Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Büller HR, Bethune C, Bhanot S, et al. Factor XI antisense oligonucleotide for prevention of venous thrombosis. N Engl J Med. DOI: 10.1056/NEJMoa1405760

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Supplement to : Factor XI Antisense Oligonucleotide for Prevention of Venous Thrombosis ClinicalTrials.gov number ,

NCT01713361

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Outcome definitions

1.Venous thromboembolism (VTE)

1.1. Asymptomatic DVT per protocol bilateral venography:

Per protocol, bilateral ascending venography will be performed at Day 10 ± 2 days post surgery

The diagnostic criteria for DVT will be a constant intraluminal filling defect of the same shape on two different images, or non-visualization of vein(s) in the presence of a sudden cut-off on venography.

The veins will be scored as: normal, constant intraluminal filling defect (DVT) or inadequate. Each leg will be reported as normal, distal DVT, proximal DVT, proximal and distal DVT, non evaluable entire leg, non evaluable distal but normal proximal veins or not performed. The final overall diagnosis will be normal, DVT, partially evaluable no proximal DVT, or non evaluable (see table below: Classification protocol bilateral venography):

Right / Left	Normal	Distal DVT	Proximal DVT	Proximal and Distal DVT	Non evaluable entire leg	Non evaluable distal but normal prox. veins	Not performed
Normal	Normal	DVT	DVT	DVT	Non evaluable	Partially evaluable, no proximal DVT	Non evaluable
Distal DVT	DVT	DVT	DVT	DVT	DVT	DVT	DVT
Proximal DVT	DVT	DVT	DVT	DVT	DVT	DVT	DVT
Proximal and distal DVT	DVT	DVT	DVT	DVT	DVT	DVT	DVT
Non evaluable entire leg	Non evaluable	DVT	DVT	DVT	Non evaluable	Non evaluable	Non evaluable
Non evaluable distal but normal prox. veins	Partially evaluable, no proximal DVT	DVT	DVT	DVT	Non evaluable	Partially evaluable, no proximal DVT	Non evaluable
Not performed	Non evaluable	DVT	DVT	DVT	Non evaluable	Non evaluable	Non evaluable

Classification protocol bilateral venography:

1.2. Clinically overt DVT

Clinical symptoms of suspected DVT with one of the following findings [1]

- a constant intraluminal filling defect of the same shape on two different images of venography, or non-visualization of veins in the presence of a sudden cut-off on venography, or in case the previous per protocol venography showed asymptomatic thrombosis, the criteria would be a new or an extension of a constant intraluminal filling defect, or a new site of non-visualization of vein(s) in the presence of a sudden cut-off on venography, or
- abnormal compression ultrasound (CUS).

If there is a suspicion of DVT, it is allowable firstly to perform a compression ultrasound unless the suspicion occurs on the day of scheduled bilateral venography. If the outcome of compression ultrasound is positive, venography is not required.The final diagnosis will be DVT (indicating the location of the thrombus), no DVT ,or not evaluable.

1.3. Clinically overt PE

Clinical symptoms of suspected PE with one of the following findings [2]

- an intraluminal filling defect in segmental or more proximal branches on sCT (spiral CT**)**
- an intraluminal filling defect or an extension of an existing defect or a new sudden cutoff of vessels more than 2.5 mm in diameter on the pulmonary angiogram,
- a perfusion defect of a segment with a local normal ventilation result (high-probability) on ventilation/perfusion lung scintigraphy
- inconclusive sCT, pulmonary angiography or lung scintigraphy with demonstration of DVT in the lower extremities by compression ultrasound or venography.
- Fatal PE:
    - PE based on objective diagnostic testing, autopsy, or
    - Death which cannot be attributed to a documented cause and for which PE/DVT cannot be ruled out (unexplained death)

The final overall diagnosis will be PE, no PE, or not evaluable.

2. Bleeding events

All bleeding events will be reviewed by the CIAC and classified as major bleeding, clinically relevant non-major bleeding, minor bleeding or no bleeding event.

If **major**, bleeding will be specified as one of the following:

1. Fatal bleeding
2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular if in a major joint*, or pericardial, or intramuscular with compartment syndrome
3. Clinically overt bleeding leading to transfusion of ≥2 units of packed red blood cells or whole blood or a fall in hemoglobin of 20 g/L or more**
4. Surgical site bleeding that requires a second intervention-open, arthroscopic, endovascular - or a hemarthrosis*** of sufficient size as to interfere with rehabilitation by delaying mobilization or delayed wound healing, resulting in prolonged hospitalization or a deep wound infection

* In the context of the present study, bleeding in the operated joint or not meeting criteria as specified in bullet 4 are excluded

** For transfusion of units of packed red blood cells or whole blood hemoglobin drop, it will be carefully evaluated whether this was really associated with the bleeding event (based on clinical description) and this will need to be distinguished from hemoglobin-drop and transfusions associated with the total knee replacement surgery in itself

*** Hemarthrosis should be objectively documented including the presence of an abundance of erythrocytes Site of bleeding will be indicated.

A **clinically relevant non-major** bleed (CNMB) is defined as overt bleeding not meeting the criteria for major bleeding but that resulted e.g. in medical examination, intervention or had clinical consequences for a patient. [3] Examples of such bleeding are:
any bleeding compromising hemodynamics, or any bleeding leading to hospitalization, or subcutaneous (skin) hematoma if the size is larger than 25 cm^2 for spontaneous bleeding , or 100 cm^2 if provoked, intramuscular hematoma, or epistaxis if it lasts for more than 5 minutes, (where the 5 min is defined as profuse epistaxis) if it is repetitive (i.e. 2 or more episodes of true bleeding, i.e. not spots on a handkerchief, within 24 hours), or leads to an intervention (packing, electrocoagulation etc), or gingival bleeding if it occurs spontaneously (i.e. unrelated to tooth brushing or eating), or if it lasts for more than 5 minutes (where the 5 min is defined as profuse gingival bleeding) or hematuria if it is macroscopic, and either spontaneous or lasts for more than 24 hours after instrumentation (e.g. catheter placement or surgery) or the urogenital tract, or macroscopic gastro-intestinal hemorrhage: at least one episode of melena/hematemesis, if clinically apparent and hemoccult positive, or rectal blood loss, if more than a few spots on toilet paper, or

hemoptysis, if more than a few specks in the sputum and not occurring within the context of PE or any other bleeding type that is considered to have clinical consequences for a patient.

All other acute clinically overt bleeding events not meeting the criteria for either major or clinically relevant non major bleeding will be classified as **minor**.

3. Mortality

For all patients who died during the study, the CIAC will assign the cause of death to one of the following:

- VTE related death (as above)
- Cardiovascular death
  - Myocardial infarction
  - Other cardiac death
  - Stroke
  - SEE (systemic embolic event)
  - Other cardiovascular death, to be specified
- Other cause of death
  - Cancer
  - Bleeding
  - Infectious disease
  - Other known cause, to be specified

4. Cerebrovascular Events

All cerebrovascular events will be reviewed by the CIAC and the conclusion will either be as specified below or 'not assessable'.

4.1 Stroke

A stroke is defined as an abrupt onset, over minutes to hours, of a focal neurological deficit in the distribution of a single brain artery that is not due to an identifiable non-vascular cause (i.e. brain tumor or trauma), and that either is associated with symptoms lasting more than 24 hours or that results in death within 24 hours of symptom onset or, if symptoms last <24 hours, a clear matching lesion on CT or MRI
CT and/or MRI scan reports, operative notes, autopsy results and other clinical data will be considered by the CIAC to support the clinical impression, and to permit sub classification of the type of stroke.
All strokes will be sub-classified as "ischemic" or "primary hemorrhagic" based on imaging data, if available, or "uncertain cause" if imaging data is not available according to the definitions below. In addition, ischemic stroke will be further sub-classified by presence or absence of hemorrhage.

Acute ischemic stroke

- Acute ischemic stroke with no hemorrhage: Stroke without focal collections of intracerebral blood on a brain imaging.
- Ischemic stroke with hemorrhagic conversion: Cerebral infarction with blood felt to represent hemorrhagic conversion and not a primary hemorrhage.

Primary hemorrhagic stroke

Primary hemorrhagic strokes will also be adjudicated and classified as bleeding (multiple locations may be checked if appropriate).

- Intracerebral Hemorrhage: Stroke with focal collections of intracerebral blood seen on a brain image (CT or MRI) or a postmortem examination, not likely to represent hemorrhagic conversion.
- Subarachnoid hemorrhage: High density fluid collection in subarachnoid space on brain images, or autopsy.

Acute stroke unknown type

Any stroke without brain image (CT or MRI), autopsy documentation, or other diagnostic information that permits sub-classification of the stroke, or if the tests are inconclusive

4.2. Transient ischemic attack (TIA)

A TIA is defined as an abrupt onset over minutes to hours of a focal non-fatal, neurological deficit in the distribution of a single brain artery that lasts less than 24 hours and that does not satisfy the definition of stroke above.

4.3 Other intracranial haemorrhage

Subdural hematoma

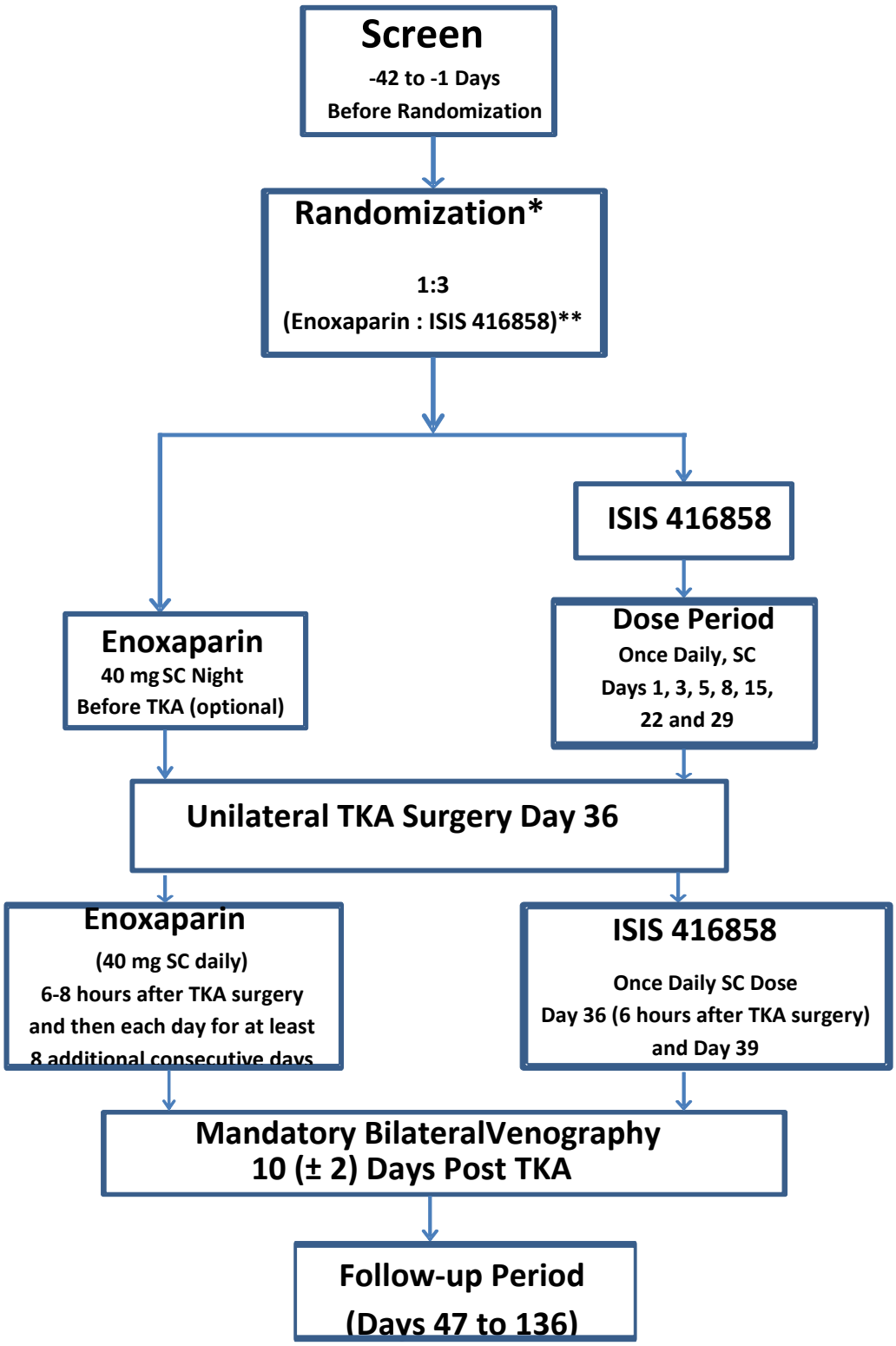
A subdural hematoma is defined as a high density fluid collection in subdural space on brain images or blood in the subdural space on autopsy.

Epidural hematoma

An epidural hematoma is defined as a collection of high density fluid collection on brain images or blood occurring between the dura mater and the skull.

NOTE: A subdural hematoma and epidural hematoma will be considered intracranial hemorrhages but will not be classified as a hemorrhagic stroke. NOTE: If the Cerebrovascular event was fatal – complete Mortality CAF.

Figure 1S: Study flow chart



*Randomization: Although the actual number of randomized patients per treatment group (as described in Figure 2) could appear to reflect an allocation schema of 2:1:1, the described randomization procedure was followed, i.e., patients were randomly allocated to one of two cohorts of FXI-ASO (200 and 300 mg) and within each of these cohorts to receive either FXI-ASO or enoxaparin in a 3:1 ratio. The protocol defined a target number of at least 70 patients per treatment arm. Randomization to one cohort (FXI-ASO 300 mg) was stopped for this reason (see footnote Figure 2) (actually enrolled 78 patients in this dose group). At that time point, in the other cohort (FXI-ASO 200 mg) only 56 patients were included and far fewer than 70

patients were assigned to enoxaparin. Therefore, randomization was continued in the 200 mg cohort in a 3:1 ratio (FXI-ASO 200 mg vs. enoxaparin) up to a target number of 70 patients in the enoxaparin group (actually achieved 75 patients) resulting in a number significantly above 70 in the 200 mg dose (actually achieved 147). Recruitment was then stopped altogether

**Study medication: ISIS 416858 was discovered and synthesized by Isis Pharmaceuticals Inc. Carlsbad, CA, USA and formulated (200 mg/ml) in sterile buffered saline for subcutaneous administration. ISIS 416858 is a single-stranded antisense oligonucleotide that is 20 nucleotides in length and incorporates several chemical modifications to improve potency, duration of action, and tolerability [4]All of the internucleotide phosphates are chemically modified with a phosphorothioate substitution, in which one of the non-bridging oxygen atoms is substituted with sulfur. Additionally the drug incorporates five 2′-O-(2-methoxyethyl) (2′-MOE) modified ribonucleosides at the 3′ and 5′ ends while retaining ten 2′-O-deoxyribonucleosides within the central portion of the molecule. ISIS 416858 was supplied in 2-ml stoppered glass vials and was administered as a single or two subcutaneous injections; the volume administered per injection was 1.0 ml and 1.5 ml for the 200 mg or 300 mg doses respectively.

Commercially available enoxaparin was provided by the Sponsor in accordance with the local regulatory requirements. All Study Centers were instructed to use the same manufacturer throughout the study. Enoxparin was supplied as 100 mg/ml prefilled syringes for subcutaneous use and was administered as a 0.4 ml volume per injection.

Figure 2S: Hemoglobin values

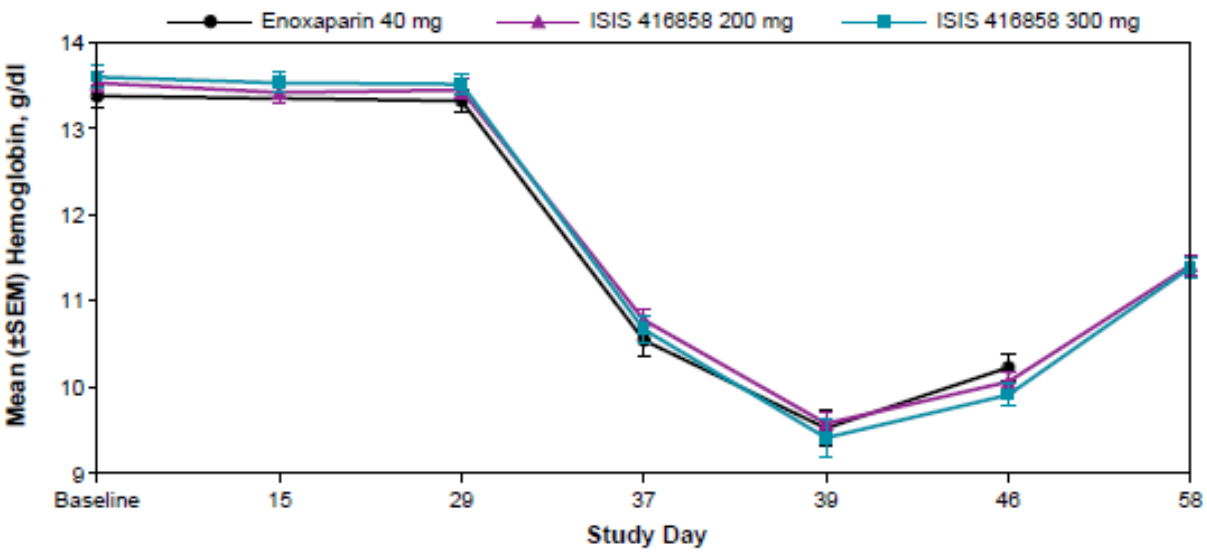


Table S1 Modified intention-to treat (mITT) population: efficacy analysis

	ISIS 416858 200 mg	ISIS 416858 300 mg	Enoxaparin
mITT population -N	139	71	71
Primary efficacy outcome : total VTE * -no.(%) (95 % CI)	36(25.9) (18.8 -34.0)	3 (4.2) (0.9 -11.9)	22 (31.0) (20.5 - 43.1)
Risk difference ISIS 416858 – enoxaparin, %(upper limit of 90% CI)	-5.1 (3.4)	-26.8 (-19.1)	-
ISIS 416858 versus enoxaparin, p-value for superiority	0.44	<0.001	
Components of the primary efficacy outcome			
Symptomatic DVT -no.(%)	2 (1.4)	0	1 (1.4)
Asymptomatic DVT – no. (%)	34 (24.5)	3(4.2)	21(29.6)
Proximal DVT– no. (%)	7(5.0)	1 (1.4)	5 (7.0)
Distal DVT – no. (%)	29 (20,9)	2 (2.8)	17 (23.9)
Extent of DVT Total number of DVT - N	36	3	22
Bilateral †	2	0	2
Confluent distal into proximal	6	0	2
Isolated proximal, extensive	0	0	2
Isolated proximal, small	0	1	0
Isolated distal, extensive	16	0	7
Isolated distal, small	12	2	9

Table S2 : Primary efficacy outcome in relation to factor XI (FXI) activity (per protocol population)

	Patients with FXI ≤ 0.2 U/ml no.(%)	VTE in patients with FXI ≤ 0.2 U/ml no./N(%)	VTE in patients with FXI > 0.2 U/ml no./N(%)	VTE in patients given Enoxaparin no./N(%)
ISIS 416858 200 mg	20/134 (14.9)	2/20 (10.0)	34/114(29.8)	
ISIS 416858 300 mg	42/71 (59.2)	1/42 (2.4)	2/29 (6.9)	
Pooled	62/205 (30.2)	3/62 (4.8) (95% CI) (1.0 – 13.5)	36/143 (25.2) (95% CI) (18.3 – 33.1)	21/69 (30.4) (95% CI) (19.9 - 42.7)
Risk difference ISIS 416858 – enoxaparin, %(upper limit of 90% CI)		-25.6 (-17.7)	-5.3 (3.2)	
ISIS 416858 versus enoxaparin, p-value for superiority		<0.001	0.42	

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