Title Page

A Prospective, Single- Center, Phase IV Interventional, Single Arm **T**rial for the **E**valuation of subcutaneous recombinant **H**irudin 15 mg (RB variant) in prophylaxis of Deep Vein Thrombosis (**DVT**) post major orthopedic operations

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Sub Investigator: Dr. Abdallah Hammad

Study Site: Alexandria University

Protocol Name: THRIVE-DVT

Protocol Number: Sub-Thromb-001

Version:1.0, amendment 1

Date: 25th of January, 2022

1. Synopsis

Title	Description
Study Title	Prospective, Single- Center, Interventional, Single Arm Trial for the Evaluation of subcutaneous recombinant Hirudin 15 mg (RB variant) in prophylaxis of Deep Vein Thrombosis (DVT) post major orthopedic operations
Study number	Sub-Thromb-001
Study Phase	Phase IV
Study Design	Prospective, Single arm, single-center
Study population	Patients undergoing major orthopedic operations
Study procedures	Drug: r-Hirudin Other Name: Thrombexx **100** Patients will be treated with r-Hirudin (Thrombexx) for a total of **15 days** beginning with **15 mg BID** s.c starting 6 hours after surgery or upon adequate hemostasis and continued until end of study.
Clinical & laboratory investigations	**For Events of Deep Vein Thrombosis (DVT)** within 15 days post-op.) All of the patients underwent Routine bilateral compression Doppler. **For Clinical PE (Pulmonary Embolism)** Events from Day of surgery and During Hospitalization period or end of study Clinical PE events PE (Pulmonary Embolism) events were confirmed by spiral CT. **For follow up:** APTT should be done before the first dose then after 4 & 8 hours of the first dose, then on days 1,8,15 post operatively.
Study duration	**Enrolment period:** 3 months duration **Follow-up duration:** Follow-up per patient will be for a period of 15 days
Eligibility Criteria	**Inclusion Criteria:** 18 years of age or older Body Weight >60 kg Patients undergoing major orthopedic operations

	Patients ready to sign informed consent form (ICF) Patients should discontinue any agents that affect haemostasis prior to the study medication use unless strictly indicated. These agents include medications such as: anticoagulants, thrombolytics, non-steroidal anti-inflammatory agents (including Ketorolac tromethamine), preparations containing aspirin, systemic salicylates, ticlopidine, dextran 40, clopidogrel, other anti-platelet agents including glycoprotein IIb/IIIa antagonists or systemic glucocorticoids. **Exclusion Criteria:** Significant bleeding injury such as solid organ laceration or intracranial bleed at discretion of attending physician Hypersensitivity to Hirudin or prior documented Allergy to its components Pregnant or breast feeding Hemorrhagic stroke in preceding 3 months abnormal baseline coagulation characterized by an INR >1.4, obtained at the discretion of the treating clinician Required therapeutic anticoagulation for atrial fibrillation, prior VTE, or mechanical heart valve Patients with a history of coagulation disorder Treatment with concomitant anti-platelet agent other than aspirin 326 mg or more daily (Platelet count< 100 X109 /dl) Active bleeding Subjects with a life expectancy less than 1 month
Study objective	The primary objective of this study is to evaluate the efficacy of r-Hirudin RB variant 15 mg in DVT prophylaxis post major orthopedic operations The secondary objective of this study is to evaluate the safety of r-Hirudin RB variant 15 mg in DVT prophylaxis post major orthopedic operations in terms of serious bleeding.
Study endpoint	**Primary endpoint:** Primary end points included new onset symptomatic thrombosis requiring medical or surgical intervention or death due to thrombosis defined as fatal PE, ischemic stroke, mesenteric thrombosis, or myocardial infarction. **Secondary endpoint:** The Secondary end point of major bleeding is defined as clinically evident hemorrhage associated with a

	hemoglobin decrease ≥ 2 g/dL that leads to a transfusion of ≥2 units of whole blood or packed red cells outside of the perioperative period (time from the start of the surgery or procedure and up to 12 hours after), or hemorrhage that is intracranial, retroperitoneal, or into a prosthetic joint. Recording serious adverse events (SAE)/ adverse events (AE) during the study duration.
Study medication	**Thrombexx ;** Because thrombin plays a central role in thrombogenesis, the goal of most treatment regimens is to block thrombin generation or inhibit its activity. 1- r-Hirudin binds directly with thrombin to its active & fibrinogen binding site. 2- r-hirudin does not require access to heparin binding site & can thus neutralize clot bound thrombin. Hence, down regulating thrombin generation & potentially enhancing early revascularization. Direct thrombin inhibitors were developed to overcome the inability of the heparin/ antithrombin complex to inactivate bound thrombin. In contrast to heparin and low-molecular-weight heparin, which catalyze the inactivation of thrombin and activated factor X (factor Xa) by antithrombin, direct thrombin inhibitors bind to thrombin and block its interaction with its substrates. II- They do not bind to plasma proteins; direct thrombin inhibitors produce a more predictable anticoagulant response than heparin. III- Unlike heparin, direct thrombin inhibitors do not interact with platelet factor 4 or high-molecular-weight multimers of von Willebrand factor. Consequently, the activity of direct thrombin inhibitors in the vicinity of a platelet-rich thrombus is not compromised. IV- Finally, thrombin bound to fibrin or fibrin degradation products is susceptible to inactivation by direct thrombin inhibitors. Because the active site of thrombin is not involved in the interaction of the enzyme with fibrin, it remains accessible to active site-directed thrombin inhibitors. Consequently, these agents inactivate fibrin-bound thrombin without displacing the enzyme from fibrin. In contrast, bivalent thrombin inhibitors, such as Hirudin and bivalirudin, displace bound thrombin during the inhibition reaction by competing with fibrin for access to exosite 1 on thrombin.

	Dose: In DVT Prophylaxis: 15 mg twice daily, subcutaneous for 15 days In case of renal insufficiency, the dose is titrated because r-Hirudin is excreted renally. According to Creatinine clearance: •Creatinine clearance › 31-60 ml/min (5 mg s.c twice daily) •Creatinine clearance ‹ 31 ml/min (1.7 mg s.c twice daily)
Sample size	100 patients
Statistical analysis	- Descriptive analysis: All statistical tests used a significance level of P=0.05, two tailed tests are performed for all analyses used statistical testing. Descriptive analysis for quantitative data will include count, mean with 95% CI, standard deviation, median, minimum and maximum. For qualitative categorical variables; frequency and percentage and 95% confidence interval will be applied.
Stopping rules	DVT PE Major bleeding Allergy APTT value increases by 2-2.5 folds According to investigator evaluation

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2. LIST OF ABBREVIATIONS

Abbreviation	Term
APTT	Activated Partial Thromboplastin Time
AE	Adverse Event
CI	Confidence Interval
CRA	Clinical Research Associate
CRF	Case Report Form **DM**
	Data Management
DNA	Deoxyribonucleic acid
DTI	Direct Thrombin Inhibitor
DVT	Deep Vein Thrombosis
GCP	Good Clinical Practices
IRB/IEC	Institutional Review Board/Independent Ethics Committee
ICH	International Conference on Harmonization
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
INR	International Normalized Ratio
PE	Pulmonary Embolism
RB	Recombinant Bacteria
S.A.E.	Serious Adverse Event
VTE	Venous Thromboembolism

3. SIGNATURE(S) PAGE

Study Title: Prospective, single Center, Interventional, Open label Trial for the Evaluation of subcutaneous recombinant Hirudin 15 mg (RB variant) in prophylaxis of Deep Vein Thrombosis (DVT) post major orthopedic operations

Contact details of the person authorized to sign the protocol and amendments

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I have read this report and confirm that to the best of my knowledge it accurately describes he conduct and results of the study.

Medical Manager Signature: **Principal Investigator Signature:**

Date of Signature: **Date of Signature:**

4. INTRODUCTION

4.1. Background

Hirudin is the most potent naturally-occurring direct thrombin inhibitor (DTI), and the first parenteral anticoagulant used on humans.[1]

Originally derived from the medicinal leech (*Hirudo medicinalis*), it consists of a 65 amino acids polypeptide chain, forming non-covalent, equimolar, non-reversible 1:1 complexes with α thrombin.[1] When hirudin-bound, thrombin-catalyzed reactions and fibrinogen clotting are blocked, and coagulation is subsequently inhibited.[2] Hirudin was previously produced in limited amounts, however, recombinant DNA technology allowed its mass production.[3] These recombinant forms bind bivalent to thrombin with pharmacokinetic and anticoagulant profile similar to that of the native form.[4]

Recombinant Hirudin is a direct inhibitor of thrombin. It binds in 1:1 ratio with thrombin thus blocking the coagulation cascade and the formation of thrombi.[5]

Unlike heparins, the action of Hirudin is totally independent from any coagulation cofactor that may interfere or alter its action. [5]

Thrombexx is useful in the prevention of thromboembolic complications in at risk patients thrombexx (15 mg) is administered by subcutaneous injection 5-15 minutes before orthopedic or general surgery (after induction of regional block anasthesia) then 15 mg twice daily for 9-12 days and in bedridden patients until patient is fully ambulant (maximum 12 days) [5]

According to the FDA Therapeutic Equivalence Evaluation, 30th Edition, the biosimilar of recombinant hirudin, Desirudin is FDA approved, therefore, the same applies to recombinant hirudin in Egypt.

4.2. Correlative studies:

1- Study of Prevention of Thromboembolic with Use of Recombinant Hirudin, Results of a Double-Blind, Multicenter Trial Comparing the Efficacy of Desirudin (Revasc) with That of Unfractionated Heparin in Patients Having a Total Hip Replacement[6] which showed that desirudin to be more effective than heparin in the prevention of deep vein thrombosis, without any increase in the requirements for transfusion or the risk of hemorrhage. The prevalences of over-all and proximal deep-vein thrombosis in the 174 patients who had been managed with desirudin and were included in the per-protocol analysis were 7 and 3 per cent (thirteen and six patients), respectively.[6]

2- Multicenter Trial of Desirudin for the Prophylaxis of Thrombosis: An Alternative to Heparin-Based Anticoagulation (DESIR-ABLE) shows that no patients experienced primary endpoints which is major bleeding and no new onset of symptomatic thrombosis requiring medical or surgical intervention or death.[7]

4.3. Study Rationale

The purpose of this study is to conduct several investigations required to evaluate the efficacy and safety of subcutaneous Thrombexx ampoules in the prophylaxis of Deep Vein Thrombosis (DVT) post major orthopedic operations

5. STUDY OBJECTIVES

5.1. Primary Objectives

To evaluate the efficacy of Subcutaneous Thrombexx® ampoules (r-Hirudin RB variant 15 mg) in DVT prophylaxis post major orthopedic operations

5.2. Secondary objectives

To evaluate the safety of Subcutaneous Thrombexx® ampoules (r-Hirudin RB variant 15 mg) in DVT prophylaxis post major orthopedic operations in terms of serious bleeding.
Predictive factors; baseline characteristics, Thrombexx® dose, duration and concomitant medications.
Study population demographics and characteristics.

6. TRIAL DESIGN

A Prospective, Single- Center, Phase IV Interventional, Single Arm conducted in Egypt to assess the efficacy and safety of patients undergoing major orthopedic operations such as Total knee & hip Arthroplasty with complete data and who had 2 weeks' follow-up.

This study will be conducted through main phases, preoperative phase which will be as a screening visit, operative phase which is the surgery will be done and a total of 3 follow-up visits which is the postoperative phase.

1- Pre-operative phase

2- Operative phase

3- Post operative phase (follow-up)

Detailed descriptions of the study design are as follows:

6.1. The pre-operative Phase

During this phase, patients will be screened for fulfillment of the inclusion and exclusion. Patient's demography, history of diseases will be collected, laboratory investigations such as Hemoglobin count, Platelets count, APTT, INR, SGPT, SGOT, Serum albumin, serum Bilirubin, serum creatinine and random blood glucose and pregnancy test for females in childbearing period.

APTT should be done before IMP administration, in addition to the patient's data of Thrombexx® administration regimen decided by the treating investigator according to the standard clinical practice or as prescribed in the usual manner in accordance with the terms of the local marketing authorization with regards to dose, population and indication (and within the approved label).

Medical history and Medication history should also be collected.

Main complaint of the patient during routine physical examination should be recorded

6.2. The operative Phase

During this phase, patient's registries for the type of surgery done, Type of anesthesia, patient position and type of prosthesis, & blood transfusion during surgery should be recorded. And IMP dispensing 6 hours after surgery or upon adequate hemostasis will be done. APTT will be done 4 & 8 hours after the first dose

6.3. The Follow-up Phase

During this phase, which is approximately 15 days, PE which is confirmed by spiral CT chest, any new DVT appears also to be confirmed by Doppler ultrasound, Bleeding assessment, APTT will be done on days 1, 8 & 15, concomitant medications, Hospital stay and any adverse event data will all be recorded

7. FLOW CHARTS:

7.1. STUDY FLOW CHARTS:

Activity	Pre-operative phase (Screening)	Operative phase (Day 0)	Follow-up 1 (Day 1)	Follow-up 2 (Day 8)	Follow-up 3/ End of Study (Day 15)
ICF Signature	×				
Inclusion/Exclusion Criteria	×				
Demographic Data	×				
Medical History	×				
Vital signs	×				
Concomitant Medication	×				
Physical Examination	×				

Laboratory investigation	×		×	×	×
Type of surgery		×			
Assessment of bleeding		×	×	×	
IMP Administration		×	×	×	×
Assessment of DVT			×	×	×
Assessment of PE			×	×	×
Assessment of therapeutic response					×
APTT	×	×	×	×	×
Adverse Event		×	×	×	×
Study completion					×

7.2. STUDY PHASES

The study consists of 3 phases:

Visit 1: Preoperative phase (screening)

- Patient Screening (Check eligibility)
- Signed ICF
- Gathering Patient Information as Demographic, Vital Signs, BMI, Physical Examination, Medical History, Concomitant medications
- Laboratory Investigations (Hb, PLt count, APTT, INR, SGPT, SGOT, Serum Albumin, Serum Bilirubin, serum creatinine, Creatinine clearance, Random blood sugar)
- Patients main complaint (Knee, Hip)

Operative phase (Day 0)

Patient Data to be collected:

- Type , Date of surgery
- Type of anaesthesia
- Patient position
- Type of prosthesis
- Blood transfusion

Start of IMP administration.

APTT 4 & 8 hours after IMP administration

Visit 2: Post operative phase (Follow-up 1(Day 1)

- Assessment of bleeding
- Type of blood transfusions
- Assessment of DVT
- Routine bilateral compression Doppler (confirmation for DVT)
- Assessment of PE
- Spiral CT (confirming PE)
- IMP administration
- Laboratory investigations (Hb%, Platelets count)
- APTT

Assessment will be done in the hospital, as post-operative period before discharge from hospital.

Visit 3: Follow-up 2 (Day 8)

- Assessment of bleeding
- Type of blood transfusions
- Assessment of DVT
- Routine bilateral compression Doppler (confirmation for DVT)
- Assessment of PE
- Spiral CT (confirming PE)
- IMP administration
- Laboratory investigations (Hb%, Platelets count)
- APTT

Assessment in day 8 will be done from outpatient clinic in most patients.

Visit 4: Follow-up 3/ End of Study (Day 15)

- Assessment of bleeding
- Type of blood transfusions
- Assessment of DVT
- Laboratory Investigations
- Routine bilateral compression Doppler (confirmation for DVT)
- Assessment of PE
- Spiral CT (confirming PE)
- APTT
- End of Study

Assessment in day 15 will be done from outpatient clinic.

8. STUDY OUTCOME MEASURES

8.1. Primary Endpoints

Primary end points included new onset symptomatic thrombosis requiring medical or surgical intervention or death due to thrombosis defined as fatal PE, ischemic

stroke, mesenteric thrombosis, or myocardial infarction.

The number of clinical PE events will be measured by spiral CT

Mean changes in APTT where it will be done before first dose, 4 & 8 hours after first dose then on days 1,8,15 post operatively

8.2. Secondary Endpoints

Major Bleeding: was defined as clinically evident hemorrhage associated with a hemoglobin decrease ≥ 2 g/dL that leads to a transfusion of ≥2 units of whole blood or packed red cells outside of the perioperative period (time from the start of the surgery or procedure and up to 12 hours after), or hemorrhage that is intracranial, retroperitoneal, or into a prosthetic joint.

Number of any reported (AE) or (SAE) during the study duration.

9. STUDY POPULATION

This is a prospective study, it is important to have a well-defined study population in place prior to the start of the study. Study populations should be defined using specific inclusion and exclusion criteria, which will be used to evaluate a potential subject's ability to participate in the study.

9.1. Recruitment strategy:

It is essential that the recruitment process take into account factors that will optimize the type and number of participants enrolled in the study while minimizing time and expense. Failure to meet target accrual goals can affect the "power" of a study, making it less successful in providing quality results.

Recruitment plan will be through explaining to patients the clinical trial objective in Alexandria University hospital; referral also will be used to increase the flow rate of recruitment.

9.2. Inclusion Criteria

Patients aged 18 years of age or older

- Body Weight >60 kg
- Patients undergoing major orthopedic operations Total knee & hip Arthroplasty
- Patients ready to sign (ICF)

Patients should discontinue any agents that affect haemostasis prior to the study medication use unless strictly indicated. These agents include medications such as: anticoagulants, thrombolytics, non-steroidal anti-inflammatory agents (including Ketorolac tromethamine), preparations containing aspirin, systemic salicylates, ticlopidine, dextran 40, clopidogrel, other anti platelet agents including glycoprotein IIb/IIIa antagonists or systemic glucocorticoids.

9.3. Exclusion Criteria

Significant bleeding injury such as solid organ laceration or intracranial bleed at discretion of attending physician

Hypersensitivity to Hirudin or prior documented allergy to its components

Pregnant or breast feeding females

Hemorrhagic stroke in preceding 3 months

Abnormal baseline coagulation characterized by an INR >1.4, obtained at the discretion of the treating clinician

Required therapeutic anticoagulation for atrial fibrillation, prior VTE, or mechanical heart valve

Treatment with concomitant anti-platelet agent other than aspirin 326 mg or more daily

Subjects with a life expectancy less than 1 month

10. STUDY PROCEDURE:

10.1. Selection of the study population:

Subjects will be selected according to inclusion and exclusion criteria, willing to sign informed consent and able to complete the study.

Subject's informed consent had to be obtained and documented in accordance with local regulations, ICH-GCP requirements, and the ethical principles that have their origin in the principles of the Declaration of Helsinki.

Prior to obtaining informed consent, information should had been given in a language and at a level of complexity understandable to the subject in both oral and written form by the investigator or designee. Each subject should have the opportunity to discuss the trial and its alternatives with the investigator. the written Informed Consent Form should have been signed and personally dated by the subject, or his legal representative, and by the person who conducted the informed consent discussion (investigator or designee). The subject or his legal representative must had received a copy of the signed and dated Informed Consent Form. As part of the consent process, each subject must have consented to direct access to his medical records for trial-related monitoring, auditing, IRB/IEC review and regulatory inspection.

10.2. Case Report Form

After the protocol approval, the site will be trained on the protocol and the data collection system by template of Case Report Form (CRF) with the appropriate documentations.

The data collection will take place through the previously mentioned phases within three months from the first patient entered till the last patient data collected with the optimum tracking and quality control for the patients' records and registries to ensure the validity of the collected data.

11. SOURCE DATA

Source documents are original documents and records from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and

previous and concurrent medication may be summarized into the CRF), clinical and office charts, laboratory and pharmacy records…etc, ...
All documents will be stored safely in a secured room. In all study-specific documents, other than the signed informed consent, the patient will be referred to by the patient initials and patient number i.e. patient ID.

12. TREATMENT OF TRIAL PARTICIPANTS

12.1. Description of Study Medication(s)

Because thrombin plays a central role in thrombogenesis, the goal of most treatment regimens is to block thrombin generation or inhibit its activity.

1- r-Hirudin binds directly with thrombin to its active & fibrinogen binding site.

2- r-hirudin does not require access to heparin binding site & can thus neutralize clot bound thrombin. Hence, down regulating thrombin generation & potentially enhancing early revascularization.

Direct thrombin inhibitors were developed to overcome the inability of the heparin/ antithrombin complex to inactivate bound thrombin. In contrast to heparin and low-molecular-weight heparin, which catalyze the inactivation of thrombin and activated factor X (factor Xa) by antithrombin, direct thrombin inhibitors bind to thrombin and block its interaction with its substrates.

II- They do not bind to plasma proteins, direct thrombin inhibitors produce a more predictable anticoagulant response than heparin.

III- Unlike heparin, direct thrombin inhibitors do not interact with platelet factor 4 or high-molecular-weight multimers of von Willebrand factor. Consequently, the activity of direct thrombin inhibitors in the vicinity of a platelet-rich thrombus is not compromised.

IV- Finally, thrombin bound to fibrin or fibrin degradation products is susceptible to inactivation by direct thrombin inhibitors. Because the active site of thrombin is not involved in the interaction of the enzyme with fibrin, it remains accessible to active site-directed thrombin inhibitors. Consequently, these agents inactivate fibrin-bound thrombin without displacing the enzyme from fibrin. In contrast, bivalent thrombin inhibitors, such as Hirudin and bivalirudin, displace bound thrombin during the inhibition reaction by competing with fibrin for access to exosite 1 on thrombin.

Dose of Thrombexx®:

In DVT Prophylaxis: 15 mg twice daily, subcutaneous for 15 days
In case of renal insufficiency, the dose is titrated because r-Hirudin is excreted renally.
According to Creatinine clearance:

Creatinine clearance › 31-60 ml/min (5 mg s.c twice daily)

Creatinine clearance ‹ 31 ml/min (1.7 mg s.c twice daily)

In case of patients who will receive spinal anesthesia; Thrombexx will be given 8-10 hours before puncture/catheter and 2-4 hours after puncture/catheter manipulation or removal, as per European Guidelines.

12.2. Concomitant Medication (if applicable)

Throughout the study Investigators might prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care except for those listed in the exclusion criteria. If these were required, the participant will be withdrawn.
Any medication, other than the study medication had taken during the study will be recorded in the CRF.

13. DATA COLLECTION

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data reliable to the study for each study participant.

Subjects who are screened but choose not to continue in the study or those that are found to be ineligible for the study will be considered “screen failures”. The reason for the failure will be recorded on the source document and in the CRF.

The Clinical Research Associate (CRA) will verify the paper CRF documentation for each subject against the source documents at the study center. Instances of missing or un-interpretable data will be brought to the attention for resolution. A copy of the source document and CRF will be placed in the Investigator’s study file and the original CRF will be kept by Minapharm at the end of the study. The CRF will be the sole property of the Minapharm.

Data reported in the paper CRF derived from source documents should be consistent with source documents or the discrepancy should be explained. Only the investigator/designee and clinical research coordinator are authorized to make entries on the paper CRF.

The investigators will be responsible to ensure the accuracy, completeness and timelines of the data reported.

All source documents and reports must be reviewed by the designated clinical team and data entry staff, who will ensure that they are accurate and complete. Clinical assessments data must be entered into source data. Adverse events must be graded, assessed for severity and causality, and reviewed by the site investigator or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. During the study, the investigator must maintain complete and accurate documentation for the study. All relevant clinical data from source documents will be entered into the paper CRF.

After completion of the paper CRFs by the investigator, monitor will review all subject paper CRFs for completeness and accuracy. Requests for clarifications or correction will be sent to the investigator, if necessary. Statistical analysis will be carried out after all necessary corrections have been done and database has been locked.

Minapharm will serve as the statistical and data management for this study and will be responsible for data management, quality review, analysis, and reporting of the study data.

The protocol and paper CRF are confidential documents. The paper CRFs will be supplied by the Minapharm. Appropriate information for the completion of paper CRF will be provided to all participating centers. The paper CRFs must accurately reflect data contained in subject's records (i.e. source documents). In order to maintain confidentiality, the subject will be identified only by his/her assigned study subject number and/or initials.

Clinical data will be entered into paper CRF from the source documents. The data system includes password protection and internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate.

Queries generated during data entry will be sent to investigator for resolution and record of the same would be kept.

15.1. DATA MANAGEMENT

All data collected in this study will be entered manually by data entry team on Oracle database specially designed to meet the needs of clinical trials prepared by Minapharm.

Edit checks will be run on the data and queries issued as needed and queries will be resolved. Full quality control data verification will be done and then data will be declared clean. Database will be locked after Minapharm medical department approval.

Extraction of data for statistical analysis will be done once database lock occurs. All data collected in the paper CRFs will be documented in subject data listings and summarized in tables, as appropriate.

15.2. AMENDMENT TO THE PROTOCOL

Modifications of the signed protocol are only possible by approved protocol amendments authorized by Minapharm. The protocol amendments will be approved by the IRB/IEC prior to implementation. The Investigator must not implement any deviation from, or change to the protocol, except where it is necessary to eliminate an immediate hazard to trial subject or when the change(s) involves only logistical or administrative aspects of the trial.

15.2.1. Protocol Deviations

The Investigator will not deviate from the protocol without prior written approval from sponsor (or designee), except in medical emergencies. In such cases, Minapharm must be notified as soon as possible. The governing IEC will be informed of all protocol deviations by the Investigator.

15.2.2. On-site Audits

Minapharm may conduct audits at the study center(s). Audits will include, but will not be limited to, study medication supply, presence of required documents, the informed consent process, and comparison of paper CRFs with source documents. The investigator agrees to participate with audits conducted at a reasonable time in a reasonable manner. These audits are for the purpose of verifying the adherence to the protocol, the completeness and exactness of the data being entered

in the CRF, and compliance with regulations. Subject confidentiality will be maintained during all audits. Regulatory authorities may also audit the investigator during or after the study. The investigator must contact Minapharm immediately if this occurs and must fully cooperate with the audits conducted at a reasonable time in a reasonable manner.

15.2.3. Study Monitoring

The clinical research associate (CRA) will undertake the study monitoring/supervision. In accordance with applicable regulations, ICH-GCP, Minapharm monitors will contact the site prior to the subject enrollment to review the protocol and data collection procedures with site staff. In addition, the monitor will periodically contact the center, including conducting on-center monitoring visits at an appropriate frequency, according to the monitoring plan.

During these contacts, the monitor will:

1. Check the progress of the study.
2. Review study data collected.
3. Conduct source document verification and CRF data entry.
4. Identify any issues and address their resolution.

This will be done in order to verify that the:

1. Data are authentic, accurate, and complete.
2. Safety and rights of subjects are being protected.
3. Study is conducted in accordance with the currently approved protocol (and any amendments), ICH-GCP, and all applicable regulatory requirements.

The investigator agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

Monitoring visits at each investigative site will be conducted by the CRAs at regular intervals. The first monitoring visit will be conducted immediately after the enrollment of first subject in the study and the subsequent monitoring visits will be conducted based on the recruitment status and activities at the site. The CRAs are responsible for applying the applicable monitoring plan. Any data clarification from Data Management (DM) will be resolved by the site. In addition, the CRAs will be in frequent contact through verbal and written communication with the investigators. It is essential that the CRAs have access to all documents (related to the study and the individual participants) at any time when these are requested. In return, the CRA will adhere to all requirements for subject confidentiality as outlined in the ICF. The investigator and his/her staff will be expected to cooperate with CRA, to be available during a portion of the monitoring visit to answer questions, and to provide any missing information.

Original subject records (source documents) will be audited or reviewed during the course of monitoring to verify the accuracy of the source documents and accuracy of data entered from these source documents into the paper CRF.

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents are original documents, data, records and subject files.

15.3. PREMATURE DISCONTINUATION OF STUDY

The sponsor may prematurely terminate the trial if any of the following situations occurs:

APTT value of the subject increased by 2-2.5 folds.

Comprehensive deficiency in the recorded data or protocol compliance so that the results of the study cannot be reliably assessed.

Occurrence of AEs necessitating termination such as major bleeding, DVT, PE, allergy or for any other reason according to investigator evaluation.

The IRB/IEC changes its opinion regarding the safety/efficacy/relevance of the compound.

Regulatory authority decision.

If the trial is prematurely terminated or suspended for any reason, the investigators and subsequently the trial subjects will be informed promptly, standard therapy and follow-up for subjects will be assured and, where required by the applicable regulatory requirement(s), the relevant regulatory authority(s) will be informed. The IRB/IEC and all trials sites will be informed promptly and provided with a detailed written explanation for the termination or suspension. As directed by Minapharm, all study materials must be collected and all CRFs completed to the greatest extent possible.

16. SAFETY REPORTING AND DEFINITIONS

Safety assessments will consist of monitoring and recording any adverse events reported, including serious adverse events and non-serious adverse events; measurement of protocol-specified safety laboratory assessments; measurement of protocol-specified vital signs; and other protocol-specified tests that are deemed critical to the safety evaluation of the study.

16.1. Adverse Events Monitoring

All AEs regardless of seriousness or relationship to IMP, spanning from the signature of the informed consent form, until the end of the study as defined by the protocol for that patient, are to be recorded on the corresponding page(s) included in the CRF and reported as described in the following sections.

For all adverse events, the investigator must pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event requiring immediate notification to Sponsor or its designated representative.

For all adverse events, sufficient information should be obtained by the investigator to determine the causality of the adverse event. The investigator is required to assess causality. For adverse events with a causal relationship to the investigational product, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and Sponsor concurs with that assessment. This may imply that observations will continue beyond the last planned visit per protocol, and that additional investigations may be requested by the Monitoring Team up to as noticed by the sponsor

Whenever possible, diagnosis or single syndrome should be reported instead of symptoms. The Investigator should specify the date of onset, intensity, action taken with respect to IMP, corrective treatment/therapy given, additional investigations performed, outcome and his/her opinion as to whether there is a reasonable possibility that the AE was caused by the IMP.

16.2. Reporting Period

For serious adverse events, the reporting period to Sponsor or its designated representative begins from the time that the subject provides informed consent, which is obtained prior to the subject's participation in the study, i.e. prior to undergoing any study-related procedure and/or receiving investigational product, through and till the end of the Follow Up Period as specified in the study protocol.

Any serious adverse event occurring any time after the reporting period must be promptly reported if a causal relationship to investigational product is SUSPECTED.

16.3. Adverse Event (AE):

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage.

Examples of adverse events include but are not limited to:

- Abnormal test findings
- Clinically significant symptoms and signs
- Changes in physical examination findings
- Hypersensitivity
- Progression/worsening of underlying disease

Additionally, they may include the signs or symptoms resulting from:

- Drug overdose
- Drug withdrawal
- Drug abuse
- Drug misuse
- Drug interactions
- Drug dependency
- Extravasation

Exposure during pregnancy
Drug Quality Defect that has impact on the patient safety

16.4. Serious Adverse Events

A serious adverse event or serious adverse drug reaction is any untoward medical occurrence at any dose that:

Results in death
life-threatening Event (Immediate Risk Of Death)
Requires inpatient hospitalization or prolongation of existing hospitalization
Results in persistent or significant disability/incapacity
Results in congenital anomaly/birth defect
Important medical event

Medical and scientific judgment should be exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject and/or may require intervention to prevent one of the other adverse event outcomes, the important medical event should be reported as serious

16.4.1. Instructions for Reporting Serious Adverse Events

In the case of occurrence of a SAE, the Investigator must immediately:

1. Sending within 24 hours, preferably by e-mail the signed and dated corresponding page(s) from the CRF to the representative of the Monitoring Team whose name, fax number and e-mail address appear on the Clinical Trial Protocol and qualified person for pharmacovigilance

2. ATTACH the photocopy of all examinations carried out and the dates on which these examinations were performed. Care should be taken to ensure that the patient's identity is protected and the patient's identifiers in the Clinical Trial are properly mentioned on any copy of source document provided to the Sponsor. For laboratory results, include the laboratory normal ranges;

3. All further documentation should be sent to the Monitoring Team within 1 working day of knowledge. In addition, any effort should be made to further document each SAE that is fatal or life threatening within the week (7 days) following initial notification

17. STATISTICAL METHODOLOGY

17.1. Determination of Sample Size

Sample size: Records for 100 patients who underwent major orthopedic surgery collected from 1 site

The sample size is based on literature review for previous studies, and a sample of 168 patients will be sufficient to detect an incidence of 12.5% of DVT with an acceptable error of 5%, and a 95% confidence level

17.2. Analysis Variables:

17.2.1. Primary analysis variables:

Proportion of patients experienced new onset symptomatic thrombosis
The number of clinical PE (Pulmonary Embolism) events will be measured by spiral CT

17.2.2. Secondary analysis variables:

Number of adverse events recorded
Proportion of patients experienced major bleeding

17.2.3. Statistical Method:

Descriptive analysis:

All statistical tests used a significance level of P=0.05, two tailed tests were performed for all analyses used statistical testing.

Descriptive analysis for quantitative data will include count, mean with 95% CI, standard deviation, median, minimum and maximum. For qualitative categorical variables; frequency and percentage and 95% confidence interval will be applied.

17.2.4. Interim Analysis

Not applicable in this Prospective study

18. ETHICS

18.1. INDEPENDENT ETHICS COMMITTEE (IEC) OR INSTITIUTIONAL REVIEW BOARD (IRB)

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC) before study start. A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC must be given to Minapharm Pharmaceuticals before study initiation. Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to Minapharm monitors and auditors and regulatory authorities as required

18.2. Ethical principles:

This Clinical Trial will be conducted in accordance with the principles laid down by the 18th World Medical Association (Helsinki, 1964) and all applicable amendments laid down by the World Medical Association and ICH guidelines for Good Clinical Practice [(E6)R1)]

18.3. Laws and regulations:

This Clinical Trial will be conducted in compliance with all international laws and regulations, and national laws and regulations of Egypt in which the Clinical Trial is performed, as well as any applicable guidelines.

18.4. PATIENT INFORMATION AND CONSENT

The Investigator (according to applicable regulatory requirements), or a person designated by the Investigator, and under the Investigator's responsibility, should fully inform the Patient of all pertinent aspects of the Clinical Trial including the written information giving approval/favourable opinion by the Ethics Committee (IRB/IEC). All participants should be informed to the fullest extent possible about the study, in language and terms they are able to understand.

Prior to a patient's participation in the Clinical Trial, the written Informed Consent Form and any other local applicable documents in accordance with Egyptian laws and regulations, should be signed, name filled in and personally dated by the patient or by the patient's legally acceptable representative, and by the person who conducted the informed consent discussion. A copy of the signed and dated written Informed Consent Form will be provided to the patient.

The Informed Consent Form used by the Investigator for obtaining the patient's informed consent must be reviewed and approved by the Sponsor prior to submission to the appropriate Ethics Committee (IRB/IEC) for approval/favorable opinion.

Minapharm Pharmaceuticals will provide investigators in a separate document with a proposed informed consent form (ICF) that is considered appropriate for this study and complies with the ICH GCP guideline and regulatory requirements. Any changes to this ICF suggested by the investigator must be agreed to by Minapharm before submission to the IRB/IEC and a copy of the approved version must be provided to the Minapharm manager after IRB/IEC approval.

18.5. DISCONTINUATION OF THE STUDY

Minapharm reserves the right to discontinue this study under the conditions specified in the clinical study agreement.

18.5.1. PREMATURE DISCONTINUATION OF STUDY

Minapharm may prematurely terminate the trial if any of the following situations occurs:

APTT value for the subject increases by 2-2.5 folds

Comprehensive deficiency in the recorded data or protocol compliance so that the results of the study cannot be reliably assessed.

Occurrence of AEs necessitating termination.

Minapharm terminates the development plan for the compound.

The IRB/IEC changes its opinion regarding the safety/efficacy/relevance of the compound.

Regulatory authority decision.

If the trial is prematurely terminated or suspended for any reason, the investigators and subsequently the trial subjects will be informed promptly, standard therapy and follow-up for subjects will be assured and, where required by the applicable regulatory requirement(s), the relevant regulatory authority(s) will be informed. The IRB/IEC and all trials sites will be informed promptly and provided with a detailed written explanation for the termination or suspension. As directed by Minapharm, all study materials must be collected and all CRFs completed to the greatest extent possible.

18.6. PUBLICATION OF STUDY PROTOCOL AND RESULTS

Minapharm assures that the key design elements of this protocol will be posted in a publicly accessible database such as clinicalstudys.gov. In addition, upon study completion and finalization of the study report the results of this study will be either submitted for publication and/or posted in a publicly accessible database of clinical study results.

18.7. STUDY DOCUMENTATION, RECORD KEEPING AND RETENTION OF DOCUMENTS

Records and documents pertaining to the conduct of this study, including CRFs, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 15 years after completion or discontinuation of the study, or for the length of time required by local health authorities, whichever is longer. After that period of time, the documents may be destroyed.

No records may be disposed of without the written approval of Minapharm. A written notification should be provided to Minapharm prior to transferring any records to another party or moving them to another location.

18.8. CONFIDENTIALITY OF STUDY DOCUMENTS AND PATIENT RECORDS

The investigator must ensure anonymity of the patients; patients must not be identified by names in any documents submitted to Minapharm. Patient enrollment log must be kept strictly confidential to enable patient identification at the center.

18.9. AUDITS AND INSPECTIONS

Source data/documents must be available to inspections by Minapharm or designee or Health Authorities.

19. References

1. Greinacher A, Warkentin TE. The direct thrombin inhibitor hirudin.Thromb Haemost. 2008;99:819–29.

2. Markwardt F. Hirudin as alternative anticoagulant–a historical review.Semin Thromb Hemost. 2002;28:405–14.

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4. Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral coagulants:antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141:e24S–43S.

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