Statistical Analysis Plan

Granulox01

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)

2022-09-23

[redacted]

[redacted]

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADE	Adverse Device Effect
AE	Adverse Event
CCC	Confirmed Complete wound Closure
CGI	Clinical Global Impression
CIP	Clinical Investigation Plan
CP	Conditional Power
CRF	Case Report Form
CVI	Chronic Venous Insufficiency
DD	Device Deficiency
EC	Ethics Committee
EOCR	Estimated Overall Closure Rate
EQ-5D-5L	EuroQol.org - 5 dimensions - 5 level instrument to describe and value health
FAS	Full Analysis Set
FCS	Full Conditional Specification
HRQoL	Health-Related Quality of Life
IARC	Interim Analysis Review Committee
IRB	Institutional Review Board
NRS	Numeric Rating Scale
PCC	Possible Complete wound Closure
PP	Per Protocol
PROs	Patient Reported Outcomes
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
QoL	Quality of Life

1 STUDY DETAILS

1.1 Study Objectives

1.1.1 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).

1.1.2 Secondary Objectives

[redacted]

1.2 Study 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, including a 14 days run-in period and an up to 20 weeks treatment period starting with randomization and allocation of treatment.

1.2.1 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 the 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 country, wound duration (<5 months; ≥5 months), and wound area after run-in period (<10; ≥10 cm^2), 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.

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.

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. 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.

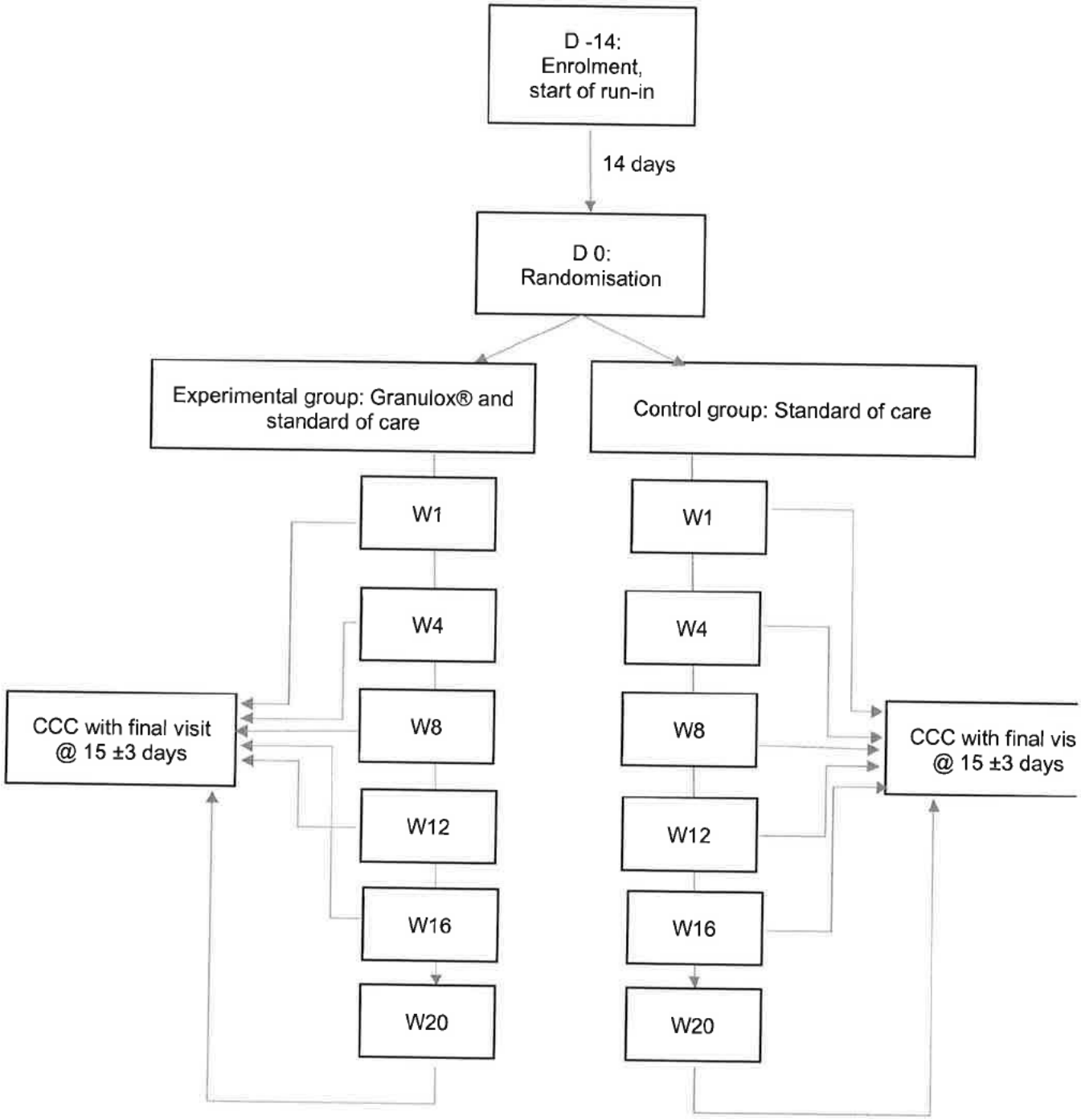
If at any time, a wound graft is required (it usually requires at 24-48 hours hospitalization), study will not be considered as completed and patients will still be followed according to the schedule of assessments (even if experimental treatment is no longer applied) up to CCC occurrence or up to W20 (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 1.5 "Definition of Study Populations".

1.2.2 *Flow Chart*



1.2.3 Schedule of Assessment

Site visits	Run-in Visit	Home care	Visit 1	Home care	Visit 2	Home care	Visit3	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	(Day 3)	W1 (Day 7 ±1 day)	Every 3rd day	W4 (Day 28 ±3 days)	Every 3rd day	W8 (Day 56 ±3 days)	Every 3rd day	W12 (Day 84 ±3 days)	Every 3rd day	W16 (Day 112 ±3 days)	Every 3rd day	W20 (Day 140 ±3 days)		
Eligibility criteria	X		X														
Randomization			X														
Demography and background data			X														
Medical and surgical history (including VLU)			X														
Wound picture and status (Tissue Analytics)	X		X		X		X		X		X		X		X	X	X
PUSH score			X		X		X		X		X		X		X	X	X
PCC					X		X		X		X		X		X	X	X
Clinical Global Impression (CGI)[b]																X	
Patient diary, completed by nurse (e.g. wound care consumables, frequency of dressing change, acceptability of therapy, easy of therapy)	X	X		X		X		X		X		X		X			
Review of patient diary and assessment of compliance			X		X		X		X		X		X		X	X	X
EQ-5D-5L and Wound-QoL[c]			X								X				X	X	

Site visits	Run-in Visit	Home care	Visit 1	Home care	Visit 2	Home care	Visit3	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	(Day 3)	W1 (Day 7 ±1 day)	Every 3rd day	W4 (Day 28 ±3 days)	Every 3rd day	W8 (Day 56 ±3 days)	Every 3rd day	W12 (Day 84 ±3 days)	Every 3rd day	W16 (Day 112 ±3 days)	Every 3rd day	W20 (Day 140 ±3 days)		
NRS (Pain)[d]			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
Reason(s) study arrest																X	

a) To be completed in any case (premature arrest, CCC)
b) To be completed after patient's study termination and based on patient-series of wound pictures.
c) EQ-5D-5L (EuroQoL health status) and Wound-QoL (Quality of Life)
d) NRS (Numerical Rating Scale) including pain and pain intensity
e) Safety monitoring including reporting of adverse events, serious adverse events, adverse device effects, serious adverse device effects, and device deficiencies.

1.3 Treatment Groups

The Granulox group will use the same standard of care as the control group but with Granulox. Granulox is a topical haemoglobin spray, which is designed to transport oxygen to the wound size.

The Control group will use the same defined standard of care as the treatment group.

1.4 Sample Size

1.4.1 Sample size calculation

[redacted]

Sample Size Re-estimation

The study will have an adaptive design approach[1] where a pre-defined re-estimation of the sample size will be done after completion of approximately 110 patients. For this purpose, an independent interim analysis review committee (IARC) will be appointed, consisting of at least one person with medical knowledge experience and one experienced statistician.

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

Based on the outcome of the CP, the IARC will form a recommendation to either terminate the study, continue the study unchanged, or increase the sample size, according to the following guidelines:

- If CP ≤30%, then terminate the study for futility.
- If 30%< CP% <80%, then adjust the sample size so that CP = 80%. The sample size adjustment will not exceed the maximum of 600 subjects.
- If CP% ≥ 80%, then do not adjust the sample size.

Only the recommended sample size adjustment (i.e. no result) will be communicated to the steering group of the study.

This procedure is detailed in [2] and guarantees type I error control.

1.5 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.

2 STUDY VARIABLES

2.1 Baseline Variables

2.1.1 Demographics and Baseline Characteristics

- Age
- Gender
- Ethnicity
- Mobility
- Subject Social Profile
  - Living situation
  - Physical activity level
  - Lifestyle
  - Require assistance
- Height
- Weight

2.1.2 History of leg ulcer

- Duration
- Wound location
- Latest ABPI value
- Recurrent ulcer
- Previous dressing
- Previous compression

2.1.3 *Medical and Surgical History*

- Relevant medical history (general and with regard to venous disease/VLU)
- Relevant surgical history (general and with regard to venous disease/VLU)

2.1.4 Wound Management

- Current wound management treatment

2.1.5 Current Wound Status

- Estimated Wound Size
- Exudate amount (before debridement)

- Predominant tissue (after debridement)
- Push Score Value
- Wound bed aspect (after debridement)
- Exudate nature

2.1.6 Prior and Concomitant Medications

2.2 Efficacy Variables

2.2.1 Primary Efficacy Variable –Confirmed Complete wound Closure (CCC)

CCC is the primary variable and main efficacy criterion. It is defined as a 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.

[redacted]

[redacted]

[redacted]

2.2.4 *Acceptability and compliance*

During clinical visits, the patient-specific diary (completed by the study nurse) will provide input to the assessment of acceptability of care, ease of care, and compliance as follows:

- Assessment of acceptability of care by means of a 5-item scale (e.g. very poor, poor, average, good, very good). This will be performed at the final visit only.
- Assessment of ease of care by means of a 5-item scale (e.g. very poor, poor, average, good, very good). This will be performed at the final visit only.
- Evaluation of patient compliant to venous compression as either:
    - Fully compliance (defined as 7/7 days)
    - Moderately compromised compliance (defined as 1, 2 or 3 days/week maximum without compression)
    - No compliance (defined as >3 days/week without compression)

2.2.5 *Exploratory Efficacy Variables*

Measured at all schedule visits.

[redacted]

2.3 Safety Variables

2.3.1 *Adverse Events*

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 the CIP.

3 STATISTICAL METHODOLOGY

3.1 General Statistical Methodology

This study will use confirmative and explorative analyses. All analyses of primary and secondary variables will be performed on FAS. Subjects without any scheduled follow-up visit will not be included in any follow-up analyses. Complementary analysis of primary and secondary variables will also be performed based on PP population.
The primary analysis will be the comparison of Confirmed Complete wound Closure (CCC) between the two randomized groups using two-sided binary logistic regression analysis 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)

The essential analyses will be the comparison between the two randomised groups: the Granulox group and the Control group for primary and secondary variables. Analyses within each of the randomized groups will also be performed but without the same scientific value. For secondary efficacy variables the main analysis will be performed on change from baseline to final visit or at final visit, but analyses of change from baseline to the other visits will also be performed at week 1, 4, 8, 12 16 and 20. Final visit is defined as week 20 for subjects that have a 20 week visit, date of first CCC visit for subjects with CCC and for drop-outs with multiple imputation for week 20.

The study includes a fixed order of sequential testing of the following secondary variables.

[redacted]

[redacted]

The study utilized stratified randomization based on country, wound size, and wound duration. This is reflected by a stratified primary analysis adjusted for these variables.
If baseline confounders are found, variables that differ between the two randomised groups and has a causal relation to the outcome variable, then complementary analyses will be performed adjusted for these variables.

For adjusted comparison between the two randomized groups, multivariable logistic regression will be used for dichotomous variables, analysis of covariance for continuous variables, Mantel-Haenszel pooling technique for ordered categorical variables and Cox proportional hazard model for time to event data.

The most important efficacy analyses both for unadjusted and adjusted analysis will be the calculation of the mean difference between the Granulox group and the Control group with 95% CI for continuous and dichotomous variables.

Continuous variable will be described with mean, SD, median, Q1, Q3, minimum and maximum and categorical variables with numbers and percentages. For dichotomous variables, for descriptive purpose, relative risk with 95% CI will also be calculated. Proc Genmod binary outcome variable and link = log. For time to event data Kaplan-Meier curves will be presented.

For analyses of change from baseline to other visits within each randomised group Fisher's non-parametric permutation test for paired observations will be used for continuous variables and Sign test for dichotomous and ordered categorical variables.

For continuous variables measured over time Box-plots will be given over visits by randomized group and Box-plots for change from baseline to each visit will be given by group. For selected secondary dichotomous and ordered outcome variables measured over time vertical bars will be given over visits by randomised group and vertical bars for change from baseline to each visit will be given by group.

All significance tests will be two-sided and conducted at the 5% significance level.

All analyses will be performed by using SAS® v9.4 (Cary, NC) or later.

3.2 Missing data.

[redacted]

[redacted]

3.3 Patient Disposition and Data Sets Analysed

The number of subjects included in each of the FAS, PP and safety populations will be summarized for each treatment group and overall. The number and percentage of subjects enrolled, randomized and treated will be presented. Subjects who completed the study and subjects who withdrew from study prematurely will also be presented with a breakdown of the reasons for withdrawal by treatment group for the FAS, PP and safety populations.

3.4 Protocol Violations/Deviations

Major protocol deviations are those that are considered to have an effect on the primary analysis. A list of potential major protocol deviations will be generated programmatically from the data captured before the clean file meeting. The clinical monitors of the study will review the list and the finalisation of the major protocol deviations will be done at the clean file meeting.

The number and percentage of patients with major protocol deviations will be summarized per treatment group.

3.5 Demographics and Baseline Characteristics

Demographics and baseline characteristics given in section 2.1 will be summarized by treatment group for the FAS and PP populations and analysed according to the methods described in section "General Statistical Methodology" above, 3.1.

3.6 Medical and Surgical History

Listings of all medical and surgical history collected by the investigator will be produced.

3.7 Prior and Concomitant Medications

Listings of all medications collected for each subject by the investigator will be produced.

3.8 Efficacy Analyses

3.8.1 Primary Efficacy Analysis

The primary analysis will be the comparison of Confirmed Complete wound Closure (CCC) between the two randomized groups using two-sided binary logistic regression analysis at

significance level 0.05, on FAS with multiple imputation including the following stratified covariates:

- Country (as fixed effect)
- Wound duration (<5 months; ≥5 months)
- [redacted]

[redacted]

Complementary analyses will also be performed for CCC between the two groups on the PP population.
Complementary analyses will also be performed for EOCR (CCC+PCC) between the two groups on the FAS population.

If baseline confounders are found, variables that differ clinically between the two randomised groups and has a causal relation to the primary outcome variable, then complementary analyses will be performed adjusted also for these variables with the same methods as for primary analysis.

Adjusted and unadjusted mean % differences between the two groups in CCC will also be given. The percentage of CCC in each group with 95% exact CI will be calculated.
Unadjusted primary analyses of CCC by country and by country and centre will be presented with percentage and mean percent difference with 95% CI.

3.8.2 Secondary Efficacy Analyses

[redacted]

3.8.3 Analyses of Exploratory Efficacy variables.

[redacted]

3.8.4 Sub-group analyses

[redacted]

3.8.5 Exploratory Interaction analyses between treatment and baseline variables

[redacted]

3.9 Safety Analyses

3.9.1 Adverse Events

All safety variables will be summarized and analysed descriptively by treatment group on the safety population.

The number of subjects reporting one or more AE/ADE/SAE/SADE/DD will be summarised by treatment group using frequency counts. If very few AE/ADE/SAE/SADE/DD these will only be listed.

The incidence (%) of AE/ADE/SAE/SADE/DD reported during the investigation will be summarised in an overview table and by treatment group.

4 INTERIM ANALYSES

[redacted]

5 CHANGES OF ANALYSIS FROM PROTOCOL

[redacted]

[redacted]

6 LISTING OF TABLES, FIGURES AND LISTINGS

6.1 Listing of Tables

Table Number	Table Title
[redacted]	

[redacted]

6.2 Listing of Figures

[redacted]

6.3 Listing of Listings

[redacted]

7 REFERENCES

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2. Mehta CR, Pocock SJ (2011) Adaptive increase in sample size when interim results are promising: a practical guide with examples. Statistics in medicine 30 (28):3267-3284. doi:10.1002/sim.4102

3. Daniel R. Gorin, MD, Paul R. Cordts, MD, Wayne W. LaMorte, MD, PhD, MPH, James O. Menzioan, MD, (1996) The influence of wound geometry on the measurement of wounds healing rates in clinical trials. J Vasc Surg 1996;23;524-8.