RESEARCH PROTOCOL

TropicALL study

Thromboprophylaxis in Children treated for Acute Lymphoblastic Leukemia with Low-molecular-weight heparin: a randomized controlled trial

A multi-center trial in cooperation with the Dutch Childhood Oncology Group

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# TABLE OF CONTENTS

1. **INTRODUCTION AND RATIONALE** ................................................................................................................................. 6
2. **OBJECTIVES** ................................................................................................................................................................. 7
3. **STUDY DESIGN** ............................................................................................................................................................... 7
4. **METHODS** .................................................................................................................................................................. 7
   4.1 Study population .......................................................................................................................................................... 7
   4.2 Randomization and Stratification ............................................................................................................................... 8
   4.3 Treatment plan ........................................................................................................................................................... 9
   4.3.1 Intervention arm A ................................................................................................................................................ 9
   4.3.2 Standard of care control arm (arm B) .................................................................................................................. 9
   4.4 Follow-up of patients .................................................................................................................................................. 10
   4.5 LMWH in the treatment arm ....................................................................................................................................... 11
   4.6 Interruption rules for thromboprophylaxis (nadroparin/LMWH) ......................................................................................... 11
   4.7 Premature discontinuation study medication ........................................................................................................ 11
   4.8 Treatment compliance ................................................................................................................................................ 12
   4.9 Management of bleeding in children ......................................................................................................................... 12
5. **INVESTIGATIONAL MEDICINAL PRODUCT** ............................................................................................................... 13
   5.1 Name and description of investigational medical product: .......................................................................................... 13
   5.2 Summary of findings from clinical studies ................................................................................................................ 13
   5.3 Summary of known and potential risks and benefits ............................................................................................... 14
   5.4 Description and justification of route of administration and dosage ......................................................................... 14
   5.5 Preparation and labelling of Investigational Medicinal Product .................................................................................. 14
   5.6 Drug accountability .................................................................................................................................................... 14
6. **OUTCOMES** ................................................................................................................................................................. 15
   6.1 Endpoints ................................................................................................................................................................. 15
   6.2 Definitions ............................................................................................................................................................... 15
   6.2.1 Venous thromboembolism (VTE) ...................................................................................................................... 15
   6.2.2 Bleeding ........................................................................................................................................................... 17
7. DATA COLLECTION

7.1 Study parameters

7.2 Blood samples

8. STATISTICAL AND POWER ANALYSIS

8.1 Power analysis

8.2 Statistical analysis

9. ADVERSE EVENTS

9.1 Section 10 WMO event

9.2 Definitions

9.2.1 Adverse events (AE)

9.2.2 Serious adverse events (SAE)

9.3 Relationship of AE to the thrombophrophylaxis

9.4 Intensity of an AE, action taken and outcome

9.5 Reporting of SAEs

9.6 Study specific exceptions to the (S)AE reporting

9.7 Suspected unexpected serious adverse reactions (SUSAR)

9.8 Follow-up of adverse events

9.9 Data Safety Monitoring Board (DSMB)

10. PREMATUOSE TERMINATION OF THE STUDY

11. ETHICAL CONSIDERATIONS

12. DATA MANAGEMENT

13. FINANCIAL ASPECTS

14. REFERENCES
SUMMARY

A. RATIONALE
Venous thromboembolism (VTE) often complicates the treatment of acute lymphoblastic leukemia (ALL), particularly during asparaginase therapy.

B. STUDY OBJECTIVES

Primary objective:
To assess the efficacy of thromboprophylaxis with high prophylactic dose LMWH as compared with standard care without systemic thromboprophylaxis in children treated for primary ALL during asparaginase treatment.

Secondary objectives:
1. To assess the safety of thromboprophylaxis using high prophylactic dose LMWH as compared with standard of care without systemic thromboprophylaxis in children treated for newly diagnosed ALL, by assessment of the incidence of major bleeding during asparaginase treatment.
2. To identify clinical risk factors or hematological biomarkers in ALL patients with and without symptomatic objectified VTE; to increase insight in the pathogenesis of coagulation disorders during ALL treatment and to establish a risk model for VTE.

C. STUDY DESIGN
A national, multi-centre, randomized controlled, open-label trial

D. STUDY POPULATION
A total of 354 children between 1 and 19 years of age with primary ALL, who are treated within or according to the DCOG ALL-11 or subsequent protocol.

E. TREATMENT PLAN AND METHODS

Intervention-arm
In the intervention arm, high prophylactic dose LMWH (nadroparin) is subcutaneously injected daily, adjusted to actual body weight with 85 IU anti-Xa/kg with a maximum of 5700 IU anti-Xa daily. Target anti-Xa level: 0.3-0.4 IU/ml

Standard of care-arm
The comparator treatment in the standard of care control arm is no systemic thromboprophylaxis, i.e. no intervention, which is the current standard during childhood ALL treatment. Placebo injections will not be applied.

F. OUTCOMES

Primary efficacy endpoint:
Incidence of symptomatic objectified VTE during childhood ALL treatment in the intervention and standard arm during asparaginase treatment.
Secondary endpoints:
1. Incidence of major bleeding in the intervention and standard arm during asparaginase treatment.
2. Incidence of the clinically relevant non-major bleeding and minor bleeding in the intervention and standard arm during asparaginase treatment.
3. Incidence of composite of asymptomatic and symptomatic objectified VTE during childhood ALL treatment in the intervention and standard arm during asparaginase treatment.
4. Identification of clinical risk factors and hematological biomarkers in consecutively included patients with and without VTE; to increase insight in the pathogenesis of coagulation disorders during ALL treatment, and to establish a risk model for VTE.
1. INTRODUCTION AND RATIONALE

Acute lymphoblastic leukemia (ALL) is diagnosed in up to 120 children in The Netherlands annually and is the most common cancer in this age group. ALL is treated with chemotherapy, aimed at inducing a lasting remission of the disease. Asparaginase is a key component of ALL therapy. Its use has increased clinical remission rates up to 98% and intensive use of asparaginase in the early phases of treatment has significantly improved cancer-free survival. Studies including asparaginase in treatment for newly diagnosed ALL have shown a survival advantage above those without this agent. Although the role of asparaginase is thus fairly undisputed, serious complications associated with its use still represent a major issue.

A frequent and serious complication during ALL treatment is venous thromboembolism (VTE). Reported VTE incidences vary from 2 to 37%. Its occurrence is mainly associated with the use of asparaginase and steroids. The background annual incidence of VTE in children is 1:100,000, illustrating the importance of ALL as a risk factor. In the Dutch Childhood Oncology Group (DCOG) ALL-10 study, the incidence of VTE, defined as symptomatic, objectively diagnosed events requiring anticoagulation treatment, hospitalization, intervention, or of fatal nature, was as high as 10% (75 of 780 children), including catheter-related VTE. Incidence might have been even higher as VTE events were not systematically registered. 53% of VTE was observed during the first weeks of remission induction treatment, in which asparaginase was particularly administered. VTE during remission induction in the ALL-10 was often located in the cerebral veins (25% of VTE), particularly the sinovenous system. A considerable proportion of VTE also occurred in the first 35 weeks of intensification in the Medium Risk group during repetitive PEG-asparaginase therapy (36% of all VTE; in 7% of Medium Risk patients). Stratification of VTE events by age groups showed that 43% occurred in children < 10 years and 57% in children ≥ 10 years.

Venous thromboembolism induced complications
Cerebral VTE is associated with a high direct mortality rate (20% in ALL-10), 10-20% severe neurological morbidity and 3% recurrence. Patients with VTE of the upper or lower limbs, also after catheter-related VTE, frequently develop a lifelong post-thrombotic syndrome (up to 50% of patients), a clinical syndrome of pain, swelling and skin changes of the affected limb. Furthermore, VTE may lead to suboptimal ALL therapy, due to the necessity to interrupt, delay (due to loss of central venous access or need for replacement of a central venous catheter), or discontinue chemotherapy. Studies have shown that optimal use of asparaginase improves ALL outcomes, while insufficient exposure to asparaginase leads to decreased survival. Moreover, VTE requires anticoagulation therapy with subcutaneous injections twice daily for 3 to 6 months, or for as long as chemotherapy is given.

Thromboprophylaxis in children with ALL
VTE reduction by efficient thromboprophylaxis can optimize treatment of childhood ALL, may improve outcome and can decrease side effects. This will enable sufficient exposure to asparaginase, consequently leading to better disease outcome. The need for and optimal approach of thromboprophylaxis during ALL treatment are widely discussed. Clinical practice differs substantially
between centers and globally in the absence of evidence-based guidelines. The most frequently used measures to reduce VTE during ALL treatment are supplementation of fresh frozen plasma (FFP) or antithrombin concentrate, or prophylactic use of low-molecular-weight heparin (LMWH). Studies on the efficacy of FFP or antithrombin are mostly retrospective or observational and report mostly negative results. Pilot studies with prophylactic LMWH show promising efficacy, especially in children with a high VTE risk, with a negligible bleeding risk. In some Europe countries, prophylactic LMWH is used in children with ALL and high risk of thrombosis. However, the efficacy and safety of thromboprophylaxis with LMWH during ALL treatment has not yet been investigated in randomized controlled trials.

2. OBJECTIVES

Primary Objective: To assess the efficacy of thromboprophylaxis with high prophylactic dose LMWH as compared with standard care without systemic thromboprophylaxis in children treated for primary ALL according to the DCOG ALL 11 or subsequent protocol.

Secondary Objectives:
1. To assess the safety of thromboprophylaxis using high prophylactic dose LMWH as compared with standard of care without systemic thromboprophylaxis in children treated for newly diagnosed ALL, by assessment of the incidence of major bleeding during asparaginase treatment.
2. To identify clinical risk factors or hematological biomarkers in ALL patients with and without symptomatic objectified VTE; to increase insight in the pathogenesis of coagulation disorders during ALL treatment and to establish a risk model for VTE.

3. STUDY DESIGN

This is a multicenter randomized controlled open-label trial comparing thromboprophylaxis with high prophylactic dose LMWH as compared with standard care without systemic thromboprophylaxis, i.e. no intervention. Placebo injections will not be applied. Thromboprophylaxis will be given in an open-label setting, without blinding for patients or physicians.

4. METHODS

4.1 Study population
The study population consists of children between 1 and 19 years of age with primary ALL, who are treated within or according to the DCOG ALL-11 or subsequent protocol. Patients will be recruited by their treating pediatric-oncologist or –hematologist.
Inclusion criteria

a. Written informed consent for TropicALL randomisation has been given
b. Newly diagnosed patients with T-lineage or precursor-B lineage ALL (patients with mature B-ALL are not eligible)
c. Age between ≥ 366 days and < 19 years
d. Diagnosis ALL confirmed by DCOG laboratory
e. Patient should be treated in a Dutch Childhood Oncology Centre

Exclusion criteria

a. Patients who are already being treated with anticoagulation upon screening (for other indications)
b. Patients with a heparin allergy (or for one of its components), a recent history (within 6 months) of heparin-induced thrombocytopenia (HIT) or any other contraindication listed in the local labeling of LMWH
c. Patients with active bleeding or high risk for bleeding contraindicating anticoagulant therapy (Thrombocytopenia is not an exclusion criterion)
d. Patients with renal insufficiency (glomerular filtration rate (GFR) < 30 ml/min/1.73m²)
e. Patients with hepatic disease which is associated with coagulopathy leading to a clinically relevant bleeding risk
f. Patients with stage 2 hypertension defined as blood pressure confirmed > 99th percentile + 5 mmHg
g. Patients with any condition that, as judged by the investigator, would place the patient at increased risk of harm if he/she participated in the study.
h. Patients who are included in the ALL-11 IVIG study
i. Patients with Ph-positive ALL (documented presence of t(9;22)(q34;q11) and/or of the BCR/ABL fusion transcript). These patients will be transferred to the EsPhALL protocol in induction according to the guidelines of the EsPhALL protocol.

A register of all patients who were eligible, but not included in the study, will be kept and reason for refusal of participation will be recorded.

4.2 Randomization and Stratification

Patients will be randomized after informed consent has been obtained. After informed consent is obtained patients will be randomized at the start of induction treatment. LMWH prophylaxis starts within the first week of ALL-treatment. Patients will be randomized to one of the two treatment arms (equal n’s in both arms):

A. intervention arm (arm A) with thromboprophylaxis by high prophylactic dose LMWH
B. standard of care control arm (arm B) without thromboprophylaxis.

Eligibility for the TropicALL study will be evaluated directly after study inclusion in the ALL-11 or subsequent protocol. In ALL-11, inclusion in the TropicALL study will take place within the first week of
ALL treatment (day 11 at the latest), and after receiving written informed consent. Randomization will take place on day 11, the day before the first PEG-asparaginase administration (day 12). Randomization of each patient will be performed by a GCP proof randomization computer program at the DCOG trial office.

The TropicALL study is an open-label study; therefore no blinding of patients / parents / caretakers, physicians or investigators is applicable. The standard of care control arm (arm B) will receive no intervention, i.e. no placebo injections are applied.

Randomization will be stratified and blocked to ensure equal distribution of patients over study arms. Stratification will be done according to:
- type of ALL (B-cell or T-cell).
- study centre

4.3 Treatment plan

4.3.1 Intervention arm A

Previous analysis showed that the majority of VTE occurred in (induction) treatment cycles with frequent use of (PEG-)asparaginase. Therefore, we will apply thromboprophylaxis to all patients in all treatment cycles with asparaginase therapy, from the day asparaginase is started until:
- 14 days (for PEG-asparaginase) or
- 7 days (for Erwinia asparaginase)
after the end of asparaginase therapy.

This is based on the pharmacokinetics of PEG- and Erwinia asparaginase, respectively; the end of the maximum therapeutic and thrombotic effect occur respectively 14 and 7 days after each administration.

In case of substitution of 1 dose PEG-asparaginase by 6 doses Erwinia asparaginase (3 times/ week) due to clinical allergy or silent inactivation: thromboprophylaxis should be given for the total duration of Erwinia asparaginase therapy until 7 days after the last administration of Erwinia asparaginase.

In ALL-11, PEG-asparaginase is administered in:
- Remission Induction Phase (cycles IA and IB):
  • in case of non-continuous asparaginase schedule:
    Induction IA (day 12, 26, 40; 1500 IU/m2);
  • in case of continuous asparaginase schedule:
    Induction IA (day 12, 26, 40; 1500 IU/m2), Induction IB (day 54, 68; with an individualized dose) and Protocol M (day 9, 23, 37, 51; individualized dose).
- Medium Risk Intensification (Medium Risk group patients (>60% of all patients)):
during Intensification, every 2 weeks for 14 times in standard non continuous arm or 8 times in continuous arm.

Both arms have the same number of total PEG-asparaginase administrations (=17 times).

In summary:
Patients treated according to the standard risk and high risk ALL-11 protocol will receive thromboprophylaxis in cycles 1A and 1B, during 3 asparaginase administrations: 6 weeks=42 days.

Patients treated according to the medium risk ALL-11 protocol will receive thromboprophylaxis until two weeks after the last of total 17 asparaginase administrations in the medium risk intensification phase: in total 34 weeks=238 days for both the non-continuous arm and the continuous arm.

Hence, in the Intervention arm (arm A), patients treated according to the MR ALL 11 protocol will receive thromboprophylaxis with high prophylactic dose LMWH according to the following outline (Table 1):

<table>
<thead>
<tr>
<th>Table 1 – Thromboprophylaxis outline Intervention arm (arm A) TropicALL study for patients treated according to medium risk ALL 11 protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>non-continuous asparaginase schedule</strong></td>
</tr>
<tr>
<td><strong>Induction</strong></td>
</tr>
<tr>
<td>- start: day 12 of Induction IA;</td>
</tr>
<tr>
<td>• continued until: day 54 (6 weeks=42 days)</td>
</tr>
<tr>
<td>in <strong>Induction IA and IB</strong>;</td>
</tr>
<tr>
<td>(until 14 days after the last PEG-asparaginase administration or 7 days after the last Erwinia asparaginase administration)</td>
</tr>
<tr>
<td><strong>Medium Risk Intensification</strong></td>
</tr>
<tr>
<td>• restart: in <strong>week 1</strong> (on day of first asparaginase administration);</td>
</tr>
<tr>
<td>• continued until: <strong>week 29</strong> (28 weeks=196 days)</td>
</tr>
<tr>
<td><strong>Total 34 weeks=238 days</strong></td>
</tr>
<tr>
<td><strong>continuous asparaginase schedule</strong></td>
</tr>
<tr>
<td><strong>Induction, Protocol M and Medium Risk intensification</strong></td>
</tr>
<tr>
<td>• start: day 12 of <strong>Induction IA</strong>;</td>
</tr>
<tr>
<td>• continued until <strong>week 17</strong> of MR intensification</td>
</tr>
<tr>
<td><strong>Total 34 weeks=238 days</strong></td>
</tr>
</tbody>
</table>

**4.3.2 Standard of care control arm (arm B)**
Patients randomized to the standard of care control arm, will not receive any form of systemic thromboprophylaxis, i.e. no intervention. Placebo subcutaneous injections are not applied. Specifically, FFP or antithrombin should not be given for the purpose of thromboprophylaxis, also not in case of
decreased laboratory values. However, if FFP is needed for other clinical indications than thromboprophylaxis, such as sepsis, it should be used as deemed necessary.

4.4 Follow-up of patients

All patients will be prospectively followed until 3 months after the last PEG-asparaginase administration, until recurrence of ALL or until death, whatever endpoint comes first.

4.5 LMWH in the treatment arm

In patients randomized to the intervention arm (arm A), a high prophylactic weight-adjusted dose LMWH (nadroparin) is injected subcutaneously once daily, aimed at an anti-Xa level of 0.3-0.4 IU/ml. Starting dose will be 85 IU/kg, with a maximum of 5700 IU once daily. Prefilled syringes of 9500 IU anti-Xa/mL, 0.3 mL, 0.4 mL or 0.6 mL, will be adjusted to the calculated weight-adjusted dose.

Peak anti-Xa levels (aim: 0.3-0.4 IU/ml) should be measured once, 3 to 5 days after the start of LMWH prophylaxis, as previous studies indicate that especially young children require higher dosages of LMWH.3 Blood samples (citrate) must be taken 4-6 hours after LMWH administration and measured for anti-Xa levels at local treatment sites.

The route of administration is by subcutaneous injections; by self-administration or by administration by their parents / caretakers or nurses. Patients or their parents / caretakers will be instructed by a trained nurse to perform subcutaneous nadroparin injections independently for the period of thromboprophylaxis in the outpatient setting. Emla cream can be applied to the skin locally for anesthesia before each subcutaneous injection.

If nadroparin is not tolerated, other LMWHs can be administered according to local availability.

4.6 Interruption rules for thromboprophylaxis (nadroparin/LMWH)

1. Thromboprophylaxis (nadroparin/LMWH) should be stopped 24 hours before lumbar puncture or other interventions, such as insertion of central venous catheters; thromboprophylaxis should be restarted after invasive procedures or surgical interventions within 24 hours, provided the clinical situation allows and adequate hemostasis has been established.

2. Thromboprophylaxis (nadroparin/LMWH) should be temporarily interrupted if platelet levels drop below 20x10^9/L; as thrombocytopenia is expected, particularly during Induction IA and IB, platelet levels should be monitored regularly (1-2x per week / upon initiation of new treatment cycles); platelet transfusions are not advised other than standard of care (transfusions only in case of bleeding);

3. Thromboprophylaxis (nadroparin/LMWH) should be temporarily interrupted in case of serious bleeding (see Management of bleeding in children 4.9)

4.7 Premature discontinuation study medication

Children can prematurely discontinue study medication:
• At their own request or at the request of their parents/legally acceptable representative without the need to provide a reason
• If, in the investigator’s opinion, study medication should be stopped for any reason
• If the patient has an indication for therapeutic dosages of anticoagulation as result of thromboembolic events

Subjects can discontinue their participation in the study at any time for any reason if they wish to do so without any consequences for their care. Patients will be taken off of thromboprophylaxis (nadroparin/LMWH) and withdrawn from the TropicALL study. These patients will be further treated according to standard of care of ALL-11 or subsequent protocol (no thromboprophylaxis).

Research will be performed according to the code of behaviour set up by the NVK “Verzet in kader van medisch-wetenschappelijk onderzoek”. This implies that research will be stopped in any case of resistance by the minor participant.

Subjects will not be replaced after withdrawal.

The investigator will ask to continue follow-up of the patients according to the protocol, i.e. until 3 months after the end of the ALL-11 or subsequent protocol treatment period, with the intention to get complete follow-up, or a minimum of vital status and the occurrence of VTE or major bleeding. If child/parent/legal representative indicate that they no longer authorize the investigator to obtain outcome data, this will be respected and documented in the source records.

In children who prematurely discontinue study treatment for other reasons than withdrawal of the informed consent, study visits will take place as planned only with the reason to collect potential study outcomes and AEs.

4.8 Treatment compliance

Instruction will be given to return all unused drugs, as well as the empty drug boxes at each visit to the hospital. A registration booklet is created on which administration of the daily injections must be registered by parents / caretakers during the entire period of thromboprophylaxis. Booklets will also include questions on the burden of daily subcutaneous injections. Information from these booklets will be recorded on CRFs by research nurses.

4.9 Management of bleeding in children

If a child has a serious bleed during study treatment, the following routine measures could be considered after consultation of a hematology specialist:

• Delay the next LMWH administration or discontinue treatment, if indicated
• Consider protamine sulfate administration
• Consider usual treatment for bleeding, including blood transfusion, and/or fresh frozen plasma
• Measure anti-Xa level of LMWH
If bleeding cannot be controlled, consider administration of one of the following procoagulants: recombinant factor VII (Novoseven®) or 4-factor concentrate (Cofact).

5. INVESTIGATIONAL MEDICINAL PRODUCT

5.1 Name and description of investigational medical product:

In children LMWH is the first choice for treatment and prevention of VTE. Therapeutic and preventive doses have been reported for most types of LMWH. In this study, nadroparin (Fraxiparin®, manufacturer GlaxoSmithKline) will be administered subcutaneously in arm A to reach a anti Xa level of 0.3-0.4 IU/ml. If nadroparin is not tolerated, other LMWHs can be administered according to local availability.

5.2 Summary of findings from clinical studies

No randomized clinical trials with nadroparin have been performed in children with ALL for the indication of prevention of VTE. Pilot studies with prophylactic doses of low-molecular-weight heparin (LMWH) showed reduced VTE incidence in pediatric ALL patients. Elhasid et al. report a prospective cohort study in which they administered LMWH prophylaxis to 41 consecutive children with ALL. LMWH was applied by enoxaparin in a median dose of 0.84 mg/kg/day starting at the first dose of L-asparaginase until 1 week after the last dose. No VTE was seen in 41 patients with enoxaparin (during 76 courses of L-asparaginase) and no bleeding episodes occurred. In a historical cohort of the same research group without enoxaparin, two VTE events occurred in 50 patients. They concluded that enoxaparin prophylaxis is safe and seems effective in the prevention of VTE in ALL patients during L-asparaginase therapy.

Meister et al. performed a prospective double cohort study in children undergoing ALL treatment comparing the effect of antithrombin substitution alone versus combined antithrombin substitution plus LMWH. Both cohorts received antithrombin supplements when plasma antithrombin levels dropped ≤ 50%. LMWH was applied by enoxaparin in a dose of 0.75-1.2 mg/kg starting at the first dose of L-asparaginase until plasma antithrombin levels resolved within 1-2 weeks after cessation of asparaginase. Nearly 60% of all children needed at least one, most needed two or three antithrombin supplementations during induction therapy. VTE occurred in 9 of 71 (12.7%) of patients with antithrombin substitution alone, while no VTE occurred in 41 patients with antithrombin plus LMWH (p=0.02). The authors concluded that enoxaparin prophylaxis was safe and effective in preventing VTE.

In a pilot study Mitchell et al. showed a significant better thrombosis free survival in children with ALL and high risk score receiving prophylactic LMWH compared to the children without prophylactic LMWH (p=0.023). LMWH started before catheter insertion and was administered until the end of induction therapy. No bleeding complications occurred.
5.3 Summary of known and potential risks and benefits
At the moment, children with ALL and thrombosis are treated with LMWH for 3 months. After this period, LMWH is mostly continued in prophylactic dose until such time as the central venous catheter is removed. In some Europe countries, prophylactic LMWH is used in children with ALL and high risk of thrombosis. However, the efficacy and safety of thromboprophylaxis with LMWH during ALL treatment has not yet been investigated in randomized controlled trials. Potential benefit of thromboprophylaxis is prevention of VTE, of which 25% is cerebral VTE in ALL patients. Harm consists of an increased risk of bleeding. However, all cohort studies about thromboprophylaxis did not show increased risk of bleeding in children. The burden is that patients will have to receive or administer subcutaneous injections for the entire duration of asparaginase therapy. Use of EMLA may diminish the pain of subcutaneous injections.

5.4 Description and justification of route of administration and dosage
LMWH is injected subcutaneously once daily. Starting dose will be 85 IU/kg, with a maximum of 5700 IU once daily. Prefilled syringes of 9500 IU anti-Xa/mL, 0.3 mL, 0.4 mL or 0.6 mL, will be adjusted to the calculated weight-adjusted dose. Peak anti-Xa levels (aim: 0.3-0.4 IU/ml) should be measured once, 3 to 5 days after the start of LMWH prophylaxis, as previous studies indicate that especially young children require higher dosages of LMWH. Blood samples (citrate) must be taken 4-6 hours after LMWH administration and measured for anti-Xa levels at local treatment sites.

5.5 Preparation and labelling of Investigational Medicinal Product
The use of the investigational medicinal product (LMWH) is open-label. Study medication will be prescribed in the usual setting of patient care and will not be reimbursed by the sponsor. No specific labelling for research purposes will be performed.

5.6 Drug accountability
This is a pragmatic clinical trial that mimics the real life setting of patient care. Nadroparin will be used from commercial stock. Drug accountability is performed on a patient named basis by registration of distributed batch numbers.
During outpatient periods (e.g. Medium Risk Intensification), nadroparin is distributed by the local (hospital) pharmacy every 2 weeks after PEG-asparaginase administrations. Distributed batch numbers are registered on a patient named basis and patients or their parents / caretakers are required to return empty drug boxes.
6. OUTCOMES

6.1 Endpoints

*Primary efficacy endpoint:*
Incidence of symptomatic objectified VTE during childhood ALL treatment in the intervention and standard arm.

*Secondary endpoints:*
1. Incidence of major bleeding in the intervention and standard arm during asparaginase treatment.
2. Incidence of the clinically relevant non-major bleeding and minor bleeding in the intervention and standard arm during asparaginase treatment.
3. Incidence of composite of asymptomatic and symptomatic objectified VTE during childhood ALL treatment in the intervention and standard arm during asparaginase treatment.
4. Identification of clinical risk factors and hematological biomarkers in consecutively included patients with and without VTE; to increase insight in the pathogenesis of coagulation disorders during ALL treatment, and to establish a risk model for VTE.

6.2 Definitions

6.2.1 Venous thromboembolism (VTE)
The following definitions are applied for a suspected episode of **symptomatic VTE**:

*Symptomatic VTE* is defined as symptomatic venous thrombosis in any component of the venous or pulmonary arterial circulations or the heart, including symptomatic cerebral sinovenous and central venous catheter (CVC)-associated VTE, requiring anticoagulation treatment, intervention, life-saving measures, ALL treatment adjustments, or of fatal nature, and objectively confirmed by standard imaging tests:

- cerebral venous thrombosis: computerized tomography (CT) venography or conventional angiography, or magnetic resonance (MR) imaging of the head with MR venography;
- upper extremity thrombosis: compression (Doppler) ultrasonography or venography;
- upper body central venous catheter-related VTE: echocardiography (Doppler), Doppler ultrasonography or venography;
- lower extremity deep vein thrombosis (DVT): compression (Doppler) ultrasonography or venography;
- pulmonary emboli (PE): CT angiography.

No screening for asymptomatic VTE should be done in any form.
However, if thrombi are incidentally found upon diagnostic imaging for other indications, these should be counted as **asymptomatic VTE events**.
To minimize a diagnostic bias for suspected symptomatic VTE in patients without thromboprophylaxis, observed symptoms and rationale for diagnostic imaging should be clearly stated in the CRF (VTE section) for all patients. All imaging tests are assessed for presence or absence of VTE by local radiologists per treatment center, who have appropriate expertise, who are not directly involved in the patient’s care, and who are blinded to treatment groups.

**Symptomatic DVT or upper extremity thrombosis** is defined as suspicion of VTE based on clinical symptoms, such as swelling, erythema, skin discoloration, increased warmth, pain, tenderness, venous distension, or presence of subcutaneous collateral veins. **Symptomatic DVT or upper extremity thrombosis** is confirmed with one of the following findings:

If there were no previous DVT investigations:
- abnormal compression ultrasound (CUS);
- an intraluminal filling defect on venography.

If there was a previous DVT investigation:
- abnormal CUS where compression had been normal or, if non-compressible during screening, a substantial increase (4 mm or more) in diameter of the thrombus during full compression;
- an extension of an intraluminal filling defect, or a new intraluminal filling defect or an extension of non-visualization of veins in the presence of a sudden cut-off on venography.

**Symptomatic PE** is defined as suspicion of PE based on clinical symptoms, such as dyspnea, tachypnea, chest pain, hemoptoe, decreased oxygen saturation, collapse, tachycardia, and increased oxygen need, and it is confirmed with one of the following findings:
- a (new) intraluminal filling defect in subsegmental or more proximal branches on spiral CT scan
- a (new) intraluminal filling defect or an extension of an existing defect or a new sudden cut-off of vessels more than 2.5 mm in diameter on the pulmonary angiogram
- a (new) perfusion defect of at least 75% of a segment with a local normal ventilation result (high-probability) on ventilation/perfusion lung scan (VPLS)

**Symptomatic cerebral (sino)venous VTE** is defined as suspicion of VTE based on clinical symptoms of increased intracranial pressure, such as persistent or extreme headache, persistent post-dural puncture headache, seizures, or focal neurological deficits and is confirmed with one of the following findings:
- a (new) intraluminal filling defect, ‘empty triangle sign’ or ‘delta-sign, or presence of a thrombus in one of the cerebral veins or sinuses on MR imaging with MR venography, CT venography or conventional angiography
- a (new) (non-)hemorrhagic cerebral infarct attributable to a cerebral VTE

**Symptomatic central venous catheter (CVC)-associated VTE** is defined as suspicion of VTE based on clinical symptoms such as swelling, erythema, skin discoloration, increased warmth, pain, tenderness,
venous distension, presence of subcutaneous collateral veins, loss of central venous catheter patency, catheter-related septicemia unresponsive to standard antibiotic treatment, or unexplained thrombocytopenia. Also, CVC-associated objectified VTE requiring CVC removal or revision are considered symptomatic. A CVC-related VTE is considered to be “objectified” if there is a constant intraluminal filling defect, absence of blood flow, or constant non-visualization of a proximal vein in the lower venous system or a vessel proximal to the axillary vein in the upper venous system, in the presence of a sudden cut-off, present on at least two projections. Extensive collateral venous circulation in the limb associated with the CVL is also accepted as confirmatory evidence of a CVL-related VTE.

*Incidental thrombi* found at the catheter tip upon CVC-removal or diagnostic imaging, without any clinical symptoms, are considered asymptomatic and should be reported as *asymptomatic VTE events*.

**Treatment of VTE events**
If VTE events are treated with therapeutic anticoagulation according to standard care, start of therapeutic anticoagulation implies (premature) discontinuation of thromboprophylaxis.

### 6.2.2 Bleeding

Bleeding is categorized as major, clinically relevant non-major or minor bleeding, according to Perinatal and Pediatric Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis criteria \(^8\)

1. **Major bleeding:**
   a. fatal bleeding;
   b. clinically overt bleeding associated with a decrease in hemoglobin of \(\geq 3.1\) mmol/L in a 24-hour period;
   c. bleeding intracranial, retroperitoneal, pulmonary, or otherwise involving the central nervous system;
   d. bleeding that requires surgical intervention in an operating suite.

2. **Clinically relevant non-major bleeding:**
   a. overt bleeding for which blood product is administered and not directly attributable to the patient’s underlying medical condition;
   b. bleeding that requires medical or surgical intervention to restore hemostasis, not in an operating suite.
3. Minor bleeding:
   any overt or macroscopic evidence of bleeding that does not fulfill above criteria for clinically overt or clinically relevant nonmajor bleeding. Menstrual bleeding resulting in medical consultation and/or intervention is classified as minor bleeding.

All cases of major bleeding will be reported as serious adverse event (SAE).

Interruption rules are addressed in paragraph 4.6
Management of bleeding is addressed in paragraph 4.9

7. DATA COLLECTION

7.1 Study parameters
The following data of all patients will be obtained at the time of randomization:
- initials, date of birth, gender, weight
- date of diagnosis, ALL diagnosis and subtype, prognostic risk classification
- center of treatment
- hemoglobin level, white blood cell count, platelet count, central nervous system infiltration of ALL, CRP
- medication other than ALL treatment
- insertion date, type of catheter, flushing method, removal and reinsertion of a CVC, as well as reason of removal, catheter dysfunction

Furthermore: clinical risk factors for VTE will be obtained at time of randomization and during the study:
- age, gender
- family history of thrombosis, presence of thrombophilia in family
- presence of CVC
- corticosteroid treatment, ALL type
- number of septicemia/bacteremia, treated with antibiotics.
- type of asparaginase therapy (PEG asparaginase or Erwinia asparaginase), asparaginase levels measured at specified time points), asparaginase antibodies (present or not present)

7.2 Blood samples
Previous studies have shown that presence of thrombophilia is associated with a higher VTE risk. Hence, one EDTA blood sample (3 ml) for DNA extraction will be collected for evaluation of genetic thrombophilic mutations (factor V Leiden and factor II mutation) upon study inclusion.

Plasma citrate samples will be consecutively collected upon study inclusion, during Induction cycles and during Intensification in Medium Risk group patients. Blood collection dates coincide with other set sample collection dates for monitoring of asparaginase activity and antibody formation in ALL-11 or subsequent protocol, and therefore do not form an extra burden for patients.
Blood sample collection dates include (appendix 2):
- at inclusion of study (within the first week, day 7 at the latest)
- Induction IA and IB: before the 2 and 3rd asparaginase administration
- Medium Risk group children: in week 1 of Intensification and on the last day of PEG-asparaginase therapy of Intensification
Each blood collection requires 5 ml of blood in citrate tubes.

Samples of patients with and without VTE will be used to explore the presence of several potential biomarkers during ALL treatment. The list of assessed biomarkers includes, but is not limited to, antithrombin, fibrinogen, D-dimer, TAT, thrombin generation assays. Biomarkers are assessed at the end of the study. The definitive list may be subject to change depending on developments in the field of research in the meantime.

Citrate samples should be locally collected and centrifuged for 15 minutes at 4000 RPM within 3 hours after withdrawal. Plasma samples should be stored at participating centers temporarily before central analysis and storage (AMC, Amsterdam). Samples will be centrally stored under anonymous codes for a maximum of 20 years.

8. STATISTICAL AND POWER ANALYSIS

8.1 Power analysis
To perform power calculations previously reported symptomatic VTE incidences are used, in particular the recently performed childhood ALL-10 study (10%; 95% confidence interval (CI) 7.7-11.8). The expected risk reduction is based on pilot studies with LMWH in childhood ALL and thromboprophylaxis with LMWH for other indications.

The efficacy analysis will be based on an intention to treat analysis. The expected loss-to-follow-up is closed to 0-1%
In the table below power computations are performed for several scenarios corresponding to different relative risk reduction (RRR).

<table>
<thead>
<tr>
<th>Relative risk reduction (RRR)</th>
<th>50%</th>
<th>60%</th>
<th>70%</th>
<th>75%</th>
<th>80%</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL-10 VTE incidence (n=780) (95% CI)</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>(7.7-11.8)</td>
<td>(7.7-11.8)</td>
<td>(7.7-11.8)</td>
<td>(7.7-11.8)</td>
<td>(7.7-11.8)</td>
<td></td>
</tr>
<tr>
<td>Expected VTE incidence (n=630) (95% CI)</td>
<td>5%</td>
<td>4%</td>
<td>3%</td>
<td>2.5%</td>
<td>2%</td>
</tr>
<tr>
<td>(3.6-6.7)</td>
<td>(2.8-5.6)</td>
<td>(1.9-4.3)</td>
<td>(1.5-4.0)</td>
<td>(1.2-3.1)</td>
<td></td>
</tr>
<tr>
<td>Necessary n of patients per arm (equal arms)</td>
<td>434</td>
<td>282</td>
<td>194</td>
<td>162</td>
<td>137</td>
</tr>
</tbody>
</table>
Considering the use of a high prophylactic dose of LMWH in this study we consider to be appropriate in symptomatic VTE incidence a relative risk reduction (RRR) equal to 75% and therefore according to the computations shown above a total of n=324 patients will be included for randomization in this study.

The expected inclusion rate of the ALL-11 study is ca. 100 patients/year. The inclusion of n=324 patients is therefore feasible within the remaining duration of ALL-11 (total n=630 patients). If this is not achieved, continuation of the TropicALL study during the subsequent ALL study is foreseen.

8.2 Statistical analysis
In this section all statistical analysis that will be performed is described.

Descriptive statistics
Patients’ characteristics at baseline data will be reported. The number of patients in each population will be reported. Continuous variables will be presented as mean with its corresponding standard deviation (SD) if normally distributed and medians with ranges if data are skewed, categorical variables will be given in percentages.

Analysis populations
All data will be analyzed according to the intention-to-treat (ITT) principle. The ITT population will consist of all patients who have been randomized to the treatment arm. All patients will be analyzed in the specific assigned treatment group, irrespective of withdrawals, dropouts or other reasons for failing to complete the study, after randomization and after having received at least one dosage of thromboprophylaxis (nadroparin/LMWH). The safety-analysis population is defined as all randomize patients who received at least one dose of study treatment. Per protocol analysis population consist of all randomized patients, who completed the total treatment period, excluding participants who failed to receive their allocated treatment.

Efficacy analysis
All efficacy analyses will be performed on the ITT population. Incidence proportions and cumulative incidences will be calculated for the primary (symptomatic objectified VTE) and secondary efficacy outcomes (composite of asymptomatic and symptomatic objectified VTE), observed from randomization up to 14 and 7 days after the last PEG asparaginase and Erwinia asparaginase, respectively.

Safety analysis
All safety analyses will be performed on the safety-analysis population. All bleeding events that occurred during study treatment or within 2 days after stop of study medication will be
summarized. Individual listings of major, clinically relevant non-major and minor bleeding events will be provided. Incidence proportions (number of children with outcome during the period divided by number of children at risk at the beginning of the period) and cumulative incidences will be estimated for the primary safety outcome (major bleeding).

**Risk factors analysis**

To identify which risks factors are associated with time to VTE a joint model for longitudinal data and survival outcome will be used. The longitudinal aspect in the data is given from the two prognostic factors levels of asparaginase and levels of antibody collected at fixed time points during the study. A joint model for longitudinal and survival data must be used since the focus here is on a survival outcomes and we need to account for the effect of time-varying risk factors. Depending on the data distribution a T-test (in case the data are normally distribute) or a Mann-Whitney U-test (in case of skewed data.) will be used to compare continuous variables between the treatment groups Categorical variables will be analyzed using the Chi-square test.

9. **ADVERSE EVENTS**

9.1 **Section 10 WMO event**

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the participating patients and parents and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects’ health. The investigator will take care that all patients and their parents are kept informed.

9.2 **Definitions**

9.2.1 **Adverse events (AE)**

AEs are defined as any undesirable complications occurring to a subject during the study, whether or not considered related to ambulatory care or other study procedures. All grade III/IV AEs according to the NCI-CTC classification (version 4) reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 **Serious adverse events (SAE)**

A Serious Adverse Event (SAE) is any undesirable sign, symptom or medical condition which:
- is fatal or life-threatening
- requires prolonged hospitalization
- results in persistent or significant disability/incapacity
• constitutes a congenital anomaly or a birth defect
• is medically significant, may jeopardize the subject and may require medical or surgical intervention
to prevent one of the outcomes listed above.

Life- threatening events are defined as:
• circulatory/cardiac insufficiency requiring catecholamines/positive inotropes
• respiratory failure requiring intubation/ventilation
• other clinical situations requiring immediate intervention, e.g.
  - gastrointestinal bleeding or perforation requiring surgery
  - cerebral abscess/bleeding requiring immediate neurosurgical intervention.

9.3 Relationship of AE to the thromboprophylaxis
The assessment of the causal relationship between an AE and the use of thromboprophylaxis is a clinical decision based on all available information at the time of the completion of the CRF and is based on whether there was a “reasonable causal relationship” to the medication.

9.4 Intensity of an AE, action taken and outcome
The intensity of an AE is assessed as mild (usually transient in nature and not interfering with normal activities), moderate (sufficiently discomforting to interfere with normal activities), and severe (prevents normal activities).

Any action on study treatment to resolve the AE is to be documented as either: thromboprophylaxis withdrawn, interrupted, dose reduced, dose not changed or dose not increased, not applicable or unknown. Other specific treatments of AEs will be documented as: none, remedial drug therapy or other. The outcome of the AE is to be documented as: recovered/resolved, recovering/resolving, recovering/resolving with sequelae, not recovered/resolved, fatal or unknown.

9.5 Reporting of SAEs
All SAEs will be reported through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 15 days after the investigator has first knowledge of the serious adverse reactions. SAEs which also comply with the definitions of a primary or secondary outcome (symptomatic VTE, major or clinically relevant nonmajor bleeding, death) have to be reported to the AMC preferably within 48 hours, but no later than 72 hours of the site’s first knowledge of the event.

If the SAE results in stopping of thromboprophylaxis (nadroparin/LMWH), the patients will be followed-up by an outpatient clinic consult at the remaining regular scheduled visits or until death, whichever comes first.

SAEs that result in death or are life threatening should be reported immediately. The immediate reporting will occur not later than 7 days after the responsible investigator has first knowledge of the
adverse reaction. This is for a preliminary report with another 8 days for completion of the report. Due to the multicenter setting, in case of SAEs the local, designate investigator or clinician will notify the principal investigator as soon as possible for further action. The principal, coordinating investigator of the DCOG ALL-11 or subsequent protocol, is ultimately responsible for reporting SAEs.

9.6 Study specific exceptions to the (S)AE reporting
The efficacy outcomes will not be reported as (S)AE.

9.7 Suspected unexpected serious adverse reactions (SUSAR)
Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator’s Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorised medicinal product). Because of the ample experience with nadroparin/ LMWH, also in children, the occurrence of SUSARs is very unlikely. The coordinating investigators will report expedited SUSARs that have arisen in this study to the AMC. Also, the coordinating investigators will report all SUSARs expedited through the web portal ToetsingOnline to the METC. The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the SUSARs. For fatal or life threatening cases the term will be a maximum of 7 days for a preliminary report with another 8 days for completion of the report.

9.8 Follow-up of adverse events
All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated.

9.9 Data Safety Monitoring Board (DSMB)
This committee has the responsibility to provide the core committee with recommendations related to the protection of the patients’ safety, including stopping recruitment and study treatment. During the study, the DSMB will semi-annually review all incidences of SAEs, symptomatic DVT and PE, and bleeding.

10. PREMATURE TERMINATION OF THE STUDY
The study can be prematurely terminated in case of unexpected dangers or significant increase serious adverse events (SAEs), for instance fatal or major bleeding, associated with the TropicALL study protocol. As pilot studies in pediatric ALL patients with LMWH have been performed safely, we do not expect an increase in SAEs.

In case the Core Committee decides to stop the study prematurely, all patients included in the study who are still on treatment will be contacted by the local study team within 14 days after notification of
this decision. Patients will be taken off study medication and will be treated according to standard patient care, at the discretion of the treating physician.

11. ETHICAL CONSIDERATIONS

Thromboprophylaxis with LMWH has been proven to be safe in children for the prevention of VTE. This study aims to assess the efficacy of thromboprophylaxis on the incidence of symptomatic VTE by use of high prophylactic dose LMWH during ALL treatment. VTE is a common complication of ALL, particularly during asparaginase therapy. The potential benefit of participating is that patients may have a reduced risk of symptomatic VTE and its consequences, while the risk for bleeding may not be increased as compared to standard of care without thromboprophylaxis. The burden is that patients will have to receive or administer subcutaneous injections for the entire duration of asparaginase therapy. A potential risk may be that patients are at additional risk for bleeding complications, especially during thrombocytopenia. Based on previous pilot studies, this risk appears to be low. Additionally, platelet levels will be monitored and thromboprophylaxis (nadroparin/LMWH) interrupted if necessary (see 4.6).

12. DATA MANAGEMENT

All data will be entered in a central database in the SKION, Netherlands. To ensure accurate, complete, and reliable data, the clinical research coordinator and the PIs together with the SKION will do the following:
- Be available for consultation and stay in contact with the study site personnel by mail, telephone and/or fax.
- Review and evaluate CRF data and use standard computer edits to detect errors in data collection.
- Verify the quality of the data.

The clinical research coordinator and the PIs together with the SKION will make yearly visits to the study sites. During that visit, the local study file will be checked. Depending on the findings, a number of CRF’s will be checked against source documents at the study site, with a minimum of one patient per visit. The method and extent of monitoring to be performed for this study is set out in the monitoring manual DCOG TropicALL study.

13. FINANCIAL ASPECTS

The study will be financed by ZonMW 80-83600-98-10186.
Appendix 1.

LMWH randomization in ALL-11 overview

ALL-11 OVERVIEW

0 10 12 14 21 57 104 156

IV SR Maintenance

ICF1 abnormalities:
- MR Intensification/Maintenance 2B with anthracyclines
- MR Maintenance 1
- MR Maintenance 2
- MR Maintenance 3

TEL/AMKL without ICF1 deletion:
- MR Intensification/Maintenance IA without anthracyclines
- MR Maintenance 1
- MR Maintenance 2

Downstream ICF1 deletion:
- MR Intensification/Maintenance IA without anthracyclines
- MR Maintenance 1
- MR Maintenance 2

Others without ICF1 deletion:
- MR Intensification/Maintenance 2B with anthracyclines
- MR Maintenance 1
- MR Maintenance 2

HR1*  HR2  HR3

HR4  HR5  HR6  HR7

MR INTENSIFICATION IA

MR MAINTENANCE IA

MR INTENSIFICATION IB

MR MAINTENANCE IB

Prot

Prot

IVC

PEG-ASP

0 10 21 57 104 156

Weeks

* Duration of HRS-MHS depends on neutropil recovery.

I IV = IVS Randomization: IVS study patients case of SI or HR stratification.

II PEG-ASP = Asparaginase randomization: only patients with VDS positive at T1S (day 30) will be randomized.

LMWH = high prophylactic dose LMWH randomization: LMWH study continues only in HR stratification after Prot IA + IB

LMWH randomization in ALL-11 PROTOCOL IA and IB

ALL-11 PROTOCOL IA and IB

LMWH
- SC B5 IE anti-Xa/kg/day

PRED
- PO 60 mg/m²/day

VCR
- IV push 1.5 mg/m² dose max 2.0 mg/dose

DNR
- IV 1 hr 30 mg/m² dose

PEG-ASP
- IV 1 hr 1,500 IU/m² dose

CPM
- IV 1 hr 1,000 mg/m² dose

ARA-C
- IV push 75 mg/m² dose

6-MP
- PO 60 mg/m²/day

MTX
- ITH acc. to age

MTX/ARA-C/DAF
- ITH acc. to age

IVIG
- IV 0.7 g/kg

Peripheral blood (no SKIN lab)

Peripheral blood ASP monitoring (no Sophia)

BMP (no SKIN lab)

LP (no SKIN lab)

Prednisone response (WBC + diff count) (no SKINlab)

IgG levels (also in control arm)

MRD time point

CRT following period

Anti-Xa level measurement (LMWH A only)

# Not in children with Down Synd or clone.

# Only children with CNS involvement, CR1, or TLP at diagnosis.

PEG-ASP A: Non-corticosteroids, no PEG-ASP.

PEG-ASP B: Individualized dose PEG-ASP.

LMWH A: Control arm, IV 3 x 1250 IU/kg/day.

LMWH B: Treatment arm, IV 1000 IU/kg/day.

LMWH A: Intervention arm, no prophylactic dose LMWH A and B.

LMWH B: Control arm, no prophylactic dose during PROTOCOL IA and IB.

TropicALL protocol versie 3.0, 19 may 2016
LMWH randomization in ALL-11 MR Intensification without anthracyclines

ALL-11 MR Intensification + MR Maintenance part 1A without anthracyclines;
For TEL/AML1 without IKZF1 deletions and for Down syndrome without IKZF1 deletions

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEXA</td>
<td>PO 6 mg/m²/day</td>
</tr>
<tr>
<td>VCR</td>
<td>IV push 2 mg/m²/dose</td>
</tr>
<tr>
<td>MTX</td>
<td>IV push 30 mg/m³/dose</td>
</tr>
<tr>
<td>PEG-ASP</td>
<td>IV 1hr Individualized dose</td>
</tr>
<tr>
<td>6-MP</td>
<td>PO 50 mg/m³/day</td>
</tr>
<tr>
<td>MTX/ARA-C/DAF</td>
<td>ITH acc. to age</td>
</tr>
<tr>
<td>LMWH</td>
<td>SC 85 IE anti-Ka/kg/day</td>
</tr>
<tr>
<td>IVIG</td>
<td>IV 0.7 g/kg</td>
</tr>
</tbody>
</table>

Peripheral blood ASP monitoring (to Sophia)
Monitoring based on dose adjustments, see table 2 § 6.7.2

BMP (to SCION lab)
LP
IgG levels (also in control arm)
CRF following period

LMWH randomization in ALL-11 MR Intensification with anthracyclines

ALL-11 MR Intensification + MR Maintenance part 1B with anthracyclines
For IKZF1 deletions and for patients without IKZF1 deletions who have no TEL/AML1 and no Down syndrome

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEXA</td>
<td>PO 6 mg/m²/day</td>
</tr>
<tr>
<td>VCR</td>
<td>IV push 2 mg/m²/dose</td>
</tr>
<tr>
<td>DOX</td>
<td>IV 1hr 30 mg/m²/dose</td>
</tr>
<tr>
<td>MTX</td>
<td>IV push 30 mg/m³/dose</td>
</tr>
<tr>
<td>PEG-ASP</td>
<td>IV 1hr Individualized dose</td>
</tr>
<tr>
<td>6-MP</td>
<td>PO 50 mg/m³/day</td>
</tr>
<tr>
<td>MTX/ARA-C/DAF</td>
<td>ITH acc. to age</td>
</tr>
<tr>
<td>LMWH</td>
<td>SC 85 IE anti-Ka/kg/day</td>
</tr>
<tr>
<td>IVIG</td>
<td>IV 0.7 g/kg</td>
</tr>
</tbody>
</table>

Peripheral blood ASP monitoring (to Sophia)
Monitoring based on dose adjustments, see table 2 § 6.7.2

BMP (to SCION lab)
LP
IgG levels (also in control arm)
CRF following period

LMWH A: high prophylactic dose LMWH 85 IE anti-Ka/kg/day, 29 weeks in case of PEG-ASP A, 17 weeks in case of PEG-ASP B
LMWH B: no prophylaxis
**Appendix 2. FLOW CHART STUDY PROTOCOL TROPICALL ALL-11 for PEG non-continuous (Arm A)**

<table>
<thead>
<tr>
<th>Study period (protocol ALL 11)</th>
<th>Screening Inclusion</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study day</td>
<td>Day 1-11 Inductie</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 12 Inductie</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 13-15 Inductie</td>
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</tr>
<tr>
<td></td>
<td>Day 26 Inductie</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 40 Inductie</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Day 54 Inductie</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Week 1, day 1 Intensif.</td>
<td></td>
</tr>
<tr>
<td>Study visit</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>8-13</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Type of contact</td>
<td>Visit</td>
<td>Visit</td>
</tr>
<tr>
<td></td>
<td>Visit</td>
<td>Visit</td>
</tr>
<tr>
<td>Eligibility screening</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Randomization</td>
<td>x</td>
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</tr>
<tr>
<td>Weight</td>
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<td>Baseline characteristics</td>
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<td>Prescribe study medication</td>
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<td>x</td>
</tr>
<tr>
<td>Anti Xa measurement</td>
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<tr>
<td>Compliance check</td>
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<tr>
<td>Stop medication</td>
<td>x&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td>Blood sample</td>
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<td>x&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>x&lt;sup&gt;d&lt;/sup&gt;</td>
<td>x&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a: Until 14 days after the last PEG-asparaginase administration or 7 days after the last Erwinia asparaginase administration
b: Blood samples (citrate tube) must be taken **4-6 hours** after nadroparin administration and measured for anti-Xa levels at local treatment site.
In case of anti Xa levels < 0.3 IU/ml or > 0.4 IU/ml dose adjustments should be done
c: Blood sample contains one EDTA blood sample of 1.3 ml for FV Leiden mutation and FVII mutation, and 1 citrate sample of 3 ml. (See d)
d: Blood sample contains each time a citrate tube of 3ml. Citrate samples should be locally collected and centrifuged for 15 minutes at 4000 RPM within 3 hours after withdrawal. Plasma samples should be stored at participating centres temporarily before central analysis and storage (AMC, Amsterdam).

TropicALL protocol versie 3.0, 19 may 2016
Appendix 2. **FLOW CHART STUDY PROTOCOL TROPICALL ALL-11 for PEG continuous schedule (Arm A)**

<table>
<thead>
<tr>
<th>Study period</th>
<th>Screening Inclusion</th>
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</thead>
<tbody>
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<tr>
<td>Randomization</td>
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<tr>
<td>Weight</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Baseline characteristics</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Prescribe study medication</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Anti Xa measurement b</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Compliance check</td>
<td>x x x x x x x x</td>
<td></td>
</tr>
<tr>
<td>Stop medication</td>
<td>x a</td>
<td></td>
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<tr>
<td>Blood sample</td>
<td>X c</td>
<td>X d</td>
</tr>
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TropicALL protocol versie 3.0, 19 may 2016
14. REFERENCES


