Statistical Analysis Plan

A Prospective, Single- Center, Phase IV 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

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Table of Contents

Table of Tables

1 Introduction:

1.1 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.

1.2 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

2 Study 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:

2.1 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, Activated Partial Thromboplastin Time (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.

2.2 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

2.3 The Follow-Up Phase

During this phase which is approximately 15 days, PE (Pulmonary Embolism) 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

3 Study Objective:

3.1 Primary Objectives:

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

3.2 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.

4 Study Outcome Measures:

4.1 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

4.2 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.

5 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.

5.1 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.

5.2 Inclusion Criteria:

Subjects meeting all of the following criteria will be considered for enrolment in the study:

1. Patients aged 18 years of age or older
2. Body Weight >60 kg
3. Patients undergoing major orthopedic operations Total knee & hip Arthroplasty
4. Patients ready to sign (ICF)
5. 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.

5.3 Exclusion Criteria:

Subjects presenting with any of the following will not be included in the study:

1. Subjects with a history of hypersensitivity to any of the active ingredients of the treatments used
2. Significant bleeding injury such as solid organ laceration or intracranial bleed at discretion of attending physician
3. Hypersensitivity to Hirudin or prior documented allergy to its components
4. Pregnant or breast feeding females
5. Hemorrhagic stroke in preceding 3 months
6. Abnormal baseline coagulation characterized by an INR >1.4, obtained at the discretion of the treating clinician
7. Required therapeutic anticoagulation for atrial fibrillation, prior VTE, or mechanical heart valve
8. Treatment with concomitant anti-platelet agent other than aspirin 326 mg or more daily
9. Subjects with a life expectancy less than 1 month

6 Assessment Schedule

Subjects will be enrolled for duration of 1 months including the screening visit

a. Preoperative phase (screening visit)
b. Operative phase (Day 0)
c. Visit 2: Post-operative phase (Follow-up 1(Day 1)
d. Visit 3: Follow-up 2 (Day 8)
e. Visit 4: Follow-up 3/ End of Study (Day 15)

7 Medicinal Products:

- Thrombexx Ampoules (r-Hirudin 15mg/ ml pro inject **S.C. only**)

8 Statistical Methodology:

8.1 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

8.2 Analyses variables

8.2.1 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

8.2.2 Secondary analysis variables:

- Number of adverse events recorded
- Proportion of patients experienced major bleeding

8.2.3 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.

8.2.4 Interim analysis

Not applicable in this Prospective study

9 Results:

9.1 Screening Visit

9.1.1 Demographic Data and Vital Signs

9.1.2 Physical Examination

9.1.3 Medical history

9.1.4 Prior Concomitant Medication

9.1.5 Lab Results

9.2 Primary End Point:

9.3 Secondary End Point:

9.4 Study Discontinuation

9.5 Concomitant Medication

9.1 Screening Visit

9.1.1 Demographic Data and vital signs

Table 1 Demographic Data and vital signs at baseline visit

	N = 100
Gender (male), N (%)	
Age (Yrs.), Mean ± SD, (Yrs)	
Pulse, mean ± SD, (beats /min)	
Systolic BP, mean ± SD, (mmHg)	
Diastolic BP, mean ± SD, (mmHg)	
Oral Temp, mean ± SD, (^{o}C)	

9.1.2 Physical Examination

Table 2 Number and percent of Physical Examination at baseline visit

		N = 100
Physical Examination	**Normal, N (%)**	
	Abnormal, N (%)	
If abnormal, please describe with details	**Examination 1, N (%)**	
	Examination 2, N (%)	
	Examination 3, N (%)	

Table 3 Number and percent of Main Complaint at baseline visit

		N = 100
Main Complaint	Hip, N (%)	
	Knee, N (%)	

9.1.3 Medical History

Table 4 Number and percent of medical history at baseline visit

	N = 100
Medical History, N (%)	
Med history 1, N (%)	
Med history 2, N (%)	
Med history 3, N (%)	

9.1.4 Prior Concomitant medication

Table 5 Number and percent of Prior Concomitant medication at baseline visit

	N = 100
Prior Concomitant medication, N (%)	
Prior ConMed1, N (%)	
Prior ConMed2, N (%)	
Prior ConMed3, N (%)	

9.1.5 Lab Results

Table 6 Mean of Lab Results at baseline visit

	N = 100
Was the lab report received? N (%)	
Hb%, Mean ± SD, (g/dL)	
Platelets Count, Mean ± SD, (x10$_3$/µL)	
APTT, Mean ± SD, (seconds)	
INR, Mean ± SD	
SGPT, Mean ± SD, (IU/L)	
SGOT, Mean ± SD, (IU/L)	
Sr. Albumin, Mean ± SD, (g/dL)	
Sr. Bilirubin, Mean ± SD, (mg/dL)	
Sr. Creatinine, Mean ± SD, (mg/dL)	
Creatinine Clearance, Mean ± SD, (ml/min)	
RBS, Mean ± SD, (mg/dL)	

Table 7 Number and percent of Surgery Information

		N = 100
Type of Surgery	**Surgery 1, N (%)**	
	Surgery 2, N (%)	
	Surgery 3, N (%)	
Type of Anesthesia	**Anesthesia 1, N (%)**	
	Anesthesia 2, N (%)	
	Anesthesia 3, N (%)	
Patient Position	**Position 1, N (%)**	
	Position 2, N (%)	
	Position 3, N (%)	
Type of prosthesis	**Prosthesis 1, N (%)**	
	Prosthesis 2, N (%)	
	Prosthesis 3, N (%)	
Blood Transfusion	**Transfusion 1, N (%)**	
	Transfusion 2, N (%)	
	Transfusion 3, N (%)	

9.2 Primary End Point

Table 8 Proportion of patients experienced new onset symptomatic thrombosis

		Follow-up 1 (Day 1)	Follow-up 2 (Day 8)	Follow-up 3/ End of Study (Day 15)	*p*-value
Was a Routine bilateral compression Doppler done?	Yes, N (%)				
	No, N (%)				
If "No", please indicate a reason	Reason 1, N (%)				
	Reason 2, N (%)				
	Reason 3, N (%)				
Is the report interpreted, signed & dated by the PI or any of the designated site staff?	(Yes), N (%)				
	(No), N (%)				
If "No", please indicate a reason:	Reason 1, N (%)				
	Reason 2, N (%)				
	Reason 3, N (%)				
If "Yes", the report interpretation shows that the subject is:	Normal, N (%)				
	Abnormal, N (%)				
If abnormal, please state the reason:	Reason 1, N (%)				
	Reason 2, N (%)				
	Reason 3, N (%)				

Table 9 The number of clinical PE (Pulmonary Embolism) events will be measured by spiral CT

		Follow-up 1 (Day 1)	Follow-up 2 (Day 8)	Follow-up 3/ End of Study (Day 15)	*p*-value
Was a Spiral CT done?	Yes, N (%)				
	No, N (%)				
If "No", please indicate a reason	Reason 1, N (%)				
	Reason 2, N (%)				
	Reason 3, N (%)				
Is the report interpreted, signed & dated by the PI or any of the designated site staff?	(Yes), N (%)				
	(No), N (%)				
If "No", please indicate a reason:	Reason 1, N (%)				
	Reason 2, N (%)				
	Reason 3, N (%)				
If "Yes", the report interpretation shows that the subject is:	Normal, N (%)				
	Abnormal, N (%)				
If abnormal, please state the reason:	Reason 1, N (%)				
	Reason 2, N (%)				
	Reason 3, N (%)				

Table 10 Mean changes in Activated Partial Thromboplastin Time (APTT) where it will be done before first dose, 4 & 8 hours after first dose then on days 1,8,15 post operatively

		Before 1st dose	After 1st dose		Post-operative			*p*-value
			4 h	8 h	Day 1	Day 8	Day 15	
Was an APTT test performed?	Yes, N (%)							
	No, N (%)							
If "No", please indicate a reason	Reason 1, N (%)							
	Reason 2, N (%)							
	Reason 3, N (%)							
If "Yes", the test result is	**APTT; Mean ± SD (sec)**							
APTT, % change								
***p*-value**								
The test interpretation shows that the subject is:	Normal, N (%)							
	Abnormal, N (%)							
If abnormal, please state the reason:	Reason 1, N (%)							
	Reason 2, N (%)							
	Reason 3, N (%)							

9.3 Secondary End Point

Table 11 Proportion of patients experienced major bleeding

		Day 1	Day 8	Day 15	*p*-value
Assessment of Bleeding:	Normal, N (%)				
	Abnormal, N (%)				
Type Of Blood Transfusions:	Whole Blood, N (%)				
	RBCs, N (%)				
	Platelets, N (%)				
	Plasma, N (%)				

Table 12 Number and percent of Adverse Event among treatment groups

	N = 100	*p*-value
Total No of AE, N (%)		
AE1, N (%)		
AE2, N (%)		
AE3, N (%)		

Table 13 Number and percent of Serious Adverse Event among treatment groups

	N = 100	*p*-value
Total No of SAE, N (%)		
SAE1, N (%)		
SAE2, N (%)		
SAE3, N (%)		

Table 14 Percent Change in mean Hb%

	Screening	Day 1	Day 8	Day 15	*p*-value
Hb%, (mean ± SD)					
% change					
***p*-value**					

Table 15 Percent Change in mean Platelets count

	Screening	Day 1	Day 8	Day 15	*p*-value
Platelets count, (mean ± SD), x 10^3/µL					
% change					
***p*-value**					

Table 16 Percent Change in mean Systolic BP

	Screening	Day 0	Day 1	Day 8	Day 15	*p*-value
Systolic BP, (mean ± SD), mmHg						
% change						
***p*-value**						

Table 17 Percent Change in mean Diastolic BP

	Screening	Day 0	Day 1	Day 8	Day 15	*p*-value
Diastolic BP, (mean ± SD), mmHg						
% change						
***p*-value**						

Table 18 Percent Change in mean Sitting Pulse Rate

	Screening	Day 0	Day 1	Day 8	Day 15	*p*-value
Sitting Pulse Rate, (mean ± SD), beats /min						
% change						
***p*-value**						

Table 19 Percent Change in mean Oral Temperature

	Screening	Day 0	Day 1	Day 8	Day 15	*p*-value
Oral Temperature, (mean ± SD), °C						
% change						
***p*-value**						

9.4 Study Discontinuation

Table 20 Number and percent of causes of treatment discontinuations

		N = 100	*p*-value
Did the patient complete the study as per protocol?	**Yes, N (%)**		
	No, N (%)		
Reason for the discontinuation from study	**Patient Withdrew Voluntarily**		
	Adverse Event		
	Lost Follow-up		
	Death		
	Other Reasons		

9.5 Concomitant Medication

Table 21 Number and percent of Concomitant medication

	N = 100
N	
ConMed1, N (%)	
ConMed2, N (%)	
ConMed3, N (%)	

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