is allocated throughout the trial.

5.2. Allocation concealment

Randomization will be coordinated by a central randomization center. Neither investigator nor subject will know their randomization allocation or can influence their allocation (=allocation concealment).

6. TRIAL TREATMENT MODALITIES

6.1. Trial Treatment

The TWO_2 group will receive topical oxygen as an adjunctive treatment to standard of care treatment. The TWO_2 treatment consists of the Topical Wound Oxygen therapy system (AOTI Ltd.) as well as an oxygen concentrator. Oxygen will be delivered to the device at a flow rate of at least 10 liters/ minute.

After connecting the TWO_2 Single patient-use leg chamber to the controller and concentrator, the controller initiates 4 phases of the treatment:

Phase 1:
The outer leg chamber is inflated. The limb with the ulcer can then be placed into the chamber.

Phase 2:
The integrated cuff is inflated to seal the inside of the chamber against the limb.

Phase 3:
The therapy phase: Cyclical pressurized oxygen is delivered to the limb inside the chamber.

Phase 4:
The cuff and outer leg chamber is deflated and removed from subject's limb.

This setup delivers cyclical pressurized oxygen to the wound site within a sealed and humidified environment.

The affected limb will be placed in the chamber for 90 minutes daily, 5 days a week, for a period of up to 12 weeks (plus an additional 2 visits, 2 weeks apart to confirm wound closure for index ulcers closing at week 9 or later) or until full closure of the wound, whichever is sooner.

All treatments can be done with or without removing the dressings.

The picture below shows the TWO_2 Single patient-use leg chamber and the controller.

6.2. Control Treatment

The control treatment will look and act exactly like the TWO2 treatment. The only difference is that the controller has technical modifications.

In phase 3, the control therapy will not supply oxygen or pressure to the affected limb during the treatment but mimics the therapy on the display with pressure values that go up and down.

All other phases of device operation are identical.

6.3. Standard Care Treatment

Subjects in both groups will receive the same standard care regime that includes sharps debridement of all callus/necrotic tissue, AMWT and off-loading, where it is required. In a multi-center trial the value of study site consistency in standard care regimens within a trial cannot be over-emphasized because of the profound effects these procedures have on clinical outcome for chronic wounds.[23] Consistency in standard care regimens is important for minimizing variability and allowing assessment of treatment effect. Every attempt will be made to minimize deviations from the procedures described in the protocol and subject compliance recorded in CRFs.

In order to minimize variability in standard of care between sites:

- All centers will perform a standardized training before the trial
- All centers will be monitored closely by a study site monitor and the study director

6.3.1. Debridement

Adequate debridement is key for wound healing in diabetic ulcers. All wounds will receive baseline sharps debridement of all callus/necrotic tissue and additional debridement as needed throughout the trial. Debridement will be performed where it is determined to be clinically necessary by the PI/SubI.

Any surgical debridement has to be documented on CRFs, reported as an adverse event and will be included in analysis of product safety and efficacy.

Standardized debridement will be part of the Investigators training. To ensure the same standard of debridement/wound preparation in all centers, photographs of all subjects will be evaluated by a central assessor.

Enzymatic debridement products, like other concomitant topical products, can confound results in topical wound-treatment product trials and therefore must be prohibited.

6.3.2. Off-Loading

Excessive pressure on the sole of the foot is one of the key factors in the pathogenesis of neuropathic plantar ulcers. Consequently pressure relief is of fundamental importance in the treatment of the diabetic foot ulcer[30] and has been intensively proven in well designed randomized clinical trials. [24,26,31,32]

Therefore all subjects in the present study will be provided with an off-loading device (for weight bearing wounds) from Salvatelli srl, Italy (Piaggesi study):

	
Optima Diab	**Optima CLHeel**

During the weekly wound assessment, subjects will be educated about the importance of the off-loading (where it is required).

6.3.3. Dressing Changes and Wound Dressing

Dressing changes will be performed a minimum of once a week according to the Investigators recommendation and consist of the following procedures:

1. Dressing removal
2. Cleansing with saline
3. Application of new dressing

At each dressing change the wound will be cleansed with saline.

Antiseptic substances like Octenisept®, Prontosan®, or similar products CANNOT be used in this trial.

All wound dressings will be from Convatec/Kendall. The wound dressings will be limited to the following:

Primary Dressing
Kendall Foam Dressing,

Wound filling dry wounds
Curafil™* Gel Wound Dressing 3 Ounce Tube

Secondary Dressing
Non-sterile Kerlix Rolls

Surgical Tape
Generic surgical tape one roll

Saline
Generic sterile saline 0.9%

The wound dressings selected by the Investigator for each subject, will be used in accordance with the manufacturer's guidelines and institutional protocols and healthcare professional's judgment.

The number of dressing changes will be documented in the CRFs.

Dressing changes can be performed by the subject, caregiver or healthcare professional (such as a home health nurse or aide).

6.3.4. Monitoring and treatment of wound infection

Infection will be assessed clinically via symptoms and signs including purulent drainage, erythema, warmth, exudation, odor, pain, fever, and leukocytosis as well as wound size and time to wound healing. In patients with diabetes, fever, pain, and leukocytosis may be absent.[37-41]

Mild and moderate infections can be included into the study. No local antimicrobial treatments are allowed in the study. Systemic antibiotic treatment of infection will be prescribed according to local standards (refer to section 3.3.4.).

An infection that needs hospitalization is regarded as a severe adverse event or device effect and the subject will be suspended from the study for up to 4 weeks, up to 2 times, and may rejoin the study on resolution and compliance with other inclusion criteria.

6.3.5. Education

Subjects will be educated during each study visit about the importance of maintaining normal blood glucose levels and encouraged on continued appropriate diabetes management including diet and medications to achieve or maintain a stable glycemic state. Subjects will also be educated on how to care for their feet.

Routine laboratory testing of blood glucose or HbA1c is included in this trial and will be drawn at the screening visit and at the end of the treatment period.

Comorbidities are documented but no special treatment of comorbidities is part of this trial.

6.4. Treatment modalities not allowed in the study

Other adjunctive or alternative treatments, such as; Hyperbaric oxygen therapy (HBO), Vacuum assisted closure (VAC), Heat therapy, Laser therapy, Mechanical constant tension, Larval/maggot therapy (Biodebridement), Electrical stimulation (ESTR), Artificial skin (Dermagraft) are not allowed during this study.

7. ASSESSED VARIABLES

7.1. Efficacy Assessment

7.1.1. Primary Efficacy Assessment

The primary endpoint is the "Incidence of complete wound closure within 12 weeks".

Only one ulcer (the index ulcer) will be assessed per subject. In subjects with multiple ulcers, this will be the largest ulcer present fulfilling all other inclusion criteria.

Closure is defined as skin re-epithelialization without drainage or dressing requirements which is confirmed at 2 consecutive trial visits 2 weeks apart.[23]

Wound closure checks will be performed weekly. The closure of the wound is a clinical judgment by the investigator. A digital picture reviewed by the central assessor will also provide evidence of wound closure.

Ulcers will be measured by the central assessor. A deidentified digital photograph of the ulcer will be taken with and transmitted by the study sites using a wifi/wireless enabled tablet device. The ulcer's surface area, circumference, maximum and minimum diameters will be recorded at baseline assessment of the subject.

7.1.2. Secondary Efficacy Assessments

Time to complete wound closure
The time (in days) from first treatment at baseline of the ulcer until it closes completely will be recorded.

Change in wound size over time
Ulcer size over time will be assessed from the digital photographs of the ulcer taken at each visit.

Incidence of recurrence
Recurrence is identified as an interruption in the epithelium following complete healing of the index ulcer. If the subject does have a recurrence, this will be documented and the re-opened ulcer will be measured and recorded

Incidence of Amputation
All amputations will be recorded. The investigator will judge whether the amputation was done because of the index ulcer or for different reasons.

Incidence of adverse events and device effects

Quality of Life

Pain - A Visual Analogue Scale will be used to determine pain levels.
Mood and Function - The Cardiff Wound Impact Schedule (CWIS) assesses levels of mood and function and is the tool used to determine Quality of Life (Appendix I). (Subjects in countries where the CWIS has not been validated will be debriefed on its use at the end of the study).

Economic Analysis
Economic assessments will be determined from the Euroqol-5D (EQ-5D), subject diary and

medical records documenting dressing changes, time, location and healthcare professional assistance and management (Appendix I).

Cost effectiveness will be determined by examining the difference in the number of healed ulcers in relation to the difference in cost in each randomized group

$$\text{Incremental cost per additional ulcer healed} = \frac{\text{Cost of treatment (TWO) - Cost treatment (Control)}}{\text{Healed ulcers (TWO) - Healed ulcers (Control)}}$$

Cost Utility will be determined by examining the difference in Quality Adjusted Life Years (QALYs) associated with the different treatment regimens and their associated costs. Health utilities will be derived from the EQ-5D tool and used to calculate QALYs in each group. This will be related to overall treatment costs using the following formula

$$\text{Incremental cost per QALY} = \frac{\text{Cost of treatment (TWO)-cost of treatment (control)}}{\text{QALY (TWO)} - \text{QALY(control)}}$$

See 7.2 Safety Endpoints

7.2. Safety Endpoints

This study is being conducted in North America and Europe. The study will align assessment, documentation and reporting of safety outcomes with the best available guidance that is derived from ICH GCP, ISO 14155:2011(E) and 21 CFR Part 812.

Each subject will be carefully monitored throughout the trial and asked for an account of adverse events and device effects at each study visit. An assessment must be made of the seriousness, intensity and relationship of each occurrence to the administration of TWO2 therapy or control therapy.

The subject population for this study is known to have a high incidence of morbidity and mortality. Known co-morbidities related to diabetes include cardiovascular disease, cerebrovascular disease, renal failure and deterioration of foot ulcers that may require hospitalization. The study will capture data on adverse events and device effects and serious adverse events and device effects related to the study's secondary outcome measures and will also record concomitant medication related to these occurrences. Serious adverse events that are anticipated in this population do not require expedited reporting, but they will be recorded as they may affect the status of subjects in the study. Detailed documentation of pharmaceutical interventions are not required for the safety evaluation of the device, but will be recorded where in the opinion of the PI/SubI they may directly or indirectly relate to the index ulcer.

7.2.1. Definition of Adverse Event

An adverse event (AE) is defined as:
An AE is: Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users or other persons, whether or not related to the investigational medical device
NOTE 1 This definition includes events related to the investigational medical device or the comparator.
NOTE 2 This definition includes events related to the procedures involved.
NOTE 3 For users or other persons, this definition is restricted to events related to investigational medical devices.
ISO 14155:2011E (3.2)

7.2.2. Definition of Adverse Device Effects

An adverse device effect (ADE) is defined as:
An adverse event related to the use of an investigational medical device.
NOTE 1 This definition includes any effect resulting from insufficiencies or inadequacies in the instructions for use or the deployment of the device.
NOTE 2 This definition includes any effect that is a result of user error (ISO 14155:2011(E) (3.1)).

7.2.3. Definition of Unanticipated Adverse Device Effects

An unanticipated adverse device effect (UADE) is defined as:
Unanticipated adverse device effect is any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects. (CFR 21 Part 812.3(s))

7.2.4. Serious Adverse Device Effect

A serious adverse device effect (SADE) is defined as:
Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event (ISO 14155:2011(E) (3.36)).

7.2.5. Definition of Serious Adverse Events

A serious adverse event (SAE) is defined as:
Any untoward medical occurrence which in any way:

- requires inpatient hospitalization or prolongation of existing hospitalization
- is life-threatening
- results in death or results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- or is considered serious by the investigator*

* Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above.

NOTE: The term 'life-threatening,' in the definition of 'serious,' refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death had it been more severe.

7.2.6. Relationship of Events to Study Treatment

The causal relationship of each AE to the investigational product will be assessed by the study Investigator using the following definitions:

Definite	Clear cut temporal association, and no other possible cause.
Probable	Good reasons and sufficient documentation to assume a causal relationship in the sense of plausible, conceivable, likely but not necessarily highly probable.
Possible	Sufficient information to accept the possibility of a causal relationship in the sense of not impossible and not unlikely, although the connection is uncertain or doubtful, e.g. due to missing data or

insufficient evidence.

None	The AE is completely independent of the trial product administration and/or evidence exists that the event is definitely related to another etiology.

7.2.7. Severity of Events and Effects

Classifications of the AE severity include the following:

Mild	Awareness of signs or symptoms, but easily tolerated and are of minor irritant type causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation; signs and symptoms are transient.
Moderate	Events introduce a low level of inconvenience or concern to the participant and may interfere with daily activities, but are usually improved by simple therapeutic measures; moderate experiences may cause some interference with functioning.
Severe	interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment; they are usually incapacitating.

7.2.8. Anticipated Adverse Device Effects

There are no known ADEs reported with the use of the TWO2 device. The following AEs are possible secondary outcomes and must be reported in the CRFs:

Mild to Moderate Adverse Events

Mild and moderate adverse events can include, but are not necessarily limited to, the following clinical signs and symptoms:

- Pain
- Dermatitis
- Cellulitis
- New or satellite areas of breakdown
- Erythema

Severe Adverse Events

Severe adverse device effects can include, but are not necessarily limited to, the following clinical signs and symptoms:

- Infection requiring hospitalization

7.2.9. Reporting Serious Adverse Effects

Any adverse device effects (ADEs) will be recorded and if an considered serious unanticipated adverse device effect (SUADEs) will be reported to the IRB/Ethics Committee within 10 working days (CFR 21 Part 812.150(a)(1)) or sooner if required by Regulatory Authorities and institutional guidelines and in accordance with the principles of ICH GCP. Any study related occurrence that could adversely affect the safety of subjects or the conduct of the trial should be reported to AOTI Ltd. and if locally required to the local IRB/Ethics Committee by telephone and fax within 24 hours of being noted. An expedited reporting form should be used.

The information reported must at least include the following:
Name, address and telephone number of the reporting investigator.

- Title of the trial.

- Study code.
- Subject identification number, sex and year of birth.
- Description of the AE, measures taken and outcome.
- Preliminary classification of causal relationship by the investigator.

Within 48 hours of sending the initial information the investigator must send a completed copy of the form to AOTI Ltd by fax or email (where local policy allows).

AOTI Ltd Reports must be addressed to:

Study Director Contact Details for Adverse Event Reporting:

Name: Mike Griffiths DProf, DMS, CRT, FCMI
Email: TWO2DFUstudy@aotinc.net
Fax: +1 760-859-3911 (US sites only)
Telephone: +1 760 672 1920

7.2.10. Follow-up of Adverse Device Effects

The investigator will initiate appropriate therapeutic measures and investigations for adverse effects(s) and concurrent illness(es) that occur during the study and must treat with established standards of care. The investigator must continue to follow all serious adverse effects and those non-serious effects possibly related to a study treatment until they are resolved or until the investigator considers them to be stable. This follow-up may extend beyond the end of the trial.

7.2.11. Reporting Safety Information

AOTI Ltd. will promptly notify the Investigator and the Regulatory Authorities of findings that could adversely affect the safety of subjects, impact on the conduct of the trial, or alter the IRB/Ethics Committee approval/favorable opinion of the trial. The Investigator must promptly inform the relevant IRB/Ethics Committee of all occurrences deemed to involve increased risks to subjects (if required).

8. TRIAL CONDUCT

8.1.1. Overview of Screening Treatment and Follow-Up Requirements

Procedure / Examination	Screening & Start Run-In	Baseline * (2 weeks later)	1	2	3	4	5	6	7	8	9	10	11	12	12 wks Follow up	38 wks Follow up
Informed Consent	X															
In-& Exclusion criteria	X	X														
Randomization		X														
Adverse Effects and Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical History/ Preexisting Conditions	X															
Record Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Compliance assessment (treatment, dressings)		X	X	X	X	X	X	X	X	X	X	X	X	X		
Height and Weight	X															
Wound History (Duration; course of healing last 12 months and previous treatment modalities)	X															
Dressing removal / wound cleansing and re-dressing	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Wound assessment and measurement (location, size)**	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Pain assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Neuropathic assessment	X															
Infection Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ABI and Secondary Vascular Assessment ***	X															
Dig. Photographs (post debridement)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Debridement if needed		X	X	X	X	X	X	X	X	X	X	X	X	X		
Cardiff Wound Impact Schedule ****	X	X												X	X	X
Lab tests	X													X		
Punch biopsies *****		X				X										
Education on diabetic foot care/off-loading*****	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Economic assessment		X				X				X				X	X	X
Device competency assessment		X				X				X						

Note: *Baseline visit window is -1/+2 days. All follow-up visit windows are +/-2 days. 12 and 38 Week follow-up +/- 2 weeks

** Measurement recorded from central assessor report plus manual measurement at baseline

*** Secondary vascular testing only needed if not within 4 months of screening date

**** Cardiff Wound Impact Schedule (debriefing of subjects week 38 Follow up where not previously validated)

***** Optional (none for sub-study)

***** Off-loading with study boot or CROW boot where required

****** Health Economic Assessment (EQ-5D, dressing history up to 8 weeks and significant related medical events)

8.1.2. Wound Closure Assessment

Procedure / Examination	**Wound Closure Visit # 1**	**Wound Closure Visit # 2**
Verify Informed Consent	X	X
Adverse Events	X	X
Record Concomitant Medications	X	X
Compliance assessment (treatment, dressings)	X	X
Confirm Wound Closure Assessment	X	X
Dressing removal / wound cleansing and re-dressing	X	
Pain assessment	X	X
Dig. Photographs (post Debridement if needed)	X	X
Debridement if needed	X	X
Cardiff Wound Impact Schedule (If at week 12 on treatment or last visit before follow up)	X	X
Education on diabetic foot care/off-loading Study dressings	X	
Education on diabetic foot care/off-loading Standard of care dressings per PI/Sub I		X
Economic assessment (if week 4, 8 or 12 on treatment or last visit before follow up)	X	X

Note:
* The wound closure visits may occur within the 12 week follow up period
* *Visit window is +/- 2 days.

8.2. Assessment by Time Point

8.2.1. Screening Assessment

Potential subjects should sign a written IRB/Ethics Committee approved informed consent. Inclusion criteria and exclusion criteria are checked. The index ulcer is chosen as the largest ulcer (in surface area) on the foot. The wound has to be measured and assessed. The vascular assessment is performed, and blood taken for the lab tests. Previous treatment modalities for the index ulcer attempted are documented.

If the subject meets all criteria for inclusion into the study the 2 week run-in period is initiated.

The following assessments will be done:

1) Obtain informed consent
2) Complete subject contact information and demographics
3) Assign subject a screening ID number
4) Verify subject eligibility by checking against inclusion/exclusion criteria
5) Quality of Life Assessment using CWIS
6) Medical history
7) Concomitant medication
8) Height and Weight
9) Tobacco use
10) Identify index ulcer
11) Wound History
12) Wound assessment: Location, size and appearance
13) Pain assessment of wound using the Visual Numerical Rating Scale
14) Neuropathic assessment
15) Infection assessment
16) Digital Photograph (post debridement)
17) Debridement if needed
18) Dress wound with study dressings
19) Vascular assessment (ABI and toe pressure or tissue pressure or TcPO2 or duplex (below knee))
20) Record lab data or blood sample taken to obtain values for:
    - Electrolytes
    - Creatinine

- HbA1c, blood glucose
- WBC
- Albumin and pre-albumin (where routinely available)
- CBC
- C-reactive protein

21) Provide and educate on off-loading boot/CROW boot, study dressings and diary card and ID card
22) Educate on diabetes management and foot care
23) Urine pregnancy for women of childbearing potential

The subject is supplied with the off-loading boot/CROW boot and treated with the dressings defined for this study for 2 weeks until the baseline assessment takes place.

8.2.2. Baseline Procedures (after 2 week run-in period)

Prospective subjects must meet all inclusion criteria to be eligible to participate in the study. Missing data for the final verification of all inclusion and exclusion criteria will be performed including HbA1c which has to be available at the baseline visit.

The following data will be collected at the baseline visit prior to randomization treatment with the TWO2 therapy or Control therapy:

1) Verify Informed Consent
2) Record adverse events
3) Record changes in concomitant medication
4) Verify subject eligibility by checking against inclusion/exclusion criteria
5) Quality of Life Assessment using CWIS
6) Collect diary card/Compliance assessment (treatment/dressings)
7) Remove dressings/wound cleansing
8) Pain Assessment using the Visual Numerical Rating Scale
9) Digital photograph (post debridement)
10) Debridement if needed
11) Wound assessment: Location, size and appearance
12) Infection assessment
13) Dress wound with study dressings
14) Record lab data if not previously recorded
15) Education on diabetes management and foot care and off-loading boot/CROW boot
16) Dispense study dressings and diary card
17) Health Economic Assessment (including EQ-5D)
18) Punch biopsies
19) Device competency assessment (Completed in subjects home or equivalent after baseline clinic visit)

8.2.3. Randomization Procedures

Subjects will be randomized to receive either TWO_2 therapy as an adjunctive therapy or a control therapy.

8.2.4. Weekly procedures during treatment period

All subjects (TWO_2 therapy and control therapy) will have a weekly assessment in the clinic until the index ulcer is closed or 12 weeks have passed – whichever is sooner.

Each Assessment Visit will include the following procedures:

1) Verify Informed Consent
2) Record adverse events/device effects
3) Record changes in concomitant medication
4) Quality of Life assessment using CWIS (week 12 only)
5) Collect diary card/Compliance assessment (treatment/dressings)
6) Remove dressings/wound cleansing
7) Pain Assessment using the Visual Numerical Rating Scale
8) Digital photograph (post debridement)
9) Debridement if needed
10) Wound assessment: Location, size, depth and appearance
11) Infection assessment
12) Blood sample taken to obtain values for **(visit 12 only)**:
    - Electrolytes
    - Creatinine
    - HbA1c, blood glucose
    - WBC
    - Albumin and pre-albumin (where available)
    - CBC
    - C-reactive protein
13) EQ-5D) (week 4, 8 and 12 only)
14) Education on diabetes management and foot care and off-loading boot/CROW boot
15) Dispense study dressings and diary card
16) Device competency assessment (completed in subject's home or equivalent. Week 4 and 8 only)

8.2.5 Punch biopsies

Punch biopsies may be taken at baseline and 4 weeks for up to 20 subjects enrolled in the study at selected centers. These subjects will have specifically agreed to this procedure on their Informed Consent Form.

The biopsies will be taken utilizing a standard 4 mm dermal punch biopsy kit and will be taken following routine debridement of the wounds to avoid collection of dead necrotic tissue. Prior to biopsy collection, the wound bed is prepared with local antiseptics. As per subject request, local anesthesia is administered. This is not standard because most subjects in this study will be insensate in the area of the wound.

In addition it is important to have some tissue samples without local anesthetic to determine whether the use of anesthetic agents impacts the molecular studies planned with the biopsy. Biopsies will be taken from the wound edge and from the center of the wound.

Samples will be collected, packed and transported per the procedure outlined by the testing laboratory instructions. The samples will be transported from clinic to the testing laboratory in properly secured biohazard-safe containers under appropriate temperature conditions.

The samples will be analyzed histologically for signs of infection, collagen and neo-angiogenesis. Additional immune-histochemical analyses for such values as VEG-F and matrix metalloproteinases (MMPs) are also envisioned.

8.2.6 Premature Withdrawal of a Subject from the Trial

Subjects are free to withdraw from the trial at any time without penalty or prejudice to their further treatment. Subjects may stop the trial treatment for any reason.

All subjects should be invited to do the End of Trial Visit to complete a final assessment, including wound status, complete quality of life questionnaire, etc.
The End of Trial Visit should occur on or as close as possible to treatment week 12. Though encouraged to do so in all cases, some subjects may still decline to attend this visit, in which case this will be recorded in the CRF (along with any reasons if given). Subjects who are withdrawn from the treatment will be asked if they consent to be followed up after withdrawal to determine whether their ulcer heals within the 12 weeks from randomization.

In addition, a follow-up after 12 weeks and/or 38 weeks will be undertaken wherever possible in those that were withdrawn to confirm whether they healed according to intention-to-treat (ITT).

8.2.7 Procedures for confirmation of wound closure

Subjects with index ulcers that are observed to close will be followed for 4 weeks to confirm wound closure. During this period, subjects will continue to receive treatment and dressing changes and wear the standard off-loading device. If wound closure occurs within the 11th week, treatment will continue for an additional 2 weeks to confirm closure (up to a maximum of 14 weeks of treatment).

The wound closure confirmation assessment will be done at confirmation visits 2 weeks after closure.

1) Verify Informed Consent
2) Record adverse events/device effects
3) Record changes in concomitant medication
4) Quality of Life Assessment using CWIS (If week 12 on treatment or last visit before follow up)
5) Collect diary card/Compliance assessment (treatment/dressings)
6) Confirm wound closure for past week
7) Pain Assessment using the Visual Numerical Rating Scale
8) Wound History: time of wound closure
9) Digital Photograph of the closed index ulcer
10) Education on diabetes management and foot care/off-loading boot/CROW boot
11) Dispense study dressings (confirmation visit 1 only) /dressings (if indicated confirmation visit 2) and diary card (confirmation visit 1 only)
12) EQ-5D (if week 4, 8 or 12 on treatment or last visit before follow up)
13) Blood sample taken to obtain values for (if at week 12 from randomization or at last closure visit if before):
    - Electrolytes
    - Creatinine
    - HbA1c, blood glucose
    - WBC
    - Albumin and pre-albumin (where available)
    - CBC
    - C-reactive protein

Subjects whose index ulcers are confirmed as closed will enter into the follow-up phase and will be terminated from further treatment with TWO2 therapy or Control therapy. Subjects will be encouraged to continue to wear the standard off-loading boot/CROW boot during the follow-up phase.

8.2.8 12 Week Follow up

All subjects will be reviewed and followed up 12 weeks after "wound closure" or the 12-week "end of treatment phase", whichever is sooner +/- 2 weeks. Data for 12 week follow up analysis will be collected per the subject's preference by telephone or in the clinic if attending a routine visit for wound care. Index ulcers not yet healed, will continue to be treated with best available therapy as determined by the treating medical provider.

The following assessments will be performed on all subjects:

1) Verify Informed Consent
2) Record adverse events
3) Record changes in concomitant medication
4) Quality of Life Assessment using CWIS
5) Pain Assessment using the Visual Numerical Rating Scale (where possible, if applicable)
6) Digital Photograph of the open index ulcer
7) Health Economic Assessment (including EQ-5D)
8) Education on diabetes management (and foot care/off-loading when indicated)

8.2.9 38 Week Follow up

The 38 week follow up will take place 26 weeks +/- 2 weeks from the time of the last study visit (12 week follow up). Data for 38 week follow up analysis will be collected per the subject's preference by telephone or in the clinic if attending a routine visit for wound care. Index ulcers not yet healed, will continue with best available therapy as determined by the treating medical provider.

1) Verify Informed Consent
2) Record adverse events
3) Record changes in concomitant medication
4) Quality of Life Assessment using CWIS
5) Pain Assessment using the Visual Numerical Rating Scale (where possible, if applicable)
6) Digital Photograph of the open index ulcer (where possible)
7) Health Economic Assessment (including EQ-5D)
8) Education on diabetes management (and foot care/off-loading when indicated)
9) Debriefing of subjects where CWIS not previously validated

8.2.10 Definition of the End of Trial

For the purposes of notifying the IRB/Ethics Committee and the Competent Authorities, the end of the trial is defined as completion of the final 38 week follow-up on the final subject remaining in the trial.

8.2.11 Premature Termination of the Trial

If AOTI Ltd. or the principal investigator discovers conditions arising during the trial which indicate that the trial should be halted, it must be terminated after appropriate consultation between AOTI Ltd. and the principal investigator. Conditions that may warrant termination include, but are not limited to:

1. The discovery of an unexpected, significant or unacceptable risk to the subjects enrolled in the trial.
2. Failure of the investigator to enter subjects into the study at an

acceptable rate.

3. Insufficient adherence to the protocol.
4. If study data at interim analysis indicates that treatment with TWO2 therapy is of no clear benefit to diabetic patients with diabetic foot ulcers.

If the trial is prematurely terminated or suspended for any reason, the investigator and subjects will be informed promptly. Appropriate therapy and follow up for subjects will be assured.

- The investigator must notify the IRB/Ethics Committee of any premature termination of the trial, within 15 days, using the “Declaration of End of Clinical Trial” form available from the EudraCT/and other relevant websites.
- AOTI Ltd. will notify the Regulatory Authorities within 15 days using the same process.

8.2.12 Subjects Lost-to-follow-up

Every effort should be made for all subjects enrolled in this study to complete all study visits and assessments including the follow-up phase. A minimum of three (3) documented telephone calls will be made to any subject who does not return for scheduled treatment or follow up visits. Should the telephone calls be unsuccessful in contacting the subject, a certified letter will be sent. Only after a minimum of four (4) attempts to get the subject to return to the clinic, will the subject be considered "lost-to-follow-up".

If the subject has to stay in the hospital for another medical condition or because of the diabetic ulcer, all reasonable efforts will be undertaken to continue the trial treatment on discharge, if the hospitalization is no longer than 4 weeks.

9 STATISTICAL ANALYSIS

9.1 Sample Size and Power Analysis

All sample size estimations in this section of the protocol were undertaken using the sampsi function in Stata 11 (Statcorp LP, Texas, USA). Comparisons of groups was made assuming no continuity correction.

Little data are available on similar studies using topical oxygen therapy within randomized clinical trials making sample size estimation problematic. Large trials of dermal substitutes and negative pressure have been undertaken that can be used to indicate the potential healing in the control group. The three large trials have control healing of between 18% and 28.9% around 12 weeks after entering the trial, with a mean of 28%.[25,27,29]

The expected improvement in healing using the active device is more problematic as there have been few comparative studies published. Aburto and Frye undertook a randomized study of 20 venous ulcers and 20 diabetic foot ulcer patients. [42] Topical oxygen demonstrated higher healing in the DFU patients after 90 days of 90% versus 40% in the control group. Blackman et al randomized 28 patients with DFUs and obtained a similar response (82.4% versus 45.5%).[22] The potential difference between treatment groups was therefore 50% and 36.9% respectively. Using these studies a total sample size of 28 and 52 would be expected based on 80% power at the 5% level of significance. Neither of these studies was blinded. It is therefore likely that the effect is likely to be substantially smaller than that experienced in these two studies.

Because of the small size of these trials and lack of blinding it has been decided to offer a more cautious approach to the calculation of sample size based on a smaller expected benefit of oxygen therapy. By combining the results of the two randomized studies already undertaken the control group achieved a healing rate of 9/21 (42.8%). In the actively treated group healing occurred in 23/27 (85.2%). Assuming an expected control rate of 43% and a difference of half that experienced in these trials of 21% (to 64%) would require a sample size of 88 patients in each group (total=176), with a power of 80% and a level of significance set at $p<0.05$.
In order to overcome the uncertainty surrounding the expected benefit, the trial will be undertaken using group sequential design (GSD) allowing for interim analysis to be performed at intervals during the trial recruitment. The interim analysis will take place after one third of subjects have been recruited, after two thirds and then a final analysis at full recruitment. The Pocock method of interim analysis will be used, which requires an equal level of significance ($p<0.022$) over the three analyses.[43] If the difference in primary outcome produces a probability less than this in any of the analyses then the null hypothesis is rejected and the alternative hypothesis of a difference in healing has been shown. In order to achieve this level of significance a total of 110 patients need to be recruited to each arm of the trial (total=220).

Since all patients will achieve a 12 week end point (healed versus unhealed) no uprating of this sample size will be made to take into consideration patients lost to follow up (see section 9.2).

9.2 General Statistical Methods

Analysis Populations
Two analysis populations will be evaluated: Intent To Treat (ITT) and Per Protocol (PP). The ITT population will be considered the primary population of interest and will include all subjects who were randomized to study treatment. The PP population will include all subjects who were randomized to study treatment and had no significant protocol violations that might affect the outcome of treatment.

All participants will be followed up to the primary end point (12 weeks) irrespective of whether they were on active treatment. Participants that withdraw from treatment continue to be followed up to the 12 week end-point. Participants that die, undergo amputation or who are lost to follow up will be considered as treatment failures.

Continuous demographic variables (age, ulcer duration, etc.) will be summarized for the study population as descriptive statistics (number, mean, median, SD, minimum and maximum value), and by randomized groups. No formal inferential statistical testing will take place of baseline values in relation to randomized groups.

Categorical demographic variables, such as gender, will be summarized as a proportion of the intention-to-treat population.

All analyses will be undertaken using the STATA 11 statistical package.

9.3 Primary outcome analysis

The primary endpoint is the “Incidence of complete wound closure within 12 weeks”.

The null hypothesis to be tested is that there is no difference between treatment groups in terms of the rate of wound healing after 12 weeks of study treatment. The alternative hypothesis is that such a difference exists.

Uncorrected Chi squared analyses will be used to determine the difference between treatment groups in terms of wound closure rate within 12 weeks, at the interim analysis. 95% confidence intervals will be estimated using the normal approximation interval.

Logistic regression analysis will be used to determine the effects of confounding on the primary outcome at the trial end. In particular, gender, age, ulcer duration, size and diabetic factors will be considered in the model. A stepwise procedure will be used to eliminate non-significant covariates from the model. The effect of different centers’ outcomes will be entered as a random effect into the model.

9.4 Secondary outcome analyses

9.4.1 Time to complete wound closure

The analysis will use the Cox proportional hazards model to determine time to event (healing). Subjects who withdraw from treatment will be entered into the analysis and censored at the time they withdraw from the trial or are lost to follow up. The analysis will consider covariates that might influence the outcome in the trial. Factors considered potentially important in predicting the outcome of treatment will be tested in a Cox model. Stepwise elimination of non significant covariates will be undertaken and only those covariates that remain statistically significant will be retained in the model. Tests of proportionality will be undertaken using graphical (Nelson Aalen) and quantitative methods (Schoenfeld test). The data will be presented graphically using Kaplan Meier graphs. The

results of the multivariable analysis will be presented as Hazard ratios together with 95% confidence intervals and p-values generated from the model.

9.4.2 Change in wound size

An important secondary outcome measure is partial wound healing, which is a function of wound area and perimeter change. Care must be taken to choose an appropriate measure for this outcome, as measures such as reduction in area or percentage reduction in area usually lead to biased comparisons. It is proposed that the trial uses the linear healing measure of Gowland-Hopkins & Jamieson[44] and described in Gilman[45]. This measure is defined as:

$d = \Delta A / p_{avg}$

where ΔA is the change in wound area between two successive visits and p_{avg} is the average of the wound perimeters measured at these two visits. 'd' will be calculated weekly after randomization to treatment.

Comparisons of the two treatment groups will be carried out in terms of d, at each of these assessments. An unpaired *t* test will be carried out at each assessment, to test the hypothesis that the treatment groups do not differ in terms of d, against the alternative hypothesis that the treatment groups differ in terms of d. A 95% confidence interval for the difference between treatment groups in terms of 'd' will be calculated, based on the *t* test results.

9.4.3 Wound recurrence rate.

The wound recurrence rates will be analyzed with a time to event strategy as in 9.4.1.

9.4.4 Incidence of amputation

Fisher's Exact Test will be used. An approximate 95% confidence interval for the difference between treatment groups in terms of amputation rate will be estimated using the normal approximation interval.

9.4.5 Safety and Tolerability

Safety and tolerability data will be presented in summary form and in detailed data listings. No significance testing will be applied to these data.

9.5 Missing and Spurious Data

No procedures are planned for the imputation of missing data. Subjects with missing data will be omitted from the relevant analyses.

If main outcomes are missing every attempt should be made to discover why and rectify wherever possible. If it is still not possible to discover the outcomes then the reason for this must be recorded e.g. death, refusal, incapacity.

For the health economic analysis it is standard procedure to impute missing data where necessary in order that an appropriate estimate can be given of the total costs of care.

9.6 Independent Data Monitoring Committee

As suggested by international guidelines on data monitoring committees, an independent Data Monitoring Committee (DMC) will be formed to decide whether the predefined criteria for early stopping of the trial are met.[46,47] The DMC will meet at the interim analysis to be performed after 73 and 146 subjects and at the end of study. In the event of new unanticipated and related adverse effects being reported, the DMC will convene for an unscheduled meeting and will determine at that time the need for additional review and or action.

The Data Monitoring Committee will consist of three to five independent experts outside of the study group. These members:

- will not be involved in the conduct of the clinical trial
- will not have any financial interest in the outcome of the study
- will have no planned authorship in publications on study results
- will not serve in parallel on the DMC of a clinical trial with diabetic patients with a different sponsor

10 DATA HANDLING

10.1 Quality Assurance and Quality Control of Data

The Investigator will permit trial-related monitoring, audits, IRB/Ethics Committee review, and regulatory inspection(s), provide direct access to source data and documents by suitably qualified and authorized persons from AOTI, Ltd. (and their representatives) and/or the bodies stated above.

The Investigator will obtain the prior written consent of the subject for such direct access. AOTI Ltd. will conduct a site visit to verify the qualifications of each Investigator, inspect the site facilities, and inform the Investigator and all trial staff of their responsibilities and the procedures for ensuring adequate and correct documentation.

The Investigator is responsible for ensuring the accuracy and credibility of the data by ensuring that a process is in place for the review of completed CRFs prior to any inspection by the study monitor.

10.2 Case Report Forms and Source Documents

All study data will be recorded in a Case Report Form. Source data will not be recorded directly onto the CRF with the exception of the EQ-5D and CWIS. Data from source documents such as paper medical records or original electronic reports shall be signed and dated by a member of the study team and must be available in the subject's file.
All Case Report Forms (CRFs) are to be completed by site personnel delegated responsibility by the Investigator and reviewed and signed by the Investigator. All data recorded in the CRFs must be consistent with the subject's source documents. Entries in the CRF for each subject will be checked against the source documents at site by the study monitor. Missing or un-interpretable data will be discussed with the Investigator for resolution.

The database used for the compilation and storage of CRFs is the property of AOTI Ltd. and will be retained by AOTI Ltd. at the end of the trial. A copy of CRF data will be retained by each site in the provided Investigator Site File.

10.3 Direct Access to Source Data

The Investigator will permit trial-related monitoring, audits, IRB/Ethics Committee review, and regulatory inspection(s), providing direct access to source data and documents by suitably qualified and authorized persons from AOTI Ltd. (and their representatives) and/or the bodies stated above.

The Investigator will obtain the prior written consent of the subject for such direct access. During the trial a study monitor representing AOTI Ltd. will regularly visit the trial site to review protocol compliance, verify entries in the CRFs with source data in the subject's medical records and ensure the trial is being conducted in accordance with the regulatory and ICH GCP requirements.

The Investigator assures the Sponsor of the necessary support at all times.

10.4 Documentation of Subject Participation

For all subjects who give written informed consent regardless of whether they receive TWO2 therapy or control therapy, the Investigator must record subject identification data in the "Subject Identification List" (full name, initials, date of birth, subject identification code). The

Subject Identification List must allow for the definite identification of any subject that takes part in the trial. The Investigator must keep the list of subject identification codes until informed in writing by the Sponsor that the list can be destroyed.

A statement acknowledging the participation of a subject in this clinical trial must be documented in the subject's medical file/notes and, where the trial physician is not the primary care physician, it is recommended that the subject's primary care physician is informed of the subject's participation in this clinical trial, provided the subject gave his/her consent.

10.5 Essential Documents for the conduct of a Clinical Trial

At the beginning of the trial, an Investigator Site File will be established at the trial site. The Investigator is responsible for maintaining the trial documents and must take measures to prevent accidental or premature destruction of these documents.

The essential documents to be included in the Investigator Site File are listed in ICH E6 Section 8.

10.6 Archiving Trial Documents

All trial documents belonging to the Investigator Site File will be archived at site (or off-site at a suitably secure location meeting ICH GCP requirements if specified and agreed in writing by the Sponsor) for a period of 15 years from the date of overall closure of the trial. This date will be communicated to all sites by the Sponsor at the end of the trial. A written record of the location of archived documents is to be forwarded to the Sponsor by each centre at the end of the trial (e.g. in the study closure report).

All trial documents belonging to the trial master file will be archived by the Sponsor at a suitably secure location meeting ICH GCP requirements, for a period of 15 years from the date of overall closure of the trial.

11 ETHICS, LEGAL AND ADMINISTRATIVE ASPECTS

11.1 Approval of Trial Documentation

All trial related documentation must be approved by AOTI Ltd. and Principal Investigator, in writing, before it is released. Prior to initiation of the trial, the protocol, informed consent form, subject information sheet and any other relevant trial documentation will be submitted to the responsible IRB/Ethics Committee and Regulatory Authorities. Written approval of the trial must be obtained and forwarded to AOTI Ltd. before the trial center can be initiated or trial materials released to the investigator.

Furthermore, any necessary extension or renewal of the IRB/Ethics Committee approval must be obtained and forwarded to the sponsor. In particular, change(s) to any aspect of the trial, such as modification(s) of the protocol, the written informed consent form, the written information provided to subjects, and/or other procedures must be approved, in writing, by the IRB/Ethics Committee.

The Investigator will report promptly to the IRB/Ethics Committee, any new information that may adversely affect the safety of subjects or the conduct of the trial. Similarly, the Investigator will submit written summaries of the trial status to the IRB/Ethics Committee annually, or more frequently, if requested by the IRB/Ethics Committee. Upon completion of the trial, the Investigator will provide the IRB/Ethics Committee with a brief report of the outcome of the trial, if required.

11.2 Ethical Conduct of the Trial

11.2.1 Good Clinical Practice

The procedures detailed in this protocol are designed to ensure that the Sponsor and Investigator abide by the principles of the Good Clinical Practice guidelines of the International Conference on Harmonization (ICH).

11.2.2 Declaration of Helsinki

The trial will adhere to the guidelines of the World Medical Association Declaration of Helsinki in its revised edition (Fortaleza, Brazil, October 2013), as well as the demands of national drug/medical device and data protection laws and other applicable regulatory requirements. A copy of the Declaration of Helsinki is supplied in the Appendices (Appendix II).

11.3 Subject Information and Consent

The Investigator is responsible for ensuring that no patient is subjected to any trial-related examination or activity before that patient has given informed consent. Written consent must be given by the patient/their legally authorized representative after the receipt of detailed information. The verbal explanation will cover all the elements specified in the written information provided for the patient.

The Investigator will inform the patient of the aims, methods, anticipated benefits and potential hazards of the trial including any discomfort that it may entail. The patient must be given every opportunity to clarify any points he/she does not understand and if necessary, ask for more information. At the end of the interview the patient may be given time to reflect if this is required, or if the patient requests more time. Patients will be required to sign and date the informed consent form. After completion, informed consent forms will be kept and archived by the Investigator in the Investigator's trial master file.

It should be emphasized that the subject is at liberty to withdraw their consent to participate at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Patients who refuse to give, or who withdraw written informed consent will not be included or continued in the trial.

11.4 Insurance

The company carries insurance to cover the use of the products for the devices for the indications defined in this trial.

11.5 Amending the Protocol

No substantial amendment or deviation should be implemented without agreement from the sponsor and prior review and documented approval/ favorable opinion from the IRB/Ethics Committee and Regulatory Authorities except where necessary to eliminate an immediate hazard(s) to trial subjects. In such case, as soon as possible, the implemented deviation or change, the reasons for it, and if appropriate, the proposed protocol amendment(s) must be submitted to AOTI Ltd. for approval and then submitted to the IRB/Ethics Committee and Regulatory Authorities for approval.

When an amendment is thought to be non-substantial, it must be confirmed by AOTI Ltd. and the Investigator in advance, in writing, and the reason for the non-substantial amendment must be documented by both the Investigator and the Sponsor.

11.6 Data Protection

To protect the subject's identity, a unique subject identification code (subject ID) will be assigned by the Investigator to each trial subject and used in lieu of the subject's name when the Investigator reports AEs and/or other trial related data. Thus, the subject identification code, and not the subject's name or hospital number, will appear on all documents.

Personal information will be treated as confidential, but may need to be reviewed by monitors from AOTI Ltd., auditors and authorized representatives of the IRB/Ethics Committee and Regulatory Authority. The subject's consent to direct access to the subject's original medical records for data verification purposes has to be obtained prior to that patient's participation in the trial.

Documents which identify the subject (e.g. signed consent forms) must not be submitted to the sponsor at the end of the trial, but must be maintained in confidence by the Investigator.

11.7 Financial Aspect of the Trial

The Sponsor, AOTI, shall provide payment for this study in accordance with the following schedule:

1) The Sponsor will pay all fees for Institutional Review Board / Ethics Committee approval fees
2) The Sponsor will reimburse directly all study related costs for TWO_2 medical devices, dressings, and Salvatelli off-loading boot

11.8 Disclosure of all Information and Results

In signing the final protocol, the Investigator agrees to keep all information and results concerning the trial confidential for as long as the data remains unpublished. The confidentiality obligation applies to all personnel involved at the investigational site.

Publication of trial results requires prior approval from AOTI Ltd.

11.9 Declaration of the End of Trial

The Investigator is responsible for notifying the IRB/Ethics Committee of the end of the trial, within 90 days of its completion. AOTI Ltd. is responsible for notifying the competent authority of the end of the trial, within 90 days of its completion.

If the trial ends prematurely the IRB/Ethics Committee and the competent authority must be informed within 15 days.

11.10 Clinical Trial Report

AOTI Ltd. is responsible for the writing of the clinical trial report. The format of the report will be in accordance with the ICH GCP guidelines (E3).

11.11 Publication plan

It is planned to publish the main paper of this trial in a premium journal like The Lancet, New England Medical Journal or Diabetes Care.

Secondary analyses will be published depending on subject and clinical relevance.

11.12 Study Schedule

Study Start Date: July 2014
Estimated First Interims Analyses: January 2017
Estimated Second Interims Analyses: May 2017
If needed Final Analyses: December 2017
Reporting/Submission date: 6 month after data analyses

12 COROLLARY SUB STUDY OF HIGH RISK POPULATION

In many cases TWO_2 has been shown to be particularly effective in severe wounds of multimorbid patients. Diabetic patients frequently suffer from ischemia, renal insufficiency or more severe wounds. A significant fraction of these patients use corticosteroids for other chronic diseases.

Due to the low chance of healing, a high mortality, and other reasons such as reimbursement issues these patients are normally excluded from clinical trials. However, as these patients represent an increasing percentage of all patients with diabetic ulcers we shall also include a sub study with high risk patients. In this sub study 24 patients will be randomized to either the active or the control group.

All procedures and outcomes will be the same as in the main arm of the study except for two differences:

- No biopsies will be taken
- Different inclusion and exclusion criteria

12.1 Inclusion and exclusion corollary substudy

All inclusion and exclusion criteria remain the same as in the main study population *except* the ones stated below:

INCLUSION CRITERIA

- ALL University of Texas Grades allowed *(Grade 1 and 2 in the main study)*
- ABI can be as low as 0.5 *(0.7 in the main study)*
- May have osteomyelitis *(excluded in the main study)*
- May have exposed bone *(excluded in the main study)*
- Subject may require renal dialyses *(excluded in the main study)*
- Subject may use chronic steroids *(excluded in the main study)*

EXCLUSION CRITERIA

(See previous exclusions that are now allowed in this substudy)

- Gangrene is still excluded

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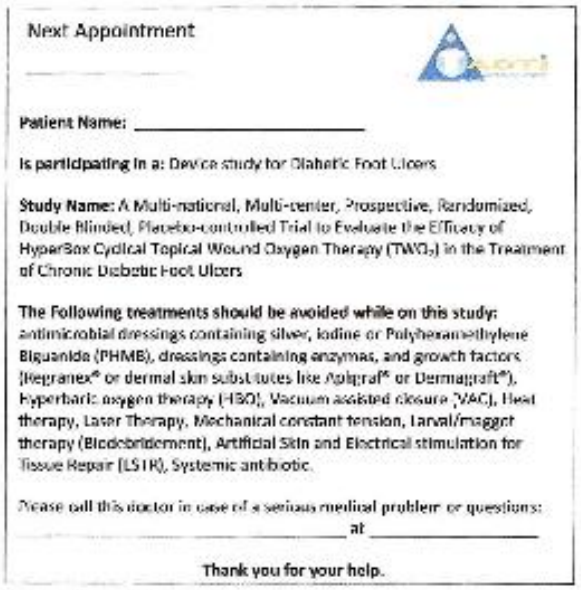
14 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse Event
ADE	Adverse device effect
ABI	Ankle Brachial Index
AMWT	Advanced Moist Wound Therapy
AOTI	Advanced Oxygen Therapy Ltd.
CE	The initials "CE" do not stand for specific words but are a declaration by a manufacturer that a product meets the requirements of applicable European Directives.
CRF	Case Report Form
CWIS	Cardiff Wound Impact Schedule
EQ-5D	Euroqol-5D
GSD	Group Sequential Design
ICF	Informed Consent Form
ICH GCP	International Conference on Harmonization Good Clinical Practice
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intention-to-treat
mbar	Millibars
NSR	Non Significant Risk
PO2	Pressure of Oxygen
PP	Per Protocol
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
TWO_2	Topical Wound Oxygen
UADE	Unanticipated Adverse Device Effect

APPENDIX I

1. Study Diary Card (Example)



Next Appointment

_____

Patient Name: _____

Is participating in a: Device study for Diabetic Foot Ulcers

Study Name: A Multi-national, Multi-center, Prospective, Randomized, Double Blinded, Placebo-controlled Trial to Evaluate the Efficacy of HyperBox Cyclical Topical Wound Oxygen Therapy (TWO_2) in the Treatment of Chronic Diabetic Foot Ulcers

The Following treatments should be avoided while on this study: antimicrobial dressings containing silver, iodine or Polyhexamethylene Biguanide (PHMB), dressings containing enzymes, and growth factors (Regranex® or dermal skin substitutes like Apligraf® or Dermagraft®), Hyperbaric oxygen therapy (HBO), Vacuum assisted closure (VAC), Heat therapy, Laser Therapy, Mechanical constant tension, Larval/maggot therapy (Biodebridement), Artificial Skin and Electrical stimulation for Tissue Repair (LSTR), Systemic antibiotic.

Please call this doctor in case of a serious medical problem or questions: _____ at _____

Thank you for your help.

FRONT

DRESSING CHANGES

Please Change your dressings:

- ☐ Monday ____ Time Taken
- ☐ Tuesday ____ Time Taken
- ☐ Wednesday ____ Time Taken
- ☐ Thursday ____ Time Taken
- ☐ Friday ____ Time Taken
- ☐ Saturday ____ Time Taken
- ☐ Sunday ____ Time Taken

TWO2 TREATMENT (5 Days)

What day did you do the treatment?

- ☐ Monday
- ☐ Tuesday
- ☐ Wednesday
- ☐ Thursday
- ☐ Friday
- ☐ Saturday
- ☐ Sunday

☐Self ☐Relative ☐Nurse ☐Home Aide ☐Doctor ☐Other

Please Initial: ____ **Date** _____ **THANK YOU**

BACK

2. University of Texas Wound Classification System

	I	II	III
A	Superficial Index Ulcer not involving tendon, capsule or	Index Ulcer penetrating to tendon or capsule	Index Ulcer penetrating to bone or joint
B	Infected	Infected	Infected
C	Ischemic	Ischemic	Ischemic
D	Infected and ischemic	Infected and ischemic	Infected and ischemic

3. Cardiff Wound Impact Schedule

Overall Quality of Life

We would like you to rate your overall quality of life during the past 7 days.

Please circle a number below

How good is your quality of life?

My quality of life is the worst possible	0	1	2	3	4	5	6	7	8	9	10	My quality of life is the best possible

How satisfied are you with your overall quality of life?

Not at all satisfied	0	1	2	3	4	5	6	7	8	9	10	Very satisfied

Overall Comments

For Office Use ☐ Total

Wound Healing Research Unit

University of Wales College of Medicine

Cardiff Wound Impact Questionnaire

Cardiff Wound Impact Schedule, English version for the USA

The following questionnaire is concerned with the effects that your wound(s) has (have) on your daily life. Please answer the questions carefully by placing a check mark in the box which most closely reflects how you feel; it should take about ten minutes to complete.

If you are unsure about how to answer a question, please mark the answer which is closest to how you feel. All answers are confidential.

Personal Details

Patient Initials **Sex** M F

Patient Number

Date of Birth M M D D Y Y

Assessment 1st ☐ 2nd ☐ 3rd ☐ 4th ☐ 5th ☐

Assessment Date M M D D Y Y **Next Assessment Due** M M D D Y Y

Wound(s) status Healed ☐ Not Healed ☐

Do you live on your own? Yes ☐ No ☐

How often do you see your family and friends?

Daily ☐ Once a month ☐

For Office Use ☐ Total

Once a week ☐ Less than once a month ☐

For Office Use ☐ Total

Social Life

How stressful has this experience been for you during the past 7 days?

	Not at all/ Not applicable	Slightly	Moderately	Quite a bit	Very
Difficulty getting out and around	☐	☐	☐	☐	☐
Relying more on others	☐	☐	☐	☐	☐
Your family/friends being overly protective	☐	☐	☐	☐	☐
Unable to enjoy your usual social life (eg hobbies)	☐	☐	☐	☐	☐
Limited contact with family/friends	☐	☐	☐	☐	☐
Not going out for fear of bumping your wound site	☐	☐	☐	☐	☐
Wanting to withdraw from people	☐	☐	☐	☐	☐

For Office Use **Total**

Social Life

Have you experienced any of the following during the past 7 days?

	Not at all/ Not applicable	Seldom	Sometimes	Frequently	Always
Difficulty getting out and around	☐	☐	☐	☐	☐
Relying more on others	☐	☐	☐	☐	☐
Your family/friends being overly protective	☐	☐	☐	☐	☐
Unable to enjoy your usual social life (eg hobbies)	☐	☐	☐	☐	☐
Limited contact with family/friends	☐	☐	☐	☐	☐
Not going out for fear of bumping your wound site	☐	☐	☐	☐	☐
Wanting to withdraw from people	☐	☐	☐	☐	☐

For Office Use **Total**

Physical Symptoms and Daily Living

How stressful has this experience been for you during the past 7 days?

	Not at all/ Not applicable	Slightly	Moderately	Quite a bit	Very
Disturbed sleep	☐	☐	☐	☐	☐
Difficulty bathing	☐	☐	☐	☐	☐
Immobility around the home	☐	☐	☐	☐	☐
Immobility outside the home	☐	☐	☐	☐	☐
Leakage from the wound(s)	☐	☐	☐	☐	☐
Pain from the wound site	☐	☐	☐	☐	☐
Discomfort from the bandaging/dressing	☐	☐	☐	☐	☐
Unpleasant odor or smell from the wound(s)	☐	☐	☐	☐	☐
Problems with everyday tasks (eg shopping)	☐	☐	☐	☐	☐
Difficulty in finding appropriate footwear	☐	☐	☐	☐	☐
Problems with the amount of time needed to care for the wound site	☐	☐	☐	☐	☐
Financial difficulties as a result of the wound(s)	☐	☐	☐	☐	☐

For Office Use **Total**

For Office Use **Total**

4. EQ-5D

Under each heading, please tick the ONE box that best describes your health TODAY

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

PAIN / DISCOMFORT

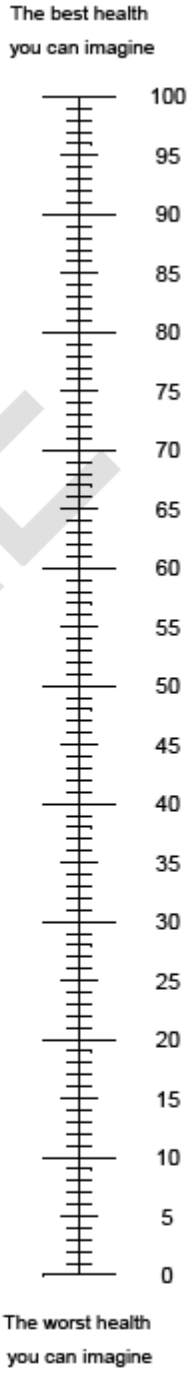
I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX II

WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the

physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.