METRO STUDY PROTOCOL

MESOGLYCAN (PRISM®) VERSUS PLACEBO IN SECONDARY PREVENTION OF SUPERFICIAL VEIN THROMBOSIS

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1. Abbreviation legend

ACCP	American College of Chest Physicians
ALT	Alanine amino transaminases
CT angiography	Vessel-optimized computed tomography
aPTT	Activated Partial Thromboblastin Time
ASA	Aceteylsalicidic acid
AST	Aspartate transaminase
CCDU	Color Coded Doppler Ultrasonography
CEAP	Clinical-Etiology-Anatomy-Pathophysiology score
CIOMS	Council for International Organizations of Medical Sciences
cps	Tablets
CRO	Contract Research Organization
CUS	Compression Ultra Sonography
DSUR	Development Safety Update Report
EDC	Electronic Data Collection
EP	Pulmonary embolism
NSAIDs	Nonsteroidal anti-inflammatory drugs
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GCS	Graduated Compression Stockings
gg	days
HR	Hazard Ratio
ISTH	International Society on Thrombosis and Haemostasis
ITT	Intention To Treat
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligrams
PCR	C-Reactive Protein
PT-INR	Prothrombin-International Normalized Ratio
rVCSS	revised Venous Clinical Severity Score
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected, Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
DVT	Deep vein thrombosis
SVT	Superficial vein thrombosis
UOC	Complex Operating Unit
VEINES/Sym-QoL	Venous Insufficiency Epidemiological and Economic Study/Symptom - Quality of Life
VTE	Venous Thromboembolic Event

2. SYNOPSIS

Mediolanum Farmaceutici S.p.A., Milan	Protocol: **METRO** Impact Code No. 50129; EudraCT No. 2016-005184-13
Investigational drug name: **Prisma®**	Development phase: **Phase IIb**
Name of the active substance: **mesoglycan**	Synopsis Date: 2017-10-24 Version: 1.7

Protocol Title:

Mesoglycan (prism®) versus placebo in secondary prevention of superficial venous thrombosis.

Objective:

To demonstrate the superiority of mesoglycan (Prisma®), compared to placebo, in reducing the incidence of thromboembolic complications (recurrence/extension of superficial vein thrombosis, deep vein thrombosis, pulmonary embolism) in patients who have completed the course of acute phase therapy after a superficial vein thrombosis (SVT).

Rationale:

SVT is a relatively common clinical condition, with recent evidence of association with the development of deep vein thrombosis (DVT) and/or pulmonary embolism (PE) that has changed the perception of the risk of this condition (with conflicting data in the literature: 3-33%). Previous retrospective or registry studies on the natural history of SVT have observed that thromboembolic complications (recurrence/extension of SVT, DVT, PE) can affect up to 10-15% of subjects at 3-6 months after the initial event, despite adequate treatment of the acute phase. There are no studies in the literature that have prospectively investigated the usefulness of drug therapy in the secondary prevention of SVT and the natural history of SVT once the initial acute therapeutic course has been completed.

Organization:

Coordinator: Dr. Giuseppe Camporese, Angiology Unit, University Hospital of Padua.

Investigators: 15-20 Italian centers with proven experience in the treatment and prevention of venous thromboembolic disease.

Independent Data and Safety Monitoring Committee will monitor the safety of the subjects and the quality of the data collected.

Sponsor and provider of the study treatment: Mediolanum Farmaceutici S.p.A., Milan. Part of the Promoter's functions will be delegated to a CRO, on express assignment and under the control of the Promoter itself.

Study population:

Inclusion Criteria:

1. Subjects of both sexes, age>=18 years,
2. With a previous diagnosis of SVT of the lower limbs documented by Color-coded Doppler Ultrasound (CCDU), which at onset was at least 5 cm in extent and at least 3 cm from the saphenous junctions (great saphenous vein and lesser saphenous vein),
3. Who have completed the initial acute therapeutic course with Fondaparinux 2.5 mg once-daily for 45 days, as recommended by the most recent international guidelines (ACCP).
4. That at CCDU initial screening do not present a concomitant involvement of the deep venous system, or an extension of the initial SVT.

Exclusion Criteria:

1. Poor compliance with SVT treatment
2. Life expectancy < 24 months
3. Anticipated lack of cooperation or inability to fill in questionnaires
4. Pregnancy, breastfeeding, or planned pregnancy during the duration of the study
5. Severe locomotor disability or prolonged immobilization
6. Participation in another study within the last 3 months
7. Post-thrombotic syndrome with "Villalta score" > 4
8. Chronic lymphedema of the lower limbs
9. Recent (< 3 months) or planned phlebological surgical intervention or percutaneous arterial trans-luminary angioplasty (PTA)
10. Ongoing dialysis treatment
11. State of malabsorption/malnutrition
12. Chronic and non-suspendable use of anticoagulants, phlebotrophics, steroids or NSAIDs, double-antiplatelet or ASA >160mg/day, centrally acting painkillers
13. Subjects with hypersensitivity to mesoglycan, heparin or heparinoids, intolerant to galactose or with lactase deficiency, carriers of diathesis and bleeding diseases

Number of subjects to be enrolled: total 650 subjects

Study design:
Phase II, multicenter, randomized, double-blind superiority study, comparing mesoglycan (Prisma®) 50 mg and placebo, both in double daily oral intake.

Study treatment:
Experimental drug: mesoglycan (Prisma®), already used in the therapy of chronic venous ulcers, is a complex mucopolysaccharide, composed of a blend of glycosaminoglycans (mainly heparan sulfate, dermatan sulfate), thrombin and Factor Xa inhibitors and active in restoring flow-mediated vasodilation.
Used in oral formulation, hard capsules, 50 mg, twice-daily, for 12 months.
Reference therapy: placebo, organoleptically indistinguishable from the investigational drug, 1 cps twice-daily, for 12 months.
Masking: the study is conducted in "double-blind".

Methodology and phases of the study:
Collection of written Informed Consent: The Investigator will inform the patient that participation in the study is voluntary and that refusal will not lead to the loss of any benefit or in any way affect the relationship with the physician. In addition, it will be made known that withdrawal from the study is possible at any time without having to provide a specific reason. Prior to being enrolled in the study, each subject will receive a full explanation of the nature and purpose of the study from the Investigator, along with a description of the benefits and risks associated with participation. Insurance coverage will also be mentioned and related procedures in case of injury will be explained. A clear information sheet covering all important aspects of the study will be given to the subject. The subject, once read, will have the opportunity to ask questions and will be given sufficient time to consider the various aspects presented, before being asked to sign and date the informed consent form. The original copy of the signed and dated informed consent form will be kept by the Investigator in the "study file" kept in the Center. Subject will receive a copy of the signed and dated informed consent form for future reference.
Screening: After written informed consent is obtained, all potential subjects will complete the screening visit. If they meet the pre-established inclusion and exclusion criteria, they will be enrolled in the study and will undergo ultrasonographic study by bilateral venous Color-Coded Doppler Ultrasound (CCDU) of the lower limbs (investigation in standard clinical practice because at the end of the acute phase treatment), to exclude a concomitant involvement of the deep venous system, or an extension of the initial SVT, which would imply the extension of parenteral anticoagulant therapy. A blood sample will be taken for the determination of complete blood count, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), creatinine, Prothrombin Time-INR (PT-INR), activated Partial Thromboplastin Time (aPTT) and C-Reactive Protein (CRP).
Randomization: centralized, via a specific application of the Electronic Data Capture website, with 1:1 allocation (stratified by age <60/>=60yy and gender; in blocks of variable size, random of 4 and 6 units) between placebo and mesoglycan. It is estimated that enrollment will be completed in 24 months.
Scheduled visits: follow-up visits at 3, 6, 9, 12 months during treatment and at 24 months with:

- global clinical assessment and treatment compliance,
- collection of information of any thromboembolic events and/or adverse events,
- evaluation of revised Venous Clinical Severity Score (rVCSS) and Venous Insufficiency Epidemiological and Economic Study (VEINES/Sym-QoL) scores
- at 12 months, CCDU and blood sampling for complete blood count, ALT, AST, creatinine, PT-INR, aPTT and CRP.
- at 24 months, blood sampling for complete blood count, ALT, AST, creatinine, PT-INR, aPTT and CRP.

The patient will be instructed to present at any time (after telephone contact) for a clinical check-up in case of signs/symptoms suggestive of thromboembolic events.
During the treatment period on days: 30, 60, 135, 225, 315 (+-5 days) from randomization, and in the follow-up period 3, 6 and 9 months after the end of treatment and then every 6 months until the end of the study, subjects will be contacted by telephone by trained personnel to assess their compliance with treatment, the possible use of drugs not allowed and the occurrence of adverse events and/or end-points of the study.
During the study, the concomitant intake of oral or parenteral anticoagulants, phlebotropics, NSAIDs in chronic and continuous treatment (except for occasional symptomatic use of NSAIDs), double-antiplatelet or ASA >160mg/day, centrally acting painkillers is not allowed,
The simultaneous use of graduated compression stockings (GCS), of which there is no evidence of real efficacy, is left to the judgment of the clinician.
Subjects will permanently exit the study if one of the primary endpoints occurs, the occurrence of one of the exclusion criteria, a serious adverse event (SAE) that realizes one of the exclusion criteria, withdrawal of consent.

Evaluation criteria:
Primary efficacy end-point: Cumulative incidence of objectively documented asymptomatic or symptomatic SVT recurrence or extension, new DVT (proximal asymptomatic or symptomatic/distal isolated symptomatic), pulmonary embolism (fatal/symptomatic non-fatal).

Secondary efficacy end-points: Cumulative incidence of the first event between: recurrence or extension, asymptomatic or symptomatic, of SVT, new proximal/distal DVT (asymptomatic or symptomatic), pulmonary embolism (fatal/symptomatic non-fatal); change in rVCSS score (efficacy) and VEINES/Sym-QoL (quality of life) score on treatment and in the following 12 months; recanalization of the affected vein at the primary thrombotic event; new development deep and/or superficial venous reflux; impact of GCS; contribution of CRP levels in predicting the onset of the primary end-point. Safety End-Point: Serious Adverse Events, Total Adverse Events. Bleeding adverse events will be graded as major or minor according to ISTH Guidelines. A copy of the Diagnostic Report of the first event considered will be mandatorily collected and sent to the coordinating center for the validation of the event.
Statistical methods: The primary analysis will be carried out in the "modified intention-to-treat" population, which will include all randomized subjects who have taken at least one dose of the study treatment. The hypothesis of superiority of the primary cumulative end-point of efficacy will be tested by multivariate Cox regression for proportional risks, using as covariates the assigned drug, age, gender, presence of additional risk factors for thromboembolisms, possible use of GCS and compliance with treatment. Analyses of the other primary and secondary end-points will be conducted with the continuity-adjusted chi-square test and the Wilcoxon test for paired data, as appropriate. Standard summaries of safety data related to adverse events will be provided
Sample size: A risk for the primary efficacy endpoint for the placebo group of 15% at 12 months and a 50% reduction in mesoglycan patients is hypothesized. A bilateral log-rank test, with a total sample of 650 subjects (1:1 randomization), achieves the power of 90%, with an alpha error of 0.025, to detect a difference of 7.0%, between 15.0% and 8.0% (hazard ratio, HR=0.51), after 12 months of treatment, taking into account a drop-out of 10.0%.

3. Background

Superficial vein thrombosis (SVT) is a relatively common, painful condition characterized by thrombosis and inflammation of a superficial vein.(1) This disease has always been considered benign.(2,3,4) However, epidemiological studies have shown that this condition is often associated with symptomatic and asymptomatic deep vein thrombosis (DVT) in 25% of cases, and in 4%, it is also linked to symptomatic pulmonary embolism (PE). (2,3,4,5,6) Additionally, in 33% of cases, an asymptomatic pulmonary embolism is already present at diagnosis. (7)

Despite numerous epidemiological studies available in the literature, the true incidence remains uncertain. Outdated research has shown that SVT had a significantly higher incidence than DVT, at 0.9‰ per year (2). However, a recent "community" study involving 32 French cities and both general practitioners and hospital doctors clearly demonstrated an SVT incidence of 0.64% per year, which is six times higher than that of venous thromboembolism (VTE). In this study, the annual diagnosis rate varied greatly with age and sex, ranging from 0.04% [95% CI, 0.00%–0.10%] in men aged 18–39 to 2.2% [95% CI, 1.59%-2.78%] in women over 75 years old. (2)

This survey also confirmed previous suspicions that SVT is not a benign disease as once thought, as it often extends to involve the deep system (25%) and the lung (4%)(2). A recent meta-analysis corroborated these findings (8), revealing an average prevalence of 18.1% and 6.9%, respectively. These events are more common when thrombophlebitis has already reached the knee, affects the thigh, or involves a perforating vein (2, 9). Moreover, among patients with isolated superficial vein thrombosis, about 8-10% develop DVT within the following three months (3). Despite limited data in the literature, the most predictive factors for thromboembolic complications in subjects with SVT have been identified as male sex, a history of previous venous thromboembolism (OR 2.53), recent onset of SVT, and severe chronic venous insufficiency (OR 4.5) (10, 11, 12).

Complications of SVT are also inversely related to female sex, young age, and pregnancy (8). Other significant risk factors include obesity, the presence of varicose veins, cancer, and prolonged orthostatism.[13] Smoking is also a major risk factor (OR 1.69), as is a family history of VTE (OR 2.28). In the third decade of life, the incidence of the disease increases to 0.05‰ per year in men and 0.3‰ per year in women, with higher rates in the eighth decade of life (1.8‰ per year in men, 2.2‰ per year in women). Additionally, more advanced CEAP classes are associated with increased risk in women.

What seems to be clear is that patients with previous SVT have a 4-fold increased risk of developing PE in the future and a 6-fold increased risk of developing DVT.[14] The presence of an acquired risk factor significantly increases the risk of DVT. (12)

This link between SVT and DVT and/or pulmonary embolism (PE) has greatly shifted the understanding of the disease's risk.

The relationship between the d-dimer test and SVT (15,17) is unclear, but currently it does not appear to be useful in diagnosing SVT due to the high number of false negatives. There are no data on whether a d-dimer remaining elevated after discontinuing therapy could cause relapses, as has been demonstrated for DVT and PE. (27)

The therapy of SVT in the acute phase has been the subject of several studies over the past decade (9,10,19,24). The data available for low molecular weight heparins do not yet allow for conclusive recommendations, although it appears that a 10-day course at therapeutic dosage, followed by an additional 20 days at intermediate doses (2/3 of the therapeutic dose), is effective in treating SVT (10,17,19). However, relapses occurring three months after discontinuation seem to happen in a percentage ranging from 7.2% to 11% in the active treatment group.

More recent studies have shown that the administration of Fondaparinux (9) at prophylactic doses (2.5 mg/day subcutaneously) for 45 days is the first-choice treatment. Compared to placebo, Fondaparinux reduces the rate of VTE and the extension and/or recurrence of SVT by about 80%, avoiding the "catch-up" phenomenon that had been described with previous studies conducted with heparin and NSAIDs, with a 77-day recurrence rate of 0.9%. (23)

Based on this data, the ACCP guidelines (24) recommend a prophylactic dose of fondaparinux (2.5 mg) or LMWH for 45 days for patients with lower limb SVT longer than 5 cm (24), identifying fondaparinux (IIB) as the first choice. Meanwhile, the NICE guidelines, supported by a recent cost-effectiveness analysis (25), suggest using fondaparinux in high-risk DVT patients, such as those with thrombosis near the saphenofemoral junction (less than 3 cm, where anticoagulation is mandatory), SVT without varicose veins, previous SVT, DVT, or PE. (26)
Despite this scientific evidence, the treatments used remain very inconsistent. In a recent survey conducted in France, only 72% of patients with SVT received acute treatment with heparin or fondaparinux, with significant variability in dosage. This inconsistency extends beyond dosage to diagnosis methods, which vary greatly—from the measurement of D-dimer to CCDU and target data. (21)

3a. Study and dosing rationale

For the secondary prevention of DVT and PE, numerous studies have demonstrated the usefulness of extending anticoagulant treatment beyond the acute phase using warfarin, low molecular weight heparins, or the new oral anticoagulants (particularly dabigatran, rivaroxaban, and apixaban), (29,30,31) or with antiplatelet therapy (27) compared to placebo or no treatment. On the other side, there are no studies in the literature that have prospectively and systematically examined the efficacy of any drug therapy for the secondary prevention of SVT once the initial treatment course of the acute phase has ended. This data, lacking in the literature, is particularly important given the results of previous retrospective and registry studies on the natural history of SVT, which have shown that thromboembolic complications (recurrence/extension of SVT, DVT, PE) can affect up to 10-15% of subjects up to 3-6 months after the initial event, despite adequate treatment of the acute phase. (3,5,17)

Mesoglycan is a complex mucopolysaccharide made up of a blend of natural glycosaminoglycans, primarily heparan sulfate and dermatan sulfate. Heparan and dermatan sulfate are thrombin inhibitors with complementary actions, mediated by antithrombin and the heparin II cofactor, respectively (40, 41); heparan sulfate also inhibits Factor Xa (40). Mesoglycan is also effective in restoring flow-mediated vasodilation in people with endothelial dysfunction (42) and has demonstrated clinical benefits in chronic venous insufficiency (43). The drug is currently approved for treating chronic venous ulcers (see Summary of Product Characteristics).

Two studies, one retrospective and one prospective, have shown the effectiveness of mesoglycan in preventing recurrence in individuals with previous DVT. The retrospective study (32) found about a 20% reduction in the relative risk of DVT recurrence five years after the initial event in the mesoglycan group, compared to the group not receiving any treatment (24.6% vs 30%, respectively). The prospective study, meanwhile, randomly assigned 90 subjects to receive mesoglycan (72 mg daily) or placebo for one year after completing a 12-week course of acute phase DVT treatment. At the end of the follow-up, the relapse rate of VTE was 6.6% in the mesoglycan group versus 11.1% in the placebo group (RRR 40%, p=ns). (33)

The use of mesoglycan as a secondary prevention strategy in individuals with previous SVT has never been studied.

The oral formulation of mesoglycan used in this study (50 mg capsules) was approved for marketing in Italy in March 1989 and has been in use for 28 years. The two-capsule-per-day dosing schedule was approved for initial use, remains authorized, and has been employed in the mesoglycan clinical trials mentioned above.

Regarding the duration of treatment, there is no data on the best management of patients with SVT after they complete the standard course of therapy during the acute phase with fondaparinux 2.5 mg/day sc for 45 days. However, a treatment period of 12 months, along with an additional 12 months of clinical follow-up, appears to be a reasonable choice to gather comprehensive data, not only on the natural history of SVT in the group that will be randomized to placebo plus follow-up but also on the effectiveness of mesoglycan in reducing SVT recurrences

during the year of treatment and possibly in the following year of follow-up, given its protective effect on the endothelium (42).

3b. Benefit/risk profile

Benefit: despite the wide availability of treatments with proven prophylactic efficacy in preventing recurrences in the field of DVT, subjects who have completed the treatment of the acute phase of SVT do not yet have a prophylaxis with proven efficacy in reducing the risk of SVT and/or DVT recurrences or clinically significant PE, despite reliance on reports in the literature that agree on a non-trivial risk (10-15%). (2,3,4,5,6,17)

As a result, patients randomized to placebo will receive care based on the current *best standard of treatment*, without deducting any therapy of consolidated efficacy, and will benefit from especially timely and precise clinical surveillance due to their participation in a clinical trial. Patients randomized to mesoglycan will receive the same level of monitoring and will undergo prophylactic treatment that, based on the considerations outlined in the previous paragraph ("Rationale"), appears to have a potential chance of efficacy.

Risk: Clinical experience with mesoglycan 50 mg since 1989 has demonstrated a low rate of adverse reactions, primarily mild abdominal pain, dyspepsia, and skin reactions. The rate of discontinuing treatment due to adverse effects is 1.2% (SmPC). In randomized, placebo-controlled trials, the risk profile of mesoglycan was similar to that of placebo (46-47). Notably, clinically significant hemorrhagic adverse events are very rare.

This study aims to evaluate a prophylactic treatment proposal that offers potential efficacy with a low occurrence and severity of adverse events, resulting in a favorable benefit/risk profile.

4. Aim of the study

The aim of the study is to demonstrate the superiority of mesoglycan compared to placebo in reducing the incidence of thromboembolic complications (recurrence or extension of SVT, DVT, PE) in subjects who have completed the initial therapeutic course with Fondaparinux 2.5 mg once-daily for 45 days, as recommended by the most recent international guidelines. (24)

5. Study Design

Phase II, multicenter, randomized, double-blind superiority study comparing mesoglycan 50 mg and placebo, both administered twice daily by oral intake. The use of a placebo as a comparator treatment is justified due to the lack of scientific evidence supporting the use of secondary prophylactic treatment for the condition of interest.

The observation of patients is divided into a treatment period and a follow-up period afterward, as specified in point 15.

Enrollment will be competitive among the various participating Centers and will conclude once the expected number of subjects is reached (see point 19). The study will be approved by the Ethics Committee of the Coordinating Center, followed by approval from each local Ethics Committee of the participating Centers.

6. Study Organization

The study will be carried out throughout Italy and will include 15 to 20 centers with proven expertise in treating and preventing venous thromboembolic diseases.

The clinical coordinator of the study is Dr. Giuseppe Camporese, from the Angiology Unit at the University Hospital of Padua.

Sponsor and provider of the study treatment: Mediolanum Farmaceutici S.p.A., Milan. Part of the Promoter's functions will be delegated to a Contract Research Organization (CRO), under explicit assignment and under the direct supervision. of the Promoter.
The formation of an "Independent Data and Safety Monitoring Committee" (DSMC) is planned, consisting of 3 clinicians with recognized expertise (whose identities will be determined before enrollment begins), who will be supported organizationally by the Department of the Clinical Coordinator of the study. The DSMC, which will convene at least every six months, will be responsible for:

- Verify the quality of the data collected through blind analysis of synthetic reports regarding their completeness and adequacy, provided by the CRO data manager, and possibly suggest procedures to improve it.

- Monitor the safety of the subjects by blinding the tables provided by the CRO data manager, which contain details of the events that constitute efficacy endpoints, safety endpoints, and adverse events in the two treatment groups. If there is a clear imbalance in the benefit/risk profile between the two groups, and to protect the safety of the enrolled subjects, the DSMC may recommend to the Sponsor and the Study Coordinator that the study be terminated early.

- Conduct a blinded interim analysis once 75% of the expected subjects have been enrolled (488 subjects), to potentially adjust the total number of subjects needed ("re-sampling") in order to achieve the planned study power of 90%. This adjustment is considered if the number of primary endpoint events observed up to that point significantly differs from expectations for the two groups, specifically if the deviation of Hazard Ratio from the estimated 50% exceeds 5% in absolute value, meaning it falls below 45% or above 55%.

- Once the study is complete, perform the final diagnostic assessment of the events that define the endpoints of efficacy and safety in a blinded manner by

reviewing the data and iconographic documentation entered into the electronic Case Report Form (eCRF) by the Investigators.

7. Study Population

Subjects of both sexes, aged 18 years or older and up to 80 years old, who are suffering from lower extremity SVT and have completed the acute phase therapy course, will be eligible for this study.
At the time of enrollment or randomization (i.e., after completing the treatment of the acute phase of SVT), subjects will undergo bilateral venous CCDU of the lower extremities (standard clinical practice investigation) to rule out concomitant involvement of the deep venous system or a possible extension of the initial SVT, which could require an extension of parenteral anticoagulation therapy.

8. Inclusion Criteria

Subjects of both sexes will be included with:

- Age of at least 18 years old and under 80 years old,
- a prior diagnosis of SVT in the lower limbs confirmed by CCDU, with the relevant diagnostic images and reports attached to the eCRF.
- a thrombus of at least 5 cm of lenght
- the proximal edge of the thrombus located at least 3 cm from the saphenous junctions (great saphenous vein and small saphenous vein).
- A complete initial treatment course for SVT with Fondaparinux 2.5 mg once-daily for 45 days, following the latest international guidelines (24).
- a CCDU performed either at the end of the therapy of the acute phase or at the time of enrollment, which is negative for DVT or extension of the initial SVT.

To ensure the reliability and consistency of CCDU assessments across clinical centers, investigators will receive proper training during the Investigators Meeting, and a consensus methodological document will be drafted.

9. Exclusion Criteria

They will be excluded subjects with:

a. poor compliance with previous Fondaparinux therapy or discontinuation before 45 days;

b. an interval of more than 15 days between stopping Fondaparinux and study enrollment;

c. anticoagulation indicated for other clinical reasons;

d. life expectancy less than 24 months;

e. anticipated lack of cooperation or inability to complete questionnaires.

f. pregnant women (confirmed by beta-human chorionic gonadotropin testing), breastfeeding individuals, those planning pregnancy during the study, or fertile women who refuse to use a highly effective contraceptive method during the study are also excluded. Female subjects are considered fertile after menarche and until menopause unless they are permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A post-menopausal state is defined as the absence of menses for 12 months without an alternative medical cause. Highly effective contraceptive measures include: hormonal contraception with the use of combined formulations of estrogen and progestogen (oral, intra-vaginal or transdermal), which are associated with inhibition of ovulation,

  - progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable or implantable),
  - intrauterine device (IUD),
  - intrauterine hormone releasing system (IUS),
  - bilateral tubal occlusion,
  - vasectomized partner,
  - sexual abstinence.
- severe locomotor disability or prolonged immobilization,
- participation in another clinical study within the last 3 months,
- overt post-thrombotic syndrome with "Villalta score"(44) >4,
- chronic lymphedema of the lower limbs,
- recent (< 3 months) or planned phlebo-surgical intervention or percutaneous arterial trans-luminary angioplasty,
- ongoing dialysis treatment,
- state of malabsorption/malnutrition,
- chronic and non-suspendable use of phlebotropics, steroids or non-steroidal anti-inflammatory drugs, double anti-aggregation or aspirin at a dose greater than 160mg once-daily, centrally acting painkillers,
- Contraindications to the use of mesoglycan:
  - hypersensitivity to the active substance or to any of the excipients (lactose monohydrate, corn starch, croscarmellose sodium, magnesium stearate, gelatin, titanium dioxide, erythrosine).
  - galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption;

- hypersensitivity to heparin and heparinoids;
- diathesis and haemorrhagic diseases.

10. Premature Withdrawal

Enrolled and randomized subjects will permanently exit the study upon the occurrence of any of the following events:

- occurrence of one of the primary endpoints (before the end of follow-up),
- occurrence of one of the exclusion criteria,
- a serious adverse event (SAE) that fulfills one of the exclusion criteria,
- withdrawal of consent to participate in the study at any time and for any reason,
- if the Investigator deems it necessary in the interest of the patient or the patient is no longer compliant with the study procedures (e.g. unavailability for scheduled follow-up visits) or in the case of a female patient who becomes pregnant,
- the breaking of the masking of the treatment assignment.

Discontinuing study treatment for reasons other than the listed eventualities will not be a valid reason for the patient to withdraw from the study. In such cases (e.g., treatment interruption due to intolerance without meeting an exclusion criterion), the patient observation will continue according to the protocol.

Subjects suspended prematurely will not be replaced.

11. Treatments

a) Investigational drugs

- *Investigational drug*: mesoglycan, currently approved for the treatment of chronic venous ulcers. It will be administered in oral hard capsules, 50 mg each, at a dosage of 1 capsule twice-daily.

- *Reference therapy*: placebo, in the form of hard capsules identical to those of the active drug and containing the same excipients, at a dosage of 1 capsule twice-daily.

- *Duration of treatment*: until one of the study exit conditions described in point 10 occurs or, failing that, until the end of the 12th month from the date of randomization.

- *Masking*: The staff responsible for conducting the study at the various participating Clinical Centers, the enrolled subjects, and the CRO staff monitoring the study will be unaware of the assigned treatment (double-blind). The study treatment packages will be identified only by a label bearing a numerical code that does not contain any references that could identify the contents. The link between the numerical code and the actual content will be known solely to the person responsible for packaging the study treatment on behalf of the Sponsor and to the CRO data manager, who will store this information securely. It will only be revealed at the end of the data analysis, after the study concludes.

The Sponsor Pharmacovigilance Service and the CRO staff involved in the study will have an emergency access to the treatment assigned to each individual patient (not to the entire randomization list) via a computerized system that supports the eCRF and also performs the patient-treatment package code matching based on the randomization list (see chapter 15).

Each access will be logged with the applicant ID, date, and reasons for requesting the blind breakage. All accesses will be included in the final report of the study.

This access may be used, in the case of the Sponsor, only to fulfill the pharmacovigilance obligations related to the management of Suspected Unexpected Serious Adverse Reactions (SUSARs) (see point 17); in the case of the CRO, to respond to any requests from investigators to understand the nature of the assigned treatment. This request may only be made in an emergency when knowledge of the ongoing experimental treatment is necessary for diagnosis and/or therapy.

If an Investigator or other clinical center personnel responsible for patient evaluation becomes aware of the treatment assigned to a specific patient (unblinded), the patient should be considered prematurely suspended from the study.

Each Center will keep an accounting of the drug under study for each patient (including quantity delivered, consumed, and returned) using a specific form.

b) Concomitant and subsequent treatments

As specified in the study exclusion criteria, concomitant use of anticoagulants, double anti-aggregation, or aspirin at a dose greater than 160 mg once-daily, phlebotropics, steroids, centrally acting painkillers, and NSAIDs for continuous use during the 12 months of study drug intake and the following 12 months of follow-up is not permitted. Occasional use of NSAIDs for symptom relief is permitted.

The use of GCS, even without solid evidence from the literature, is left to the discretion of the Investigator.

12. Primary efficacy end-point

Occurrence during the observation period of the first of the following events:

- Symptomatic recurrence or extension of SVT, objectively confirmed with CCDU, with photographic diagnostic documentation to be attached to the eCRF.

- new DVT (asymptomatic or symptomatic proximal, symptomatic isolated distal), objectively confirmed with CCDU, with photographic diagnostic documentation to be attached to the eCRF.

- new PE (fatal/symptomatic non-fatal), confirmed by CT angiography, with textual and photographic diagnostic documentation to be attached to the eCRF.

13. Secondary Efficacy End-Points

1. Occurrence during the treatment period with the study drug of recurrence or extension, symptomatic or asymptomatic, of CCDU objectively confirmed SVT, whose photographic diagnostic documentation will be attached to the eCRF.

2. Occurrence during the follow-up period of the first of the following events:

    - recurrence or extension, symptomatic or asymptomatic, of CCDU objectively confirmed SVT, the photographic diagnostic documentation of which will be attached to the eCRF.

    - new proximal or distal DVT (asymptomatic or symptomatic), objectively confirmed with CCDU, whose photographic diagnostic documentation will be attached to the eCRF.

    - new PE (fatal / symptomatic non-fatal), confirmed by CT angiography, whose textual and photographic diagnostic documentation will be attached to the eCRF.

3. Change in the rVCSS score (34,35,36), validated as a clinical evaluation indicator of treatment efficacy (annex 1, Italian version), during the study period,

4. Modification of the VEINES/Sym-QoL(37,38,39) scores, validated indicators of the subject's satisfaction and quality of life (annex 2, Italian version), during the study period,

5. Evaluation, at the end of the treatment period, of the recanalization of the affected vein during the primary thrombotic event (relative increase of the recanalized venous lumen, by means of CCDU), with photographic documentation attached to the eCRF.

6. Evaluation, at the end of the treatment period, of new development of deep and/or superficial venous reflux (by means of CCDU), with photographic diagnostic documentation attached to the eCRF.

7. Assessment of the impact of GCS on primary and secondary efficacy end-points.

8. Assessment of how well CRP levels predict the onset of the primary end-point.

14. Prmary safety end-points:

Serious adverse events and total adverse events. Adverse events of a hemorrhagic nature will be classified as "major" if they involve fatal and/or symptomatic hemorrhage in a critical area or organ (e.g., intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, pericardial, and intramuscular with associated compartment syndrome); and/or if there is hemorrhage due to a drop in hemoglobin of at least 20 g/L or requiring transfusion of two or more units of packed red blood cells (ISTH Guidelines 2012) (45).

15. Phases of the study

The flow-chart (Annex 3) and Table 2 (point 20), describe the phases and procedures of the study:

- ***Collection of written Informed Consent***:

When requesting informed consent, the Investigator will inform the patient that participation in the study is voluntary and that refusing to participate will not result in any loss of benefits or affect the relationship with the physician. Additionally, it will be made clear that withdrawal from the study can be done at any time without needing to provide a specific reason. Before enrollment, each participant will receive a thorough explanation of the nature and purpose of the study from the Investigator, along with details about the benefits and risks of participation. Insurance coverage will also be discussed, and procedures in case of injury will be explained. A comprehensive information sheet covering all key aspects of the study will be provided to the participant. After reading it, the participant will have the opportunity to ask questions and will be given ample time to consider the information before being asked to sign and date the informed consent form. The Investigator will keep the original signed and dated consent form in the "Investigator Study File" at the Center. The participant will also receive a copy of the signed and dated consent form for their records.

- ***Screening***

After obtaining written informed consent, all potential subjects will complete the screening visit. If they meet the pre-set inclusion and exclusion criteria, they will be enrolled in the study and will undergo:

  - bilateral venous CCDU of the lower limbs (standard clinical practice investigation), for:

- exclude concomitant deep venous system involvement or initial SVT extension, which would indicate the need for extended parenteral anticoagulant therapy.
- check for the presence of deep and/or superficial venous reflux.
- blood sampling for the determination of blood counts, ALT, AST, creatinine, PT-INR, aPTT and PCR. These tests will be performed by the local laboratory at each participating Center.

- ***Randomization***:

It will be centralized through a specific application of the EDC platform, which is based on a pre-generated randomization list created using validated software (nQuery v7). The allocation will be 1:1 between placebo and mesoglycan and stratified by age (<60 / ≥60 years) and gender (male/female). Randomization blocks will vary in size (4 or 6 units). Patient enrollment in the study is expected to be completed within 24 months.

- ***Treatment***

It will last for 12 months or until the patient leaves the study, whichever comes first. Each study participant will have scheduled follow-up visits at 3, 6, 9, and 12 months after enrollment, including the following:

- global clinical assessment,
- compliance with processing,
- concomitant therapies,
- collection of information on thromboembolic events and/or adverse events,
- determination of rVCSS and VEINES/Sym-QoL scores,

- at the 12-month check-up, or at the time of the patient's exit from the study, whichever comes first: CCDU and blood sampling for blood count, ALT, AST, creatinine, PT-INR, aPTT, PCR.

- ***Follow-up***

It will have a minimum duration of 12 months from the end of treatment and will end at the closure of the study. Each study participant will undergo a scheduled follow-up visit after 24 months of enrollment, including the following:

- global clinical assessment
- therapies carried out
- collection of information on thromboembolic events and/or adverse events
- determination of rVCSS and VEINES/Sym-QoL scores
- blood sampling for blood count, ALT, AST, creatinine, PT-INR, aPTT

During the treatment period, in addition to the scheduled clinical visits, trained personnel will contact enrolled subjects by phone at 30, 60, 135, 225, and 315 days (± 5 days) after randomization to assess:

- the occurrence of adverse events and/or study endpoints,
- compliance with treatment,
- concomitant therapies and possible use of drugs not allowed.

During the follow-up period, in addition to the clinical checkup already scheduled at 12 months, the enrolled subjects will be contacted by trained personnel by phone at 3, 6, and 9 months after the end of treatment to evaluate:

- the occurrence of adverse events and/or study endpoints,
- therapies carried out and possible use of drugs not allowed.

- ***End of the study***

The study will conclude when the last enrolled subject completes the scheduled follow-up visit at 24 months after enrollment, and the related data will be stored in the eCRF.

The Sponsor may terminate this study early, either entirely or at any study site, for reasonable and documented reasons, with written notice provided in advance of the intended termination. The investigator may also end the study at their site for reasonable reasons, after giving written notice to the Sponsor before the planned end. Neither party is required to give notice if the study is interrupted due to safety concerns. If the Sponsor terminates the study for safety reasons, they must notify the investigators immediately by telephone and then provide written instructions for closing the study. Reasons for early closure of a participating site by the Sponsor or Investigator may include, but are not limited to, the failure of the Investigator to follow the protocol, local health authority requirements, or Sponsor procedures; or inadequate patient recruitment.

16. Diagnosis and adjudication of efficacy end-points

Enrolled subjects will receive instructions to present themselves at the centers after telephone contact, even outside scheduled visits, if symptoms or signs suggestive of VTE events occur during the treatment period or in the subsequent follow-up.

A suspicion of DVT and/or SVT arising during the treatment or afterward will be confirmed by CCDU based on the following findings:

- new superficial venous incompressibility in territory previously free of SVT or proximal progression of the initial SVT, both extended for at least 5 cm, but not beyond 3 cm from the saphenofemoral or saphenous-popliteal junction,
- new incompressibility of the proximal (common femoral vein, superficial and deep femoral vein, popliteal vein, large and small saphenous vein less than three cm from the sapheno-femoral or saphenous-popliteal junction) and/or distal (anterior and posterior tibial veins, peroneal veins, twin and soleal veins) deep venous circulation.

Suspected PE will be confirmed by CT angiography of the pulmonary circulation based on the presence of an intraluminal filling defect affecting the main, lobar, or segmental branches of the pulmonary artery. Additionally, a CCDU of the lower limbs will be performed.

Patients who remain asymptomatic for SVT and/or DVT until the end of the treatment period will undergo a CCDU at the 12-month follow-up. Any detection of an asymptomatic SVT and/or DVT will be based on the criteria outlined above.

A copy of the report and diagnostic images of the events must be sent to the coordinating center for "blinded" validation by the "Independent DSMC."

In case of death of a patient, the event will be determined based on the autopsy report, if performed, or in its absence, on a clinical basis.

The criteria for classifying hemorrhagic adverse events will follow the internationally standardized ones (see point 14).

17. Safety and Adverse Events

The safety assessments (safety variables) of the study will be:

- vital signs (blood pressure, heart rate) collected at each study visit and at an additional follow-up at the patient's request or on the recommendation of telephone follow-up,

- medical-angiological examinations (general and segmental on the occasion of each study visit and on the occasion of an additional check-up at the patient request or on the recommendation of the telephone follow-up,
- haematological laboratory tests (haemoglobin, haematocrit and platelets) and biochemical tests (AST, ALT, PT-INR, aPTT and CRP) at the baseline visit and at the 12 and 24-month check-up and at an additional check-up at the patient request or on the recommendation of the telephone follow-up. All female patients of childbearing potential will have the beta-HCG test carried out at the baseline visit.
- Telephone follow-up: during the study, in addition to the clinical checks already planned, the enrolled subjects will be contacted by telephone by trained personnel at a distance of 30, 60, 135, 225, 315 days (± 5 days) from randomization and 3, 6 and 9 months after the end of treatment and then every 6 months until the end of the study to assess the occurrence of adverse events and/or end-points of the study, compliance with treatment and concomitant therapies and possible use of drugs that are not allowed.
- adverse events

17.1 *Adverse Events, Definitions*

- *Adverse event (AE)* means any harmful clinical occurrence that occurs in a patient or a clinical trial subject administered a medicinal product and that does not necessarily have a causal relationship with the treatment.
  An adverse event may therefore be a harmful and undesirable sign (e.g., tachycardia, liver enlargement, including an abnormal laboratory test result), a symptom (e.g., nausea, chest pain), or illness, regardless of the judgment of causal relationship with the study treatment. Conditions that pre-exist the patient entry into the study are excluded from the concept of AE.
- *Adverse reaction (ADR)*: any AE that is believed to be related to the study treatment. The definition implies the reasonable possibility of a causal relationship between the AE and the study treatment.

The causal relationship will be assessed by the Investigator in a dichotomous manner (relationship at least possible / relationship excluded). The definition of ADR also includes administration errors and use of the study drug other than described in the protocol, including inappropriate use, overdose and abuse.

- *Serious adverse event (SAE)* means any AE that meets one or more of the following criteria:
  - Fatal outcome
  - It endangers (in the immediate) the life of the subject
  - Requires hospitalization or prolongs current hospitalization*
  - Provokes persistent or significant disability or incapacity
  - Causes a congenital anomaly or birth defect
  - It is considered an important medical event in the judgment of the Investigator (i.e. an event that requires active intervention to prevent any of the characteristics/consequences mentioned above).

    **An AE is not considered a SAE if hospitalization a) was already scheduled prior to the subject enrollment or b) if it did not result in the subject overnight stay.*

- *Serious adverse reaction (SADR)*: any SAE that is believed to be related to the study treatment.

- *Serious and unexpected adverse reaction (SUSAR*): means a SADR that is unexpected because it is inconsistent, in nature, severity or outcome, with the reference information on the safety of the investigational medicinal product. This information consists of the Summary of Product Characteristics (SmPC) for the medicinal product mesoglycan in the case of the present study.

17.2 *Collection, Evaluation, and Recording of Adverse Events (AEs)*

At each visit scheduled according to the protocol, the patient will be asked to report any signs or symptoms that have arisen since the previous visit. The patient will

also be instructed to contact the Centre promptly if new signs or symptoms occur between visits. The Investigator must in fact ensure the collection of all AEs that occurred to the patient during his stay in the study, and not only of the events observed/reported during the scheduled visits.

The Investigator will assess whether a certain medical condition observed in or reported by the subject can be considered an AE based on the interview with the patient, the physical examination, and if necessary on the laboratory tests and instrumental examinations of the case.

When the Investigator has identified an AE, he or she will record it in the outpatient medical record and in the appropriate section of the *Electronic Data Capture (EDC) platform*. In this section of the EDC the Investigator will describe the AE either with a free description ("verbatim") or with a coding according to standard medical terminology (MedDRA).

In addition, the Investigator for each AE will define:

- whether it is serious (indicating the criterion(s) of seriousness that characterizes it - see section 17.1) or not serious;
- the degree of intensity (not to be confused with the "seriousness" mentioned above)
    - *mild* – perception of disorders that do not interfere with normal daily activities
    - *moderate* - presence of disorders that create discomfort and interfere with normal daily activities
    - *severe* – presence of disorders that make it impossible to perform normal daily activities;
- whether or not it is related to the study treatment (at least possible relationship / excluded relationship).

Time Period for Collection and Recording of Adverse Events (AEs)

The Investigator will collect and record each AE arising from the signing of informed consent until the end of the clinical trial follow-up period.

The Investigator will follow all recorded AEs until they are resolved or until, in his or her judgment, it is no longer possible to have further information on both the event and the final outcome.

After the closure of the clinical trial, the Investigator will notify the Sponsor of any new SADRs, or any new information relating to previously registered AEs, of which he/she becomes aware.

Pre-existing clinical conditions

The clinical conditions present at the time of signing the informed consent will be recorded as "pre-existing" in the appropriate section of the EDC platform.

Any new clinical condition, or worsening of a pre-existing clinical condition, arising after the signing of the informed consent, will be recorded as AEs, with the exception of clinical events identified in this protocol as efficacy endpoints.

Abnormal laboratory values

Abnormalities in laboratory values that require clinical intervention (i.e., therapeutic measures) or further investigation (other than simple retesting) or that are considered clinically significant by the Investigator, will be recorded as AEs.

Pregnancies

If pregnancy occurs, the patient will stop treatment and prematurely end her participation in the trial. Pregnancy by itself is not considered an AE unless there is a suspicion that the investigational treatment interfered with the efficacy of contraceptive systems.

In any case, the pregnancy must be registered and must be followed until its conclusion.

Efficacy end-point

Clinical events identified in this protocol as efficacy endpoints will not be recorded as AEs.

17.3 Adverse Event Reporting

Serious adverse events (SAEs)

The Investigator is required to register each SAE in the EDC platform within 24 hours of becoming aware.

For each registered SAE, the EDC platform will automatically send an alert email to the Sponsor and the designated CRO.

The CRO will verify the consistency of the reported data and send the SAE reporting form to the Sponsor within 24 hours of the Investigator recording of the event. In case of deficiencies in the information on the SAE, the CRO will contact the Investigator to obtain an update which will be transmitted to the Sponsor within 3 calendar days of the first report.

If the SAE is fatal or has endangered the patient life, the CRO will contact the Investigator to obtain a detailed clinical report on the event, to be transmitted to the Sponsor within 24 hours of receipt.

Previously recorded SAE follow-up information will follow the same timing as the initial information.

Non-serious adverse events (non-SAE)

The Investigator is also required to record in the appropriate section of the EDC all non-SAE that occurred during the clinical trial (including the follow-up period).

Pregnancies

Any cases of pregnancy, and any new information on pregnancies already reported, must be registered and consequently transmitted to the CRO and the Sponsor within 24 hours of becoming aware of it.

17.4 Promoter's Liability

The Trial Sponsor will record all SAEs received through the CRO in its Pharmacovigilance database.

SUSAR

The Sponsor will determine which of the SAEs considered to be related by the investigators are to be considered unexpected and therefore constitute serious and unexpected adverse reactions (SUSARs).

The Sponsor will transmit the SUSARs to the National Competent Authority (AIFA) by inserting them into the *EudraVigilance Clinical Trial Module* (EVCTM) system:

- within 7 days from the time the Investigator inserts them into the EDC for fatal or life-threatening SUSARs
- within 15 days from the time the Investigator inserts them into the EDC for all other SUSARs.

The Promoter will also send each SUSAR in CIOMS-I format to the CRO, which in turn will transmit them to the Ethics Committee of the coordinating Centre, within the same timeframe as above.

The Sponsor will also periodically send cumulative lists of SUSARs to the CRO, which in turn will transmit them to all participating Investigators.

Development Safety Update Report (DSUR)

Once a year for the duration of the clinical trial, the Sponsor will prepare a safety report (DSUR) in accordance with the format established by the "ICH guideline E2F on Development Safety Update Report" and will transmit it to the National Competent Authority (AIFA) within 60 calendar days of the applicable "Data Lock Point".

The Sponsor will also send the DSUR to the CRO for forwarding to all Ethics Committees involved in the trial within 60 calendar days of the "Data Lock Point".

17.5 Management of double blindness

Procedures for addressing blindness in patients receiving treatment are outlined in point 11. These procedures adhere to the European guideline "CT-3" on Pharmacovigilance from clinical trials (2011/C 172/01).

17.6 Data and Safety Monitoring Committee

During the clinical trial, the "Data and Safety Monitoring Committee (DSMC)" will arrange periodic sessions (at least every six months) for safety monitoring. To this end, the CRO will provide the DSMC with a tabular description of the frequencies and types of adverse events observed, stratified by treatment group but without its label, i.e., without indication of the nature of the treatment assigned to each group (blinded). In the event of an evident imbalance in the safety profile between the two treatment groups, the DSMC may recommend to

the Sponsor and the Study Coordinator that the study be terminated prematurely (see section 6 for details).

18. Electronic Data Collection

An "Electronic Data Capture" (EDC) system based on "Research Electronic Data Capture" (REDCap, produced and distributed by Vanderbilt University and the "REDCap Consortium") will be used for data collection at the participating centers.

The eCRF (clinical data collection forms) can be reviewed as a document that accompanies the protocol.

Data protection

The personal data of enrolled subjects will be handled with the highest confidentiality and in accordance with Legislative Decree 211/2003 and subsequent amendments and additions. Specifically, the personal data will be accessible only to the Investigator and their collaborators who oversee the patient and obtain their consent for treatment. Each enrolled subject will be identified by a unique numerical code.

The use and maintenance of the REDCap platform are overseen by an administrator who manages users with a flexible and detailed authorization system. REDCap enforces permissions for each user who accesses it through a web browser using SSL encryption (with a personal "username" and "password"), enabling certain functions, tabs, links, and buttons based on the granted privileges and the group or center they belong to.

REDCap provides a comprehensive "audit" of user procedures by recording all data activities, including viewing and exporting. The operational control log (log) notes the date, time, and user performing each action, enabling full review and remote monitoring of the clinical study if needed.

All users with access to the EDC platform, including Investigators, their staff, CRO staff, and the Sponsor Pharmacovigilance Team, must have attended a training session to ensure the reliability, quality, and integrity of the data entered into the EDC platform.

REDCap implementations ensure compliance with the most common industry standards, EMA (GCP, Privacy-IT: D.L. 211/2003 and subsequent amendments), and FDA (21-CFR2-Part 11) requirements.

Every effort is made to ensure compliance with the "GCDMP" (Good Clinical Data Management Practice, published by the Society for Clinical Data Management, 2013) guidelines, particularly regarding the reliability, quality, integrity, and security of the data recorded in the EDC platform, both from procedural and IT perspectives, using state-of-the-art solutions.

19. Statistical analysis plan and methodology

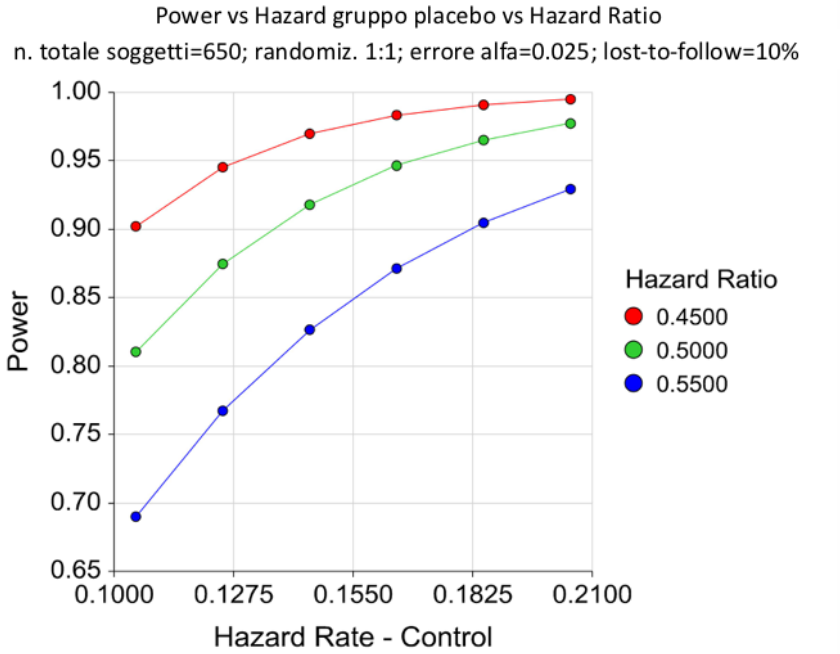
Calculation of Sample Size

Based on data available in the literature (see introduction and particularly bibliographic references 3, 5, 17), an assumed risk of 15% for the primary composite end-point in subjects in the placebo group is considered (meaning 85% of subjects remain event-free), along with a 50% reduction in risk for subjects treated with mesoglycan (Hazard Ratio, HR = 0.5).

A bilateral log-rank test with an overall sample size of 650 subjects (325 in the placebo group and 325 in the mesoglycan group) achieves 90% power at a significance level (alpha) of 0.025 to detect a 7.2% difference between 15.0% and 7.8% (the proportions of subjects with primary endpoints in the placebo and mesoglycan groups) after 12 months of treatment. This corresponds to a HR of 0.50. Subjects will be enrolled over a 24-month period, with 50% of enrollments completed by month 12. The calculation considers a 10.0% dropout rate during follow-up in both the placebo and mesoglycan groups.

Then, 650 subjects will enrolled and randomly assigned in a 1:1 ratio to the two groups: placebo and mesoglycan.

This number appears large enough to ensure the study has adequate "power" (>0.80), even if the risk of observing the primary end-point in the placebo group (Hazard Rate-Control) and the efficacy difference between the two treatments (Hazard Ratio) are significantly different from those hypothesized (see simulation graph).



However, an "interim analysis" is prepared once 75% of the expected subjects (488) are enrolled so that the DSMC can potentially adjust the number of subjects to be enrolled ("re-sampling") to achieve the study predetermined 90% power if the observed number of primary endpoint events significantly deviates from the expected for the two groups. This is particularly relevant if the deviation of HR from the planned value (50%) exceeds 5% in absolute terms, though a smaller deviation appears tolerable based on the above simulation. The alpha error is therefore conservatively set at 0.025 using the O'Brien-Fleming method.

For the interim analysis, a table showing the frequency of the primary efficacy end-point will be provided to the DSMC, broken down by treatment group but without labeling the groups (i.e., without identifying the treatment assigned to each group).

Statistical considerations

All variables will be analyzed descriptively using appropriate statistical methods: categorical variables will be examined with frequency tables, and continuous variables will be summarized with sample statistics (e.g., mean, median, standard deviation, minimum and maximum values, 25th and 75th quartiles).

All statistical tests will be two-tailed and conducted at a significance level of 5%, unless otherwise specified.

All efficacy and safety analyses, both primary and secondary, will be conducted in the modified intention-to-treat (ITT) population, which includes all randomized subjects who have received at least one dose of study drug.

An additional supportive analysis will be conducted on the "per-protocol" population, which includes all subjects who were randomized and compliant with the treatment, meaning they took at least 65% of the expected dosage units of the study drug.

Subjects who are randomized and have taken any amount of the study drug will not be replaced for any reason. Subjects who are randomized but revoke consent prior to receiving the study drug may be replaced, although they will not lose their unique identification code.

Subjects who leave observation without reaching the end-point will be considered censored and will remain at risk during the observation period.

For subjects who do not attend clinical evaluations at 12 and 24 months, every effort will be made to determine a potential outcome of interest for the study and to make them assessable for the end-points.

Treatment compliance

Oral treatment compliance can be used as a stratification variable (<65% and >= 65%) and is defined as:

Compliance = (total number of tablets actually taken / total number of tablets expected) * 100.

The count will be based on the individual accounting of the drug mentioned in point 11.

Final analysis of the primary end-points

The primary cumulative efficacy end-point will be the occurrence of the first event among the considered event types (see point 12). The hypothesis of superiority of the primary cumulative end-point of efficacy will be tested using multivariate Cox regression for proportional hazards, with covariates including the assigned drug, age at randomization, gender, treatment compliance, and additional risk factors for SVT and DVT/PE that are significantly associated in univariate analysis (such as obesity, varicose veins, cancer, orthostatism or prolonged immobilization, recent surgery, serious infections, previous DVT/PE, thrombophilia, chronic heart failure NYHA III-IV, and use of estrogen-progestogens). All variables participating in the model will be assessed for the assumption of proportionality of hazards using the standard graphical approach. A subset of variables will be selected based on the Wald method and “forward stepwise".

The crude and adjusted cumulative percentage of event-free participants over time in each treatment group will be depicted by Kaplan-Meier curves.

Final analysis of secondary endpoints

For the analysis of secondary efficacy end-points, it will be used:

1. the Kaplan-Meier estimate of cumulative occurrence over time and the log-rank test used to compare the two treatment groups,

2. For the rVCSS scores, analysis of variance will be used for repeated measures (all available assessments for intra-subject effects and the two treatment groups as a between-subject effect).

3. For VEINES/Sym-Qol scores, analysis of variance will be used for repeated measures (all assessments available for intra-subject effects and the two treatment groups as an inter-subject effect).

4. For the relative increase in venous recanalization, a repeated measures ANOVA will be used for the analysis of variance (the two assessments for intra-subject effects and the two treatment groups as the inter-subject effect).

5. The development of reflux in the two treatment groups will be tested using the chi-square test.

6. The impact of GCS at different end-points will be assessed by including it as a stratification factor in the related analyses.

7. The predictive value of CRP levels, divided into tertiles, on the primary efficacy endpoint will be assessed by including it as a stratification factor in the related analyses.

Final analysis of safety endpoints

Analysis for the primary safety end-points will be conducted using the continuity-adjusted chi-square test.

Standard summaries of safety data concerning AE and notable changes in laboratory data will also be provided.

Final report and publication of results

The study results will be included in a final integrated report that combines statistical and clinical data, following GCP-ICH guidelines. The content of the report will be jointly approved by the study Clinical Coordinator and the Sponsor.

The same joint approval will cover the contents of the initial publication and the first public presentation of the study results, which the Sponsor commits to carry out even if the evidence is negative. Any publications and/or secondary presentations will be notified in advance by the Coordinator to the Promoter or vice versa, with the right for the recipient to comment.

20. Study Procedures

Flow of procedures	Screening	3 months	6 months	9 months	12 months, end of treatment, o early exit	24 months
Date of visit	X	X	X	X	X	X
Information and consent	X					
Demographics	X					
Subject selection	X					
Clinical evaluation	X	X	X	X	X	X
CCHR	X				X	
Randomization	X					
Safety Assessment	X	X	X	X	X	X
Treatment compliance		X	X	X	X	
Blood sampling	X				X	X
Collection of scores (rVCSS and VEINES)	X	X	X	X	X	X
Study drug distribution	X	X	X	X		
Collection of concomitant treatments	X	X	X	X	X	X

21 Ethical aspects and good clinical practice

The latest revision of the Declaration of Helsinki and the Oviedo Declaration form the basis for the ethical conduct of the study.

The study protocol is designed and will be carried out to ensure compliance with the principles and procedures of Good Clinical Practice and to meet Italian laws, as outlined in the following documents and accepted by the study investigators.

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice (http://www.ich.org/products/guidelines.html)

2. REGULATION (EU) No 536/2014 on clinical trials on medicinal products for human use

3. Legislative Decree no. 211 of 24 June 2003.

4. Legislative Decree no. 200 of 6 November 2007.

5. Ministerial Decree of 21 December 2007.

6. AIFA Resolution 20 September 2012

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