CLINICAL INVESTIGATION PLAN (CIP)

INVESTIGATIONAL DEVICE:

Granulox®

INVESTIGATION TITLE:

Multicentre, prospective, randomized, open-label, assessor blinded study to evaluate Granulox® used as adjunct therapy to defined standard of care vs. defined standard of care for the treatment of predominantly chronic venous leg ulcers (VLUs)

SINGLE IDENTIFICATION NUMBER:

Granulox01

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CLINICAL INVESTIGATION PLAN (CIP) SYNOPSIS

INVESTIGATION TITLE:

Multicentre, prospective, randomized, open-label, assessor blinded study to evaluate Granulox® used as adjunct therapy to defined standard of care vs. defined standard of care for the treatment of predominantly chronic venous leg ulcers (VLUs)

Objectives

Primary Objective

The primary objective of this study is to compare wound healing of chronic venous leg ulcers (VLUs) managed using standard of care, with or without added Granulox®, at up to 20 weeks post therapy initiation. The main efficacy criteria is Confirmed Complete wound Closure (CCC).

Secondary Objectives

[redacted]

Clinical Investigation Design

This is a N = 254 patient, European, multi-centre, assessor blinded, 2-arm, randomised controlled study. The study will include multiple European countries (e.g. France, Germany, UK, Poland, Hungary, Croatia and Czech Republic), with approximately 2-7 clinics per country.

The study will run with a two-phase set-up, with a 14 days run-in period and an up to 20 weeks treatment period starting with randomization and allocation of treatment.

Endpoints

Efficacy Endpoints

The following efficacy endpoints will be assessed and evaluated in the study:

- Confirmed Complete wound Closure (CCC); derived by two blinded quantifications of 100% re-epithelialization by PictZar®, 15 days (+/- 3 days) apart.
- [redacted]

[redacted]

Safety Endpoints

Adverse Events (AE) and Serious Adverse Events (SAE) will be collected in the study to measure safety associated to study treatments from ICF signature until study completion. AEs will be assessed according to criteria for Adverse Device Effect (ADE), SAE, Serious Adverse Device Effect (SADE), and Device Deficiencies (DD).

Inclusion Criteria

1. Signed consent to participate.
2. No planned hospitalization in the forthcoming 20 weeks.
3. Male or female (women of childbearing age must have an acceptable method of birth control).
4. Age >18 years.

5. Recent (less than 12 months) doppler or duplex colour ultrasonography compatible with chronic venous insufficiency.
6. ABPI (less than 3 months) ≥0.7 for both legs. If ABPI >1.4, then big toe pressure >60 mmHg is required or an alternative measurement verifying normal distal arterial flow.
7. At least one or more supra- or peri-malleolar leg ulcers and no foot ulcer or another chronic wound.
8. In case of two ulcers, select ulcer with the highest PUSH score at randomization and treat the non-selected ulcer in the same way as ulcers in the control group.
9. In case of two ulcers on the same limb, ensure that the distance between each ulcer is 3 cm as measured from the margins of each ulcer that are closest to one another.
10. Wound duration >8 weeks and <60 months.
11. At randomization, the ulcer area should be 3 cm^2 - 70 cm^2 and should not have decreased by more than 30% during the 2-week run-in period.
12. Less than 50% of the wound area should be covered with fibrinous tissue at randomization and after debridement.
13. Patients considered as compliant/adherent to the compression device during the run-in period (i.e. no more than 2 days without compression over the 2-week run-in period).
14. No clinically significant comorbidities requiring the use of systemic steroids or any cytotoxic or immunosuppressive agents.

Exclusion Criteria

1. Infected ulcer according to the judgment of the investigator defined as any wound condition requiring the prescription or continuation of systemic antibiotic therapy at randomization.
2. Circumferential wounds.
3. Wound covered fully or partially by necrotic tissue (black tissue).
4. Patients who will have problems following the protocol, especially compression therapy.
5. Patients included in another on-going clinical investigation, or patients who have participated in a clinical investigation during the past 30 days.
6. Wounds treated with dressings containing an active component (e.g. silver, nano-oligosaccharide factor (NOSF), charcoal, chlorhexidine, iodine or ibuprofen) 14 days prior to study enrollment.
7. Patient with a systemic infection not controlled by suitable antibiotic treatment.
8. Current treatment with radiotherapy, chemotherapy, immunosuppressant drugs or high doses of oral corticosteroids (>10 mg Predinsolone or equivalent) if any.
9. Patient with deep vein thrombosis within 3 months prior to inclusion.
10. Known allergy/hypersensitivity to the ingredients of the dressings and/or Granulox®.
11. Malignant wounds.
12. Endovenous surgery planned or performed within the past 30 days.

13. Primary lymphoedema caused by congenital/developmental defect, i.e. Milroy's disease (congenital lymphedema), Meige's disease (lymphedema praecox), or Late-onset lymphedema (lymphedema tarda).

Investigational Device and Comparator

Granulox®

Granulox® is a CE marked medical device manufactured by HÄLSA Pharma GmbH. It is a topical haemoglobin spray, which is designed to transport oxygen to the wound site. In the study, Granulox® will be used as intended, in addition to defined standard care. The following procedural steps should be followed:

1. After wound bed preparation (e.g. debridement and cleaning) Granulox® should be sprayed onto the cleaned wound until the wound is completely coated with a thin film. That is, hold the nozzle about 5 to 10 cm from the wound and spray until the wound is completely coated with a thin film. 1 to 2 seconds are normally sufficient to cover a wound area of 2 x 3 cm.

2. After Granulox® has been applied, the wound should be covered with a breathable wound dressing containing no active ingredients.

3. Granulox® should be applied again every time the dressing is changed, but at least every three days.

Standard of care:

VLU standard of care treatment will be performed according to local practice requirements but reapplied at least every third day until complete epithelization. Standard of care will include the following:

1. Wound bed preparation, i.e.:

- Careful wound cleansing with water, saline, Ringer’s solution or preserved mechanical wound cleansers (i.e. polyhexanide (Prontosan, Lavanid), or hypochlorous (Granudacyn, Microdacyn) irrigation)
- Wound debridement if necessary and as per standard of care (e.g. mechanical or surgical debridement)

2. Application of appropriate breathable wound dressing which must be selected among the following categories and with regard to routine local care, level of exudate, and healing stage:

- Hydrofiber: Exufiber, Aquacel Extra
- Foam dressings: Mepilex XT, Mepilex Lite, Biatain Soft Hold, Biatain Silicone lite, Aquacel Foam, UrgoTul Lite
- Wound Contact layer: Mepitel, Mepitel One, Adaptic Touch, UrgoTul
- Superabsorbant: Mextra, Kiliniderm Superabsorber, Zetuvit Plus/Resposorb

NB: Dressings including active components are not allowed. Within a clinic, there must be consistency in the choice in dressings, i.e. the same sequence of dressings should be kept for all the patients.

3. Application of appropriate compression therapy which must be selected among the following categories and with regard to routine local care, level of exudate, and healing stage:

- Multilayer compression: [redacted]
- Hosiery and stockings compression: [redacted]

- Short stretch bandages

NB: A multilayer compression system is preferred and consistency in compression therapy throughout the study is mandatory, unless patient compliance issues necessitate change. Patients must be withdrawn from study in case of severe non-compliance to compression therapy.

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LIST OF ABBREVIATIONS

ADE	**Adverse Device Effect**
AE	**Adverse Event**
CCC	**Confirmed Complete wound Closure**
CIP	**Clinical Investigation Plan**
CP	**Conditional Power**
CRF	**Case Report Form**
CVI	**Chronic Venous Insufficiency**
DD	**Device Deficiency**
EC	**Ethics Committee**
EQ-5D-5L	**EuroQol.org - 5 dimensions - 5 level instrument to describe and value health**
FAS	**Full Analysis Set**
HRQoL	**Health-Related Quality of Life**
IARC	**Interim analysis review committee**
IRB	**Institutional Review Board**
NRS	**Numerical Rating Scale**
PCC	**Possible Complete wound Closure**
PP	**Per Protocol**
PRO	**Patient Reported Outcome**
PUSH	**Pressure Ulcer Scale for Healing**
RA	**Regulatory Authority**
SAE	**Serious Adverse Event**
SADE	**Serious Adverse Device Effect**
WAR	**Wound Area Regression**
50WAR	**50% Wound Area Regression**

90WAR	**90% Wound Area Regression**
VLU	**Venous Leg Ulcer**
Wound-QoL	**Wound Quality of Life questionnaire**

1. INTRODUCTION

Venous leg ulcer (VLU) is a severe clinical manifestation of chronic venous insufficiency (CVI) and is responsible for about 70% of chronic ulcers in the lower limbs.[1] VLUs are a major health problem because of their high prevalence and associated high cost of care.[2]

Compression therapy is the most widely used conservative treatment for VLUs as the application of external pressure to the calf muscle raises the interstitial pressure resulting in improved venous return and reduction in the venous hypertension. Published healing rates of VLUs obtained with compression therapy vary widely from 40-88%.[3-6] Over a quarter of patients with VLUs fails to heal despite standard of care including compression therapy, and those who do heal often take 6 months or longer.[7] Risk factors, such as wound age and size, have been identified to be correlated with the failure of VLUs to heal, showing VLUs present for at least 5 months prior to appropriate management and whose area is 10 cm² or larger, have less than 20% chance to heal at 24 weeks.[8]

Granulox® is a haemoglobin spray that can be added to standard of care of chronic wounds. It is expected to improve the tissue repair process by increasing tissue oxygen supply. In a previous randomized controlled trial (RCT) comparing Granulox® to a control group in VLUs, a significant mean reduction of the wound surface area was observed at 13 weeks with a decrease of 53.4% ($p<0.0001$) for Granulox® treated wounds.[9] Use of Granulox® was associated with significant pain score reduction compared to standard wound care alone thus improving patients´ quality of life.[9] Furthermore, use of Granulox® as an adjunct therapy led to a significant reduction of slough and recurrence compared with standard care alone.[9]

This study is intended to assess the healing benefits of Granulox® therapy in combination with standard of care for the management of chronic VLUs over a period of 20 weeks, compared to VLU standard of care treatment without Granulox®.

2. OBJECTIVES AND HYPOTHESES

2.1 Primary Objective

The primary objective of this study is to compare wound healing of chronic VLUs managed using standard of care, with or without added Granulox®, at up to 20 weeks post therapy initiation. The main efficacy criteria is Confirmed Complete wound Closure (CCC).

2.2 Secondary Objectives

[redacted]

3. INVESTIGATION PLAN AND PROCEDURES

3.1 Clinical Investigation Design

This is a N = 254 patient, European, multi-centre, assessor blinded, 2-arm, randomised controlled study. The study will include multiple European countries (e.g. France, Germany, UK, Poland, Hungary, Croatia and Czech Republic), with approximately 2-7 clinics per country.

The study will run with a two-phase set-up, with a 14 days run-in period and an up to 20 weeks treatment period starting with randomization and allocation of treatment.

3.2 Procedures and Assessments

This study starts with a 14 days run-in period to verify two of the required inclusion criteria, i.e. that the wound area does not decrease more than 30% during run-in period and compliance to compression therapy part of standard of care.

After the 14-days run-in period during which time patients will receive standard of care, (D-14 to D0) patients meeting the selection criteria will be randomly allocated (on D0) to experimental (i.e. standard of care plus Granulox®) or control (i.e. standard of care only) groups. Randomization will be stratified on study site, wound duration (<5 months; ≥5 months), and wound area after run-in period (<10; ≥10 cm²), to ensure equal distribution of test and control in these sub-groups. This, as wound duration and wound area may significantly impact outcomes, and as minor variations in standard of care between study sites may still be present in spite of the measures taken to ensure standardization. See details on standardization of standard of care in Section 3.5.

Each patient will be followed to wound closure, or up to 20 weeks (whichever occurs first), with scheduled clinical visits at 1 week (W1), 4 weeks (W4), 8 weeks (W8), 12 weeks (W12), 16 weeks (W16), and 20 weeks (W20). Additional visits are allowed in between the per-protocol scheduled visits. If suspected wound closure occurs at any point during the study, a final assessment visit will be scheduled 15 days (±3 days) subsequent to the suspected wound closure observation to verify CCC. If wound closure is confirmed, the study is completed for this patient. If wound closure is not confirmed, the patient should continue to be followed according to the schedule of assessments. See study Flow Chart at Section 3.2.1 and Schedule of Assessment at section 3.3.8.

In between clinical visits, wound management will follow standard of care. Home visits will be performed by study nurses who are responsible for changing dressing, reapplying Granulox® (for patients randomized to the treatment group only), and reapplying compression therapy. See details in Section 3.5.1.1. Home visits frequency will follow local practice requirements but must take place at least every 3rd day until epithelization is complete. The study nurse will record information in the patient-specific diary. If considered necessary, nurses can ask for an additional patient visit to the investigating centre. This is mandatory at suspected complete wound closure.

If at any time, a wound graft is required (it usually requires at 24-48 hours hospitalization), study will not be considered as completed and patients will still be followed according to the schedule of assessments (even if experimental treatment is no longer applied) up to CCC occurrence or up to W20 (whichever occurs first).

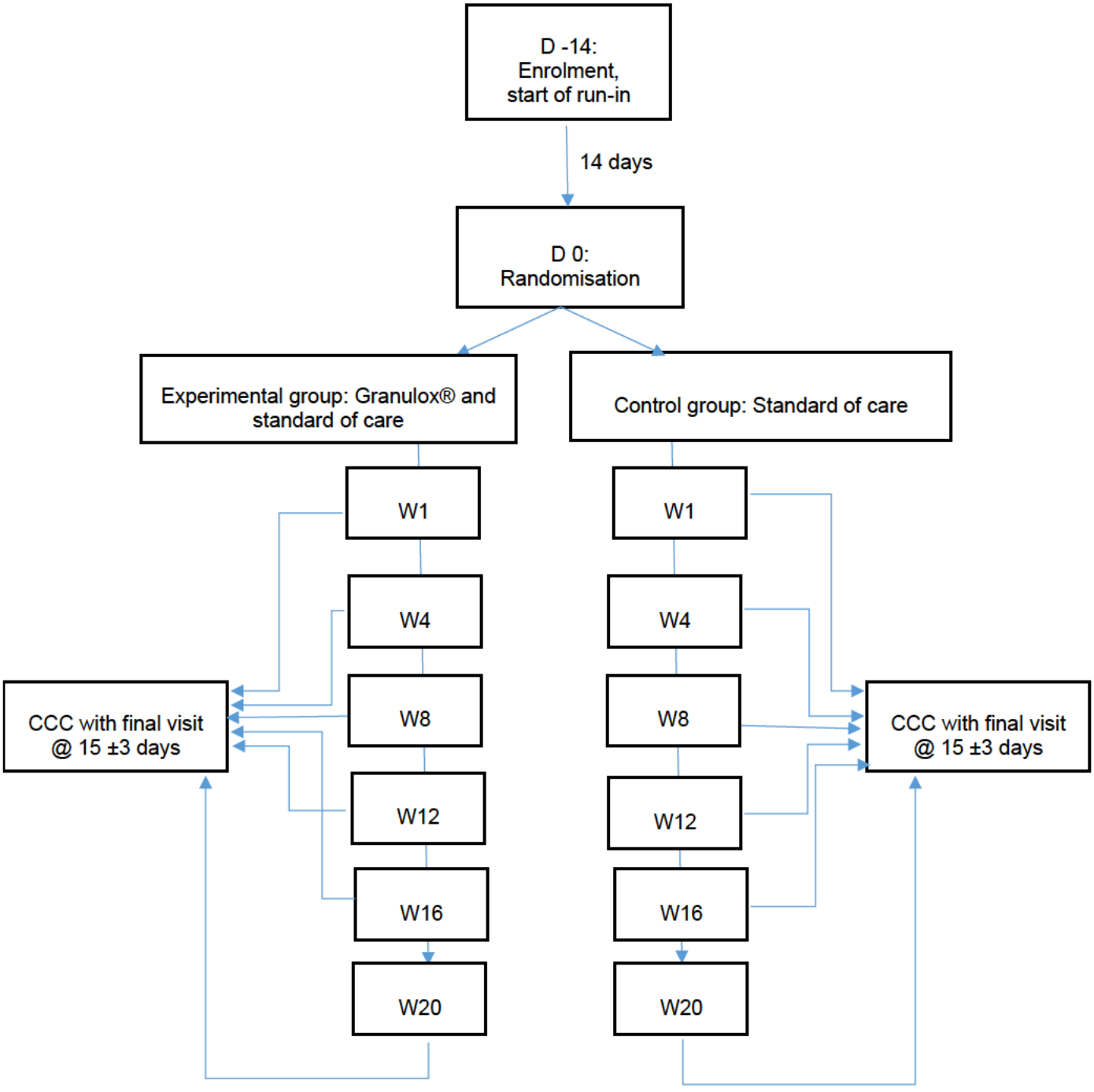
The study will be stopped before W20 for a given patient in the event of:

- Consent withdrawal
- Impossibility to follow the patient (i.e. lost to follow-up)

- Hospitalization of more than 48h for any reason other than wound graft
- Death
- Reach of endpoint and study completion, i.e. CCC.

In any other cases, including the cessation of experimental treatment for any reason, patient will continue study visits as per schedule of assessment. See details on analyses sets in Section 5.2.1.

3.2.1 Flow Chart



NB. Wound management as per standard of care, at least every 3rd day until complete epithelization, will take place between visits D0-W20. Extra clinical visits between visits D0-W20 are allowed and should be issued in the event of suspected wound closure.

3.3 Efficacy and Safety

3.3.1 Subject Characteristics

Demography and background data with potential impact on wound healing will be collected at the initial visit, i.e. :

- Date of birth
- Gender
- Ethnicity
- Mobility
- Vital signs (e.g. height/weight)
- Social profile (e.g. autonomy, lifestyle, social support)
- Relevant medical history (general and with regard to venous disease/VLU)
- Relevant surgical history (general and with regard to venous disease/VLU)
- Current wound management treatment
- Current wound status

3.3.2 Primary Efficacy Measurement and Variable

Digital photographs, taken after wound bed preparation at each clinical visit, will be used as a basis for the primary and secondary efficacy measurements and variables.

3.3.2.1 Confirmed Complete wound Closure (CCC)

CCC is the primary variable and main efficacy criterion. It is defined as an blinded assessment and observation of 100% re-epithelialization, confirmed by a second visit 15 days later (+/- 3 days). If a wound graft is required, patients will be followed to observation of CCC.

Blinded quantification of each wound picture will be made using PictZar® Digital Planimetry Software.

CCC will be derived from two measurements of 100% re-epithelialization by PictZar®, 15 days (+/- 3 days) apart.

3.3.3 Secondary Efficacy Measurements and Variables

[redacted]

[redacted]

[redacted]

3.3.7 Safety Measurements and Variables

Adverse Events (AE) and Serious Adverse Events (SAE) will be collected in the study to measure safety associated to study treatments from ICF signature until study completion.

The definition of AE, Adverse Device Effect (ADE), SAE, Serious Adverse Device Effect (SADE), and Device Deficiencies (DD) and procedures for reporting SAE and SADE and DD that could have led to a SADE are presented in section 11 of this CIP. All AE, ADE, SAE, SADE and DD must also be recorded in the appropriate section of the CRF. It is of utmost importance that all staff involved in the clinical investigation is familiar with the content of section 11. It is the responsibility of the Principal Investigator to ensure this.

3.3.8 Schedule of Assessment

Site visits	Run-in Visit	Home care	Visit 1	Home care	Visit 2	Home care	Visit 3	Home care	Visit 4	Home care	Visit 5	Home care	Visit 6	Home care	Visit 7	Final Visit[a)]	Extra visit
Time frames	D-14	D-14-D0	D0	(At least every 3rd day)	W1 (Day 7 ±1 day)	At least every 3rd day	W4 (Day 28 ±3 days)	At least every 3rd day	W8 (Day 56 ±3 days)	At least every 3rd day	W12 (Day 84 ±3 days)	At least every 3rd day	W16 (Day 112 ±3 days)	At least every 3rd day	W20 (Day 140 ±3 days)		
Informed consent	X																
Eligibility criteria	X		X														
Randomization			X														
Demography and background data			X														
Medical and surgical history (including VLU)			X														
Wound picture	X		X		X		X		X		X		X		X	X	X
PictZar®	X		X		X		X		X		X		X		X	X	X
[redacted]			X		X		X		X		X		X		X	X	X
					X		X		X		X		X		X	X	X
																X	
	X	X		X		X		X		X		X		X			
			X		X		X		X		X		X		X	X	X
																X	
			X								X				X	X	
			X		X		X		X		X		X		X	X	
Adverse events [e)]			X		X		X		X		X		X		X	X	X
Medications	X		X		X		X		X		X		X		X	X	X

Site visits	Run-in Visit	Home care	Visit 1	Home care	Visit 2	Home care	Visit 3	Home care	Visit 4	Home care	Visit 5	Home care	Visit 6	Home care	Visit 7	Final Visit[a)]	Extra visit
Time frames	D-14	D-14-D0	D0	(At least every 3rd day)	W1 (Day 7 ±1 day)	At least every 3rd day	W4 (Day 28 ±3 days)	At least every 3rd day	W8 (Day 56 ±3 days)	At least every 3rd day	W12 (Day 84 ±3 days)	At least every 3rd day	W16 (Day 112 ±3 days)	At least every 3rd day	W20 (Day 140 ±3 days)		
Reason(s) study arrest																X	

a) To be completed in any case (premature arrest, CCC).
b) Assessment of wound healing and wound progress/evolution completed after patient's study termination and based on patient-series of wound pictures.
[redacted]
e) Safety monitoring including reporting of adverse events, serious adverse events, adverse device effects, serious adverse device effects, and device deficiencies.

3.4 Subject Population

The study population is comprised of N = 254 adult patients presenting with VLUs who meet the study's inclusion and exclusion criteria. Approximately equal proportions of male and female patients will be enrolled in the study.

3.4.1 Inclusion Criteria

All of the following criteria need to be fulfilled for inclusion in the study:

1. Signed consent to participate.
2. No planned hospitalization in the forthcoming 20 weeks.
3. Male or female (women of childbearing age must have an acceptable method of birth control).
4. Age >18 years.
5. Recent (less than 12 months) doppler or duplex colour ultrasonography compatible with chronic venous insufficiency.
6. ABPI (less than 3 months) ≥0.7 for both legs. If ABPI >1.4, then big toe pressure >60 mmHg is required or an alternative measurement verifying normal distal arterial flow.
7. At least one or more supra- or peri-malleolar leg ulcers and no foot ulcer or another chronic wound.
8. In case of two ulcers, select ulcer with the highest PUSH score at randomization and treat the non-selected ulcer in the same way as ulcers in the control group.
9. In case of two ulcers on the same limb, ensure that the distance between each ulcer is 3 cm as measured from the margins of each ulcer that are closest to one another.
10. Wound duration >8 weeks and <60 months.
11. At randomization, the ulcer area should be 3 cm^2 - 70 cm^2 and should not have decreased by more than 30% during the 2-week run-in period.
12. Less than 50% of the wound area should be covered with fibrinous tissue at randomization and after debridement.
13. Patients considered as compliant/adherent to the compression device during the run-in period (i.e. no more than 2 days without compression over the 2-week run-in period).
14. No clinically significant comorbidities requiring the use of systemic steroids or any cytotoxic or immunosuppressive agents.

3.4.2 Exclusion Criteria

If any of the following criteria applies, the patient will be excluded from participation:

1. Infected ulcer according to the judgment of the investigator defined as any wound condition requiring the prescription or continuation of systemic antibiotic therapy at randomization.
2. Circumferential wounds.

3. Wound covered fully or partially by necrotic tissue (black tissue).
4. Patients who will have problems following the protocol, especially compression therapy.
5. Patients included in another on-going clinical investigation, or patients who have participated in a clinical investigation during the past 30 days.
6. Wounds treated with dressings containing an active component (e.g. silver, nano-oligosaccharide factor (NOSF), charcoal, chlorhexidine, iodine or ibuprofen) 14 days prior to study enrollment.
7. Patient with a systemic infection not controlled by suitable antibiotic treatment.
8. Current treatment with radiotherapy, chemotherapy, immunosuppressant drugs or high doses of oral corticosteroids (>10 mg Predinsolone or equivalent) if any.
9. Patient with deep vein thrombosis within 3 months prior to inclusion.
10. Known allergy/hypersensitivity to the ingredients of the dressings and/or Granulox®.
11. Malignant wounds.
12. Endovenous surgery planned or performed within the past 30 days.
13. Primary lymphoedema caused by congenital/developmental defect, i.e. Milroy's disease (congenital lymphedema), Meige's disease (lymphedema praecox), or Late-onset lymphedema (lymphedema tarda).

3.4.3 Method of Assigning Subjects to Treatment Groups

At initial screening and after an informed consent signature is obtained (day-14), an enrolment number is assigned to each patient who agrees to take part in the study. Following the run-in period, and after all eligibility criteria have been confirmed (day 0), randomisation is initiated and treatment allocated to either standard of care only (control group) or standard of care and Granulox® (test group).

Randomisation is centralized and stratified according to:

- Wound duration (<5 months; ≥5 months)
- Wound area after run-in period (<10 cm²; ≥10 cm²)
- Country

3.4.4 Withdrawal of Subjects from Treatment or Assessment

Subjects are free to discontinue participation in the study at any time, and without prejudice to further treatment. Subjects who withdraw from the study should always be asked about the reason(s) for their discontinuation and about the presence of any Adverse Event/Adverse Device Effect or Device Deficiency and, if possible, be assessed by the Principal Investigator. Each reported Adverse Event/Adverse Device Effect will be followed up.

Subjects may be withdrawn from the study at any time, at the discretion of the Principal Investigator.

Incorrectly enrolled or randomised subjects will be withdrawn from the study. A subject may, however, continue study involvement under special circumstances (i.e. if continuation of study-related treatment or follow-up actions are necessary for the subject's safety and well-being, or if only a follow-up period remains, and the continuation of the subject's study involvement is not expected to be associated with any risk or discomfort for the subject).

3.5 Investigational Device

3.5.1 Summary description of the Investigational Device(s) and Comparator(s)

3.5.1.1 Investigational Device

The investigational device is Granulox®, a topical haemoglobin spray, which is designed to transport oxygen to the wound site. Granulox® is intended to increase oxygen supply to the wound. Granulox is a CE marked medical device manufactured by HÄLSA Pharma GmbH (report number: 12911FS06F). It is an innovative product for the treatment of chronic wounds, such as venous leg ulcer, arterial leg ulcer, mixed leg ulcer, diabetic foot ulcers, secondary healing of surgical wounds and pressure sores. Granulox can also be used on sloughy and infected wounds.

The mode of action is that once Granulox® is sprayed, the haemoglobin starts to bind oxygen from the atmosphere and the process of oxygen transportation begins. Oxygen promotes the formation of new blood vessels and subsequently the growth of new skin. Granulox provides the wound with the required oxygen by means of diffusion. The active substance haemoglobin supplies the base of the wound with external oxygen. The improved oxygen supply to the base of the wound supports wound healing.

Granulox® should not be used simultaneously with topical medicines for your wound, such as application of local antibiotics, as interactions have not yet been studied sufficiently. Disinfectants are known to impair the efficacy of Granulox®. Therefore, after application of a disinfectant, the area must be subsequently flushed thoroughly with a physiological solution. Thorough flushing with a physiological solution is necessary after use of proteolytic (enzymatic) debridement.

In this study, Granulox® will be used as intended, in combination with an appropriate foam or wound contact layer wound dressing and in addition to defined standard care.

Granulox®:

The instructions for use for Granulox® should be consulted before initiating treatment. The following procedural steps should be followed:

1. After wound bed preparation (e.g. debridement and cleaning) Granulox® should be sprayed onto the cleaned wound until the wound is completely coated with a thin film. That is, hold the nozzle about 5 to 10 cm from the wound and spray until the wound is completely coated with a thin film. 1 to 2 seconds are normally sufficient to cover a wound area of 2 x 3 cm.
2. After Granulox® has been applied, the wound should be covered with a breathable wound dressing containing no active ingredients.
3. Granulox® should be applied again every time the dressing is changed, but at least every three days.

Defined standard of care:

VLU standard of care treatment will be performed according to local practice requirements but reapplied at least every third day until complete epithelization. Standard of care will include the following:

1. Wound bed preparation, i.e.:

- Careful wound cleansing with saline/water or Ringer's solution or preserved mechanical wound cleansers (i.e. polyhexanide (Prontosan, Lavanid), or hypochlorous (Granudacyn, Microdacyn) irrigation)
- Wound debridement if necessary and as per standard of care (e.g. mechanical or surgical debridement)

2. Application of appropriate breathable wound dressing which must be selected among the following categories and with regard to routine local care, level of exudate, and healing stage:

- Hydrofiber: [redacted]
- Foam dressings: [redacted]
- Wound Contact layer: [redacted]
- Superabsorbant: [redacted]

NB: Dressings including active components are not allowed. Within a clinic, there must be consistency in the choice in dressings, i.e. the same sequence of dressings should be kept for all the patients.

3. Application of appropriate compression therapy which must be selected among the following categories and with regard to routine local care, level of exudate, and healing stage:

- Multilayer compression: [redacted]
- Hosiery and stockings compression: [redacted]
- Short stretch bandages

NB: A multilayer compression system is preferred and consistency in compression therapy throughout the study is mandatory, unless patient compliance issues necessitate change. Patients must be withdrawn from study in case of severe non-compliance to compression therapy.

All performed local care has to be documented in patients' medical records.

3.5.1.2 Comparator

The control group will use the same defined standard of care as the treatment group (see section 3.5.1.1) but without Granulox®.

3.5.2 Labelling

3.5.3 Device Accountability

Mölnlycke and the Principal Investigator will keep records documenting the location of all investigational devices from shipment of investigational devices to the investigation site until return. This will be documented by a shipment log stored by Mölnlycke and in a device accountability log at the investigation site. The device accountability log at each investigational site will include information on; date, serial and batch number of delivered devices, date and subject identification for used devices, date, serial and batch numbers of devices returned.

The Site Monitor will verify the accountability process at each site during the site monitoring visits.

3.5.4 Storage conditions

During use, Granulox® can be stored at room temperature (max. 25 °C) until it is empty or up to 6 weeks. For long-term storage, place Granulox® in a refrigerator at temperatures from 2 °C to 8 °C.

Storage conditions for other components used in the study should follow instructions for use specific to each component.

3.6 Concomitant Treatments

Medication and treatment, which is considered necessary for the subject's safety and well-being, may be given at the discretion of the Principal Investigator. All concomitant medication and relevant treatment must be recorded in the appropriate section of the Case Report Form (CRF).

The following restrictions and specific considerations apply for the study:

- No use of local antiseptics will be authorized.
- Peri-wound skin cares may use emollients if necessary.
- Use of antibiotics and analgesics must be recorded on the appropriate CRF during the study.

3.7 Risks and benefits of the Investigational Device and clinical investigation

The following events have been identified as anticipated effects of Granulox®:

- In the overall evaluation of safety, the use of Granulox® for the treatment of chronic hypoxic wounds with poor blood supply as well of burn wounds and infected or critically colonized wounds is found to be safe and well-tolerated.[14-18]
- Tests for cytotoxicity, sensitization, irritation and subcutaneous reactivity did not identify any hazardous properties and thus confirmed the safety of the topical haemoglobin spray.[19] Tissue tolerability has also been tested using the semi-in-vivo method HET-CAM (hen's egg test on the chorioallantoic membrane) without any signs of irritation.[20] The risk of inhaling aerosols when using Granulox® spray has been evaluated by Holzer et al. who concluded that there is no risk of inhaling nanoparticles or being exposed to harmful concentrations and that all ingredients can be degraded and excreted by the human body through natural pathways.[21]
- Only one of the reviewed publications from previous clinical trials reported of adverse events (one event of infection) associated with topical administration of Granulox®.[18] The majority of the evaluated publications explicitly stated no occurrence of adverse events.[e.g.15,22,23]
- Within the framework of the clinical investigation carried out at the Hospital Civil de Ciudad Victoria by the Mexican health government, the haemoglobin spray was applied almost 1,500 times on chronic wounds. In all cases, the spray was well tolerated and there were no undesirable events that might have resulted due to the application of the haemoglobin spray. In addition, Granulox® was rated as very easy to handle from the participants of the clinical trials under review.[17,24]

The possible benefits associated with Granulox® are as follows:

- Higher healing rate / wound closure.
- Pain reduction.

- Reduced recurrence of slough.
- Improved health-related quality of life.

In summary, the single ingredients of Granulox® are established as safe for use in topical applications and the general safety and tolerability of Granulox® is assessed as very good.

4. DATA QUALITY ASSURANCE

4.1 Monitoring, Audits and Inspections

During the investigation, the Site Monitor will have regular contacts with the investigation site. These contacts will include monitoring visits, conducted in accordance with a clinical investigation specific monitoring plan, to confirm that the facilities remain adequate to specified standards and that the investigation site team is carrying out the procedure as stated in the Clinical Investigation Plan and supporting the Principal Investigator appropriately. All data must be accurately recorded in the CRF. Source data verification (a comparison of data in the CRF with the subject's hospital/practice and other records at the investigation site) with direct access to records will also be performed.

The Site Monitor or other Mölnlycke personnel will be available between visits if the Principal Investigator or other staff at the site needs information and/or advice.

Authorised representatives of Mölnlycke and/or a Regulatory Authority (RA) and/or the Ethics Committee (EC)/Institutional Review Board (IRB) may visit the investigation site to perform audits/inspections, including source data and informed consent verification.

4.2 Training of Staff

The Principal Investigator will ensure that appropriate training relevant to the investigation is given to the medical, nursing and other staff at the sites involved and that new information of relevance to the performance of this investigation is forwarded to the staff involved in a timely manner.

4.3 Data Management

The Data Management process includes all activities related to data handling regarding:

- Compilation of a CRF
- Randomisation
- Set-up of eCRF/database
- Specification of on-line checks
- Data entry/data editing
- Export of data from eCRF/database to analysis software
- Creation of post-entry checks and listings
- Reconciliation of Serious Adverse Event (SAE), Serious Adverse Device Effect (SADE), Adverse Devise Effect (ADE) and Device Deficiency (DD)
- Clean-file process including execution of post-entry checks and listings
- Post clean-file tasks

A web based electronic CRF system, will be used to capture data in this investigation. The eCRF system complies with FDA Title 21 CFR part 11 (ER/ES) requirement.

eCRF training will be given to appropriate personnel at initiation of the investigation site(s).

The Principal Investigator and other authorized personnel at the site(s) will perform data entry. When entering data on-line checks are incorporated for consistency and validation. Support will be provided for system user questions.

When data has been entered, authorized personnel at Mölnlycke can immediately view the data, send queries if necessary and lock eCRF pages when correct data entry has been verified.

Photos will be uploaded in the eCRF and are marked with the subject code. Uploaded photos shall not contain any information that can reveal the identity of the subject. All uploaded photos will be reviewed by personnel at Mölnlycke and stored in the company database. All data entered in the eCRF will be encrypted. The physical database will be stored in Sweden.

Programs for post-entry checks and data listings will be created and executed for validation of data.

Authorized personnel at Mölnlycke will check each finalised eCRF for completeness so that there are no unexplainable empty fields in the eCRF. This is done in order to prevent incomplete data entry.

A clean-file meeting will be held prior to database lock. All decisions on the evaluability of the data from each individual subject will be made and documented before locking the database for the statistical analysis.

4.3.1 Data Retention

The medical records of clinical investigation subjects must be retained in accordance with local legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

The Principal Investigator must retain all clinical investigation records during the clinical investigation and for the period required by the applicable regulatory requirements or for at least 20 years after the premature termination or completion of the clinical investigation, whichever is the longer. The Principal Investigator must take measures to prevent accidental or premature destruction of these documents. The Principal Investigator should contact Mölnlycke prior to destruction of any records or reports pertaining to the clinical investigation in order to ensure they no longer need to be retained. In addition, if the Principal Investigator leaves the hospital, he/she should provide Mölnlycke with the name and address of the person who will look after and be responsible for the clinical investigation-related records. If the records will be transferred to another person/party, the transfer must be documented at the investigation site or at Mölnlycke.

5. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE AND STATISTICAL ANALYSES

5.1 Determination of Sample Size

[redacted]

[redacted]

[redacted]

5.1.1 Independent interim analysis review committee (IARC)

An independent interim analysis review committee (IARC) will be appointed to follow the safety monitoring and sample size adjustment of the study.

5.1.2 Sample size re-estimation

After approximately 110 patients have completed the study, the IARC will perform an unblended interim analysis on the primary efficacy variable CCC incidence. From this analysis, the IARC will calculate the conditional power (CP).

The IARC will form a recommendation to either terminate, continue, or adjust sample size, based on the following guidelines:

- Futility: If CP ≤30%, then terminate the study for futility.
- Promising outcome: If 30%< CP% <80%, then adjust the sample size so that CP = 0.80. The sample size could be increased to maximum 600 subjects.
- Favorable outcome: If CP% >80%, then do not adjust the sample size.

The sample size re-estimation analysis will not require adjustments of the final primary statistical analysis.[28]

5.2 Statistical Analyses

5.2.1 Definition of Study Populations

Full Analysis Set (FAS): All randomized patients with at least one ordinary follow-up visit will be included in the FAS. The final decisions regarding the FAS population will be taken at the Clean File meeting before database lock.

Per-Protocol (PP) Population: All patients in FAS with no significant protocol violations will be included in the PP population. The final decisions regarding the PP population will be taken at the Clean File meeting before database lock.

Safety Population: All enrolled subjects who received at least application of randomized investigational product (IP) will be included in the safety population.

5.2.2 General statistical methodology

This study will use confirmative and explorative analyses. All confirmative analyses will be based on FAS. Confirmative analysis of primary variables will also be performed based on PP.

Imputing of missing data in outcome variables will generally be used. For confirmative analyses, multiple imputations with Fully Conditional Specification (FCS) will be used for primary efficacy analysis. Confirmative secondary analysis and exploratory analyses will use stochastic imputation with FCS. Missing data on demography and background variables will not be replaced.

The study include testing analysis of the primary variable and sequential testing of selected secondary variables for confirmative analysis.

The study utilized stratified randomization based on site, wound size, and wound duration. This is reflected by a stratified primary analysis adjusted for these variables. For adjusted comparison between the two randomized groups, multivariable logistic regression will be used for dichotomous variables and analysis of covariance for continuous variables.

Continuous variable will be described with mean, SD, median, minimum and maximum and categorical variables with numbers and percentages. For unadjusted and adjusted analyses, mean differences with 95% CI will be calculated. For dichotomous variables, for descriptive purpose, relative risk with 95% CI will also be calculated. All significance analyses will be two-sided and conducted at the 5% significance level.

For unadjusted comparison between the two randomized groups Fisher's exact test will be used for dichotomous variables, Fisher's non-parametric permutation test for continuous variables, Mantel-Haenszel chi-square test for ordered categorical variables, and Chi-Square test for non-ordered categorical variables.

Applicable descriptive statistics and non-parametric statistical test methods are used to describe and explore secondary variables. For explorative analyses, no multiple-test considerations are made. For time to endpoint outcomes the Kaplan-Meier model and logrank test are used unadjusted and Cox proportional hazard model for adjusted analyses.

5.2.2.1 Primary efficacy analysis

The primary analysis of comparison of CCC between the two randomized groups will be using binary logistic model at significance level 0.05, on FAS with multiple imputation including the following covariates:

1. Country (as fixed effect)
2. Wound duration (<5 months; ≥5 months)
3. Wound area after run-in period (<10 cm^2; ≥10 cm^2)

Sensitivity analysis will be comparison between the two randomized groups unadjusted on CCC with Fisher's exact test without imputation.

5.2.2.2 Secondary efficacy analyses

[redacted]

5.2.2.3 Sub-group analyses

Preplanned sub-group analyses will be performed for the primary analysis variables on the following variables:

- Country
- Wound duration at inclusion (<5 months; ≥5 months)
- Wound area at inclusion (<10 cm^2; ≥10 cm^2)

Additional explorative sub-group analyses will be defined in SAP.

5.2.2.4 Interim analysis

Interim analyses will only be performed by the IARC group.

5.2.2.5 Statistical Analysis Plan (SAP)

A Statistical Analysis Plan (SAP) will be written with detailed description of all statistical analysis.

6. AMENDMENTS TO THE CLINICAL INVESTIGATION PLAN

No change in the clinical investigation procedure will be implemented without the mutual agreement of the Principal Investigator and Mölnlycke.

An amendment to the Clinical Investigation Plan may require notification or approval from an EC/IRB and, in many countries, also the RA before implementation. Local requirements must be followed.

Mölnlycke will distribute Clinical Investigation Plan amendments to the Principal Investigator who is responsible for the distribution of these documents to the EC/IRB and staff concerned at his/her site. The distribution of these documents to the RA will be handled according to local practice.

7. DEVIATIONS FROM THE CLINICAL INVESTIGATION PLAN

A deviation is a failure to follow, intentionally or unintentionally, the requirements of the Clinical Investigation Plan. Every effort should be made to comply with the requirements of the Clinical Investigation Plan and the Principal Investigator is not allowed to deviate from the plan. Furthermore, the use of waivers from the Clinical Investigation Plan is prohibited.

As required by national regulations, or guidelines, requests for deviations and reports of deviations will be provided to the EC/IRB if the deviation affects subject's rights, safety or well-being, or the scientific integrity of the clinical investigation.

Under emergency circumstances deviations from the Clinical Investigation Plan may proceed without prior approval by Mölnlycke and the EC/IRB if the rights, safety and well-being of human subjects need to be protected. Such deviations will be documented and reported to the Mölnlycke and EC/IRB as soon as possible in accordance with national regulations.

When the Site Monitor or Mölnlycke identifies that the Principal Investigator is out of compliance, this will be communicated to the Principal Investigator in writing, with a request to correct the source of the deviation immediately. Corrective action(s) will be implemented to avoid repeated non-compliance, which will usually include re-training and may include terminating the clinical investigation at the site.

Mölnlycke is responsible for analysing deviations and assessing their significance. Corrective action will be implemented to avoid repeated deviations, which may include suspending the clinical investigation, and/or disqualifying the Principal Investigator.

8. STATEMENTS OF COMPLIANCE

8.1 Ethics

8.1.1 Ethics review

The final Clinical Investigation Plan, including the final version of the Subject Information and Consent Form, must be approved or given a favourable opinion in writing by an EC/IRB before enrolment of any subject into the clinical investigation. The Principal Investigator is responsible for informing the EC/IRB of any amendment to the Clinical Investigation Plan as per local requirements.

Any additional requirements imposed by the EC/IRB or RA must be followed.

8.1.2 Ethical Conduct of the Investigation

This clinical investigation will be performed in accordance with the ethical principles that have their origin in the most recent version of the Declaration of Helsinki, and applicable regulation.

8.1.3 Subject Information and Consent Form

The Principal Investigator must ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risks and benefits of the clinical investigation and must verify that the subject has understood the information. Subjects must also be notified that they are free to discontinue participation in the investigation at any time. The withdrawal of the informed consent must not affect the activities already carried out and the use of data obtained based on informed consent before its withdrawal. The subject must be given the opportunity to ask questions and adequate time for consideration prior to providing their consent to take part in the study. The subject's signed informed consent has to be obtained before conducting any procedure specifically for the investigation. The original signed copy of the Informed Consent Form must be filed by the Principal Investigator. A copy of the Subject Information including a copy of the signed Consent Form should be given to the subject.

A sample of the Master Subject Information and Consent Form is enclosed (Appendix B). If modifications are made to either of these documents according to local requirements, Mölnlycke must approve amended version(s) of the document(s).

If any new important information occurs during the clinical investigation the subject will be informed both orally and in writing.

8.2 Regulatory and standards

8.2.1 Regulatory review

If applicable, the final Clinical Investigation Plan, including the final version of the Subject Information and Consent Form, must be approved or given a favourable opinion in writing by a RA before enrolment of any subject into the clinical investigation. Mölnlycke is responsible for informing the RA of any amendment to the Clinical Investigation Plan as per local requirements.

8.2.2 Standards and other

The most recent version of ISO 14155 is followed in addition to national regulations.

8.2.3 Subject Data Protection

The written Subject Information explains that the data will be stored in a computer database, maintaining confidentiality in accordance with national data legislation and that authorised representatives of Mölnlycke and/or a RA and/or EC/IRB, may require direct access to those

parts of the hospital/practice records relevant to the clinical investigation, including medical history, for verification of data. All data computerized by Mölnlycke will be identified by subject number only.

8.3 SUBJECT PROTECTION PROCEDURES

8.3.1 Procedures in Case of Medical Emergency

The Principal Investigator is responsible for ensuring that procedures and expertise are available to cope with medical emergencies during the clinical investigation.

8.3.2 Insurance

Mölnlycke Health Care AB has product liability insurance, which also covers devices being investigated in a clinical investigation.

9. INVESTIGATION TIMETABLE AND TERMINATION

The study duration per patients is approximately 22 weeks, i.e. treatment duration of up to 20 weeks and follow up 2 weeks after closure.

Planned investigation start: Q4 2019

Planned inclusion completion: Q3 2022

Planned last subject out: Q4 2022

The clinical investigation could be prematurely discontinued if the dropout rate is higher than 20% and/or the investigation site is unable to fulfil the inclusion period according to the Clinical Investigation Agreement.

10. PRINCIPAL INVESTIGATOR(S) AND INVESTIGATION ADMINISTRATIVE STRUCTURE

Specified in separate list.

11. DEFINITIONS AND PROCEDURES FOR REPORTING OF ADVERSE EVENT, ADVERSE DEVICE EFFECT, SERIOUS ADVERSE EVENT, SERIOUS ADVERSE DEVICE EFFECT AND DEVICE DEFICIENCY

DEFINITIONS

Device Deficiency (DD)
Inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety or performance.

Note: Device Deficiencies include malfunctions, use errors, and inadequate labelling.

All Device Deficiencies that might have led to a Serious Adverse Device Effect shall be reported in accordance with Serious Adverse Event reporting procedures, as specified below.

Adverse Event (AE)

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:

- This definition includes events related to the investigational medical device or the comparator.
- This definition includes events related to the procedures involved.
- For users or other persons, this definition is restricted to events related to investigational medical devices.

Adverse Device Effect (ADE)
Adverse Event related to the use of an investigational medical device

Note:

a) This definition includes adverse events resulting from insufficient or inadequate instructions for use, deployment, implantation, or operation, or any malfunction of the investigational medical device.

b) This definition includes any event resulting from use error or from intentional misuse of the investigational medical device.

Serious Adverse Event (SAE)
Means any adverse event that led to any of the following:

a) death,

b) serious deterioration in the health of the subject, that resulted in any of the following:

1) a life-threatening illness or injury, or

2) a permanent impairment of a body structure or a body function, or

3) hospitalization or prolongation of patient hospitalization, or

4) medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function, or

5) chronic disease

c) foetal distress, foetal death or a congenital physical or mental impairment or birth defect.

Note: Planned hospitalization for a pre-existing condition, or a procedure required by the Clinical Investigation Plan, without serious deterioration in health, is not considered a serious adverse event.

Serious Adverse Device Effect (SADE)

Adverse Device Effect that has resulted in any of the consequences characteristic of a Serious Adverse Event.

PROCEDURES FOR SAE AND/OR SADE REPORTING OR REPORTING OF DD THAT COULD HAVE LED TO A SADE

The investigator is responsible for the reporting of AEs, ADEs; SAEs, SADEs and DD that occur in the control group in accordance with local requirements to authorities and if applicable manufacturers of these products.

The Principal Investigator must inform Mölnlycke, within 1 calendar day of awareness of the event. When a SAE/SADE has been entered into the eCRF by the Principal Investigator/authorised site staff, the eCRF system will automatically generate a report to: Clinical_Investigations_Event_Reporting@molnlycke.com.

In case of problem with the eCRF, a paper-based version of the SAE/SADE report form (available in the Investigator Site File) shall be used and sent by email to: Clinical_Investigations_Event_Reporting@molnlycke.com.

All SAEs/SADEs that occur during the Clinical Investigation shall be reported, whether or not they are considered causally related to the investigational device.

Device Deficiencies that might have led to SADE if either a) suitable action had not been taken, b) if intervention had not been made, or c) if circumstances had been less fortunate must be reported as a SADE.

The Principal Investigator is responsible for informing the EC/IRB and/or the RA of the SAE/SADE as per local requirements.

PROCEDURES FOR DD REPORTING

All DD shall be reported to Mölnlycke as soon as possible, without unjustified delay. If the DD might have led to a SADE the reporting requirements for SADE described above must be followed. DDs can be either subject related or non-subject related depending on if the investigational device was used by a subject or not. Separate forms are used for subject related and non-subject related DDs.

When a subject related DD has been entered into the eCRF by the Principal Investigator/authorised site staff, the eCRF system will automatically generate a report to: Clinical_Investigations_Event_Reporting@molnlycke.com

Non-subject related DDs are reported using the paper-based report form located in the Investigator Site File. The completed form shall be sent by email to: Clinical_Investigations_Event_Reporting@molnlycke.com

PROCEDURES FOR ADE REPORTING

All ADE shall be reported by the Principal Investigator to Mölnlycke as soon as possible without unjustified delay. When an ADE has been entered into the eCRF by the Principal Investigator/authorised site staff, the eCRF system will automatically generate a report to: Clinical_Investigations_Event_Reporting@molnlycke.com

This includes also ADEs occurring in the comparator arm.

CAUSALITY ASSESSMENT

The relationship between the use of the investigational device and the occurrence of each AE/SAE shall be assessed by the Principal Investigator and Mölnlycke and classified as device related or not related to the device.

Report by Mölnlycke Health Care to Regulatory Authorities

Mölnlycke will report to RA where the clinical investigation has commenced in accordance with the local requirements for market authorised medical devices.

12. PUBLICATION POLICY

The clinical investigation will be registered in a publicly accessible database before recruitment of the first subject.

A final Clinical Investigation Report (CIR) of the investigation will be completed, even if the clinical investigation is prematurely terminated. The CIR will be prepared by Mölnlycke in accordance with the guideline presented in Annex D of ISO 14155.

All publications and presentations must be based upon the CIR.

All information supplied by Mölnlycke in connection with this investigation will remain the sole property of Mölnlycke and is to be considered confidential information. No confidential information will be disclosed to other parties not involved in this clinical study without obtaining prior written consent from Mölnlycke. Confidential information associated with this study will not be used except in the performance of this investigation.

Mölnlycke may choose to publish or present data from this clinical investigation. If a Principal Investigator is offered first authorship, he/she will be asked to comment and approve the publication. Mölnlycke has the right to use the results of this study for registration, internal presentation, and for promotion.

13. LITERATURE REVIEW AND REFERENCES

In order to determine the scientific background for this clinical investigation as well as to assess risks/benefits of the device, a literature review was conducted. The below literature is listed as references and was critically evaluated before serving as background information.[1-12,14-19,22,23,30-35]

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