Alemtuzumab as remission induction for adult patients with acute lymphoblastic leukemia in relapse

A randomized phase II study

PROTOCOL

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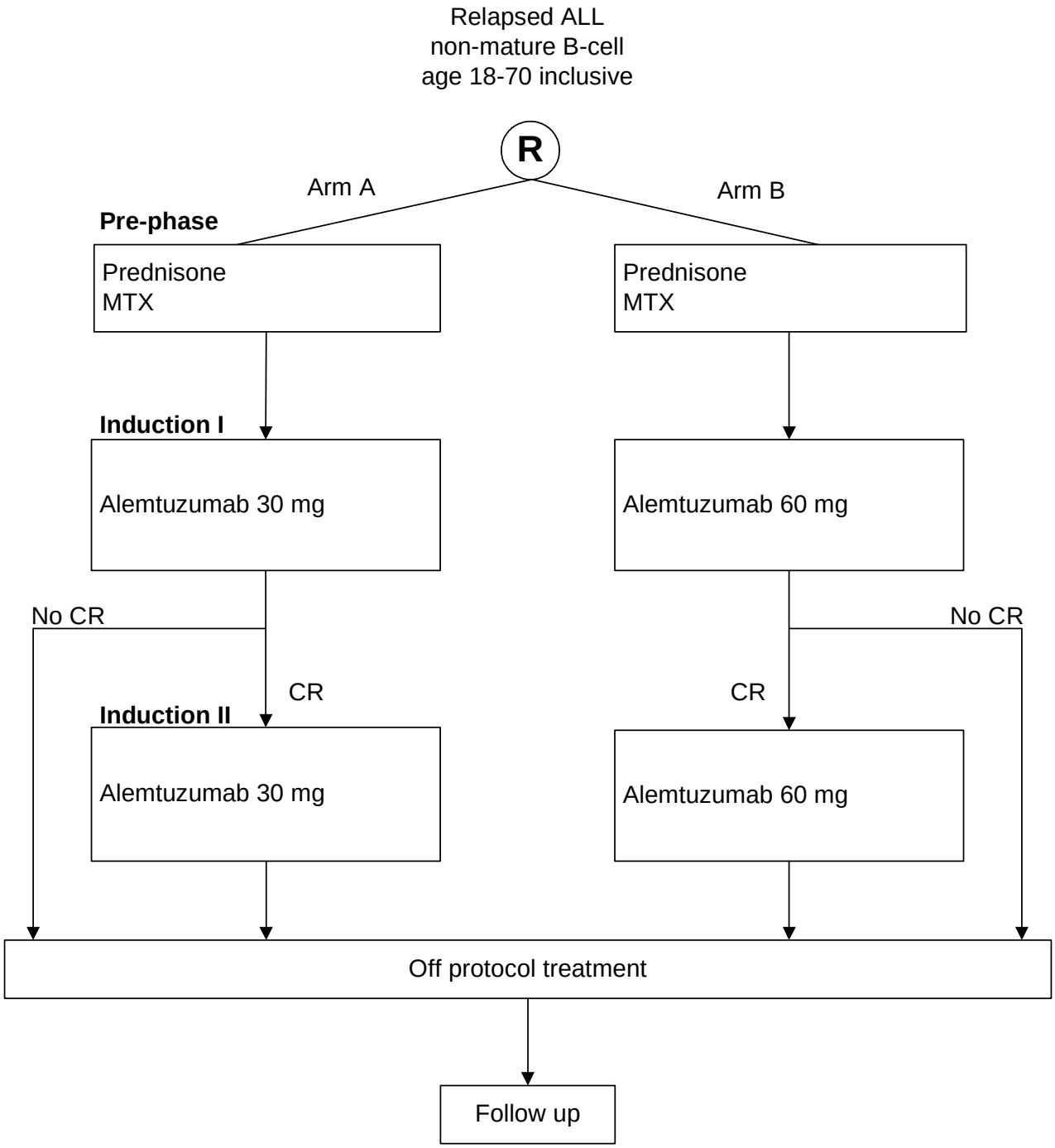
1 Scheme of study



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3 Synopsis

Study phase	Randomized phase II
Study objectives	Evaluation of feasibility of alemtuzumab in adult patients with relapsed ALL after a 1st or 2nd CR
Patient population	Patients, age 18 – 70 years inclusive, with relapsed ALL, non-mature B-cell
Study design	Prospective, multicenter, randomized
Duration of treatment	Expected duration of treatment will be approximately 11 weeks
Number of patients	120 patients registered and randomized
Adverse events	Adverse events will be documented if observed, mentioned during open questioning, or when spontaneously reported
Planned start and end of recruitment	Start of recruitment: IV 2005 End of recruitment: IV 2007

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Study Coordinators	R. Willemze	Leiden University Medical Center
	J.P. Marie	Hotel Dieu Paris
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Cytological and immunophenotype review	M.B. van 't Veer	Hematocytology Review Committee, Erasmus MC, Rotterdam
Statistician	B. van der Holt	HOVON Data Center, Rotterdam
Data management	J.G.M. Melssen	HOVON Data Center, Rotterdam
Serious Adverse Events (SAEs) notification	HOVON Data Center	Fax: +31.10.4391028

4.1 Cytological review

Review by the HRC will be performed at diagnosis of relapse only.
4 unstained blood and 6 unstained bone marrow smears should be sent together with a filled out HRC cytology form and a copy of the report of the immunological marker analysis to Dr. M.B. van 't Veer, Hematocytology Review Committee, Erasmus MC – Daniel den Hoed, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands, at the time of registration. Confirmation of diagnosis is not necessary for registration or start of treatment.

4.2 Cytogenetic review

For the HOVON 74 study cytogenetic review data obtained from previous HOVON ALL trials (HOVON 18, 37, 70 and 71) will be used. For patients who have not been treated within HOVON trials before, central review will be performed for cytogenetic analysis at diagnosis of relapse. Each cytogeneticist, responsible for the cytogenetic analysis of the patients in a hospital will be notified automatically by email of the registration of a patient from that hospital in the study. For patients with no cytogenetic review data (from previous HOVON trials) available a filled out cytogenetic form together with 2 representative karyotypes and a copy of the original cytogenetic report is requested to be sent within 3 months to the HOVON Data Center for central review (all Dutch centers). The participating GIMEMA centers will perform cytogenetic review within the Italian network. For EORTC centers cytogenetic review will be performed within the EORTC network (ms Prof. A. Hagemeijer, UZ Gasthuisberg, Centrum voor Menselijke Erfelijkheid, Herestraat 49, B-3000 Leuven, Belgium, e-mail:anne.hagemeijer@med.kuleuven.ac.be).

If additional FISH analysis was performed, a filled out FISH form together with a copy of the original FISH report is also requested to be sent with the cytogenetic data for central review.

5 Introduction

5.1 Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is a malignant disease that originates from a B- or T-lymphocyte precursor. Malignant transformation is a consequence of somatic mutation in a single lymphoid progenitor cell. This mutation might occur at different stages of B- or T-cell development. The level of differentiation of dominant leukemic clone is well established by immunophenotyping of the leukemic cells. The clonal origin of ALL could be determined by cytogenetic analysis and analysis of T-cell receptor gene or immunoglobulin gene rearrangements (1).

Treatment regimens for ALL have evolved empirically into complex schemes that use numerous agents in various doses, combinations, and schedules (2,3). Unfortunately few of the individual treatment components have been tested rigorously in randomized trials. Thus, it is difficult to analyze critically the absolute contribution of each drug or dose schedule to the ultimate outcome. Numerous nonrandomized trials have attempted to answer these questions, but multiple alterations in study design between sequential trials have made it difficult to assess the exact merit of each modification.

5.2 Remission induction

During the last 20 years the treatment results in adolescent and adult patients have slightly improved. At the beginning CR rate and long term disease free survival were in the range of 40-50% and 20%, respectively (4). Current combinations of vincristine, corticosteroids, an anthracycline, asparaginase and cyclophosphamide result in a complete remission rate of more than 80% in adult patients (5,6). In vitro and in vivo sensitivity to corticosteroids in the remission induction phase predicts prognosis (EORTC childhood ALL, GIMEMA) (7-9). Although in children dexamethasone gave better results than prednisone, for adults we have to await the results of the EORTC ALL-4 trial to answer that question. Induction therapy has been further intensified by increasing the dose of cyclophosphamide, cytarabine, asparaginase and methotrexate or by adding a new drug. The attempt of such approach is to destroy leukemia cells before they develop selective drug resistance. In childhood ALL, L-asparaginase appears to prolong DFS (10). E Coli appeared to be more active than Erwinia asparaginase (11). Longer-acting and potentially less immunogenic enzymes, such as PEG-asparaginase and recombinant asparaginase, are now in clinical trials.

5.3 Post-remission therapy and relapse

To improve the prognosis of adult patients with ALL the current consensus is not to use a single protocol for all ALL patients but to use, after achievement of complete remission, different protocols according to well defined risk groups (13-14). More rapid cytoreduction during the induction course may not increase the already high CR rate, but may produce longer durations of the remission.

Since the beginning of the eighties the EORTC Leukemia Group performed 4 successive randomized studies on adult ALL. The ALL-3 study included high dose Ara-C consolidation and a randomization between an intensive maintenance regimen and autologous stem cell

transplantation (4). Meta-analysis with the more or less similar French LALA trial did not show a difference in DFS and survival between the two arms. The most recent study (ALL-4) included for patients in complete remission a randomization for a consolidation/ maintenance phase (autologous stem cell transplantation followed by low maintenance versus repeated chemotherapy reinduction courses for 2 years). For the consolidation maintenance therapy a meta-analysis with the current French LALA-trial is planned. Current complete remission rate after the remission induction regimen in the ALL-4 is approximately 75% and after "consolidation" approximately 85%. Grade 3/4 toxicity (prolonged cytopenia, toxic death, prolonged delay of the start of the consolidation course) was observed in approximately 15-25% of patients. Long-term survival is in the range of 35-40%. The relapse risk during the first 2 years of remission is high. Second remissions are usually obtained with standard or more intensified chemotherapy based induction schedules in approximately 40-60%. Duration of the first complete remission is a prognostic factor for the achievement of a second CR, but most patients relapsed again within a year and finally died from the disease. Innovative options for the treatment of relapsed patients have been non-existing for a long time. Currently the development of monoclonal antibodies against molecules on the cell membrane of lymphoid cells may offer new opportunities.

5.4 Investigational agents, alemtuzumab (Campath-1H)

Campath-1H (Cambridge Pathology) is a genetically engineered, humanized, IgG1 kappa monoclonal antibody, which is specific for a 21-28 kD lymphocyte surface glycoprotein (CD52) expressed primarily on the surface of normal and malignant B and T cell lymphocytes. This antigen is present on at least 95% of all human peripheral blood lymphocytes and monocytes/macrophages (15). Antibody against CD52 antigen effectively lyses lymphocytes with complement. But, this does not appear to be the case for monocytes/macrophages. The antigen is also present on granulocyte subpopulation (<5%), and it was not found on erythrocytes, platelets and bone marrow stem cells. CD52 antigen is expressed more intensely in B-cell malignancies than in T-cell malignancies. The antigen is present on lymphocyte or their precursors in patients with CLL, non-Hodgkin's lymphoma (NHL) or acute lymphoblastic leukemia of both B-cell and T-cell origin (16). The therapeutic use of antibodies against CD52 should not compromise normal hematopoiesis.

In the early 1980s a series of rat Campath antibodies was produced by Waldmann et al in Cambridge U.K. The antibody caused cell lysis using host effectors mechanism such as complement fixation and antibody-dependent cell mediated cytotoxicity (ADCC). The first antibody, an IgM, Campath-1M, was successfully used, but only ex vivo, as treatment of donor

bone marrow to remove T-cells and thus to prevent graft versus host disease (17). A rat IgG2b, Campath-1G, was subsequently selected which was, on intravenous administration, able to deplete lymphocytes from blood, bone marrow and spleen of patients with lymphoid malignancies and from bone marrow transplantation recipients. Pilot studies have shown that the Campath-1 antibodies are active in a variety of diseases including graft-versus-host-disease, organ transplant rejection, rheumatoid arthritis and other autoimmune diseases, as well as NHL and leukemias (18-23).

To reduce the risk of an anti-globulin response and to further optimize the effector function Campath-1G was reshaped by introducing the six hyper-variable regions from the rat antibody into a human IgG1 framework. This led to the development of the humanized antibody, initially called Campath-1H (alemtuzumab) with similar biological activity and affinities as Campath-1G. Alemtuzumab appeared to be effective in a variety of studies with heavily pretreated CLL patients. Objective responses were found in 33% of them. Some very preliminary data in ALL patients showed that alemtuzumab might be effective in destroying leukemic blasts (23). Other diseases that were successfully treated were T-cell prolymphocytic leukemia and Sezary cell leukemia/lymphoma (18-24). Side effects were similar to earlier Campath preparations and included rigors, fever, nausea, vomiting, urticaria, dyspnea, coughing, pruritus, headache, diarrhea and hypotension. Due to the lymphocyte depletion opportunistic infections occur commonly. Alemtuzumab levels have been measured after intravenous administration of dosages in the range of 100 mg in the context of stem cell transplantation. In contrast to the halflife (12-24 hours) measured during treatment of active CLL, alemtuzumab appeared to be still detectable in the plasma up to 2 months after stem cell transplantation (25). These differences may be explained by the number of target cells available at the time of the alemtuzumab infusion. For treatment of relapsed chronic lymphocytic leukemia (CLL) a dose of 30 mg every other day has been selected because of the favourable balance between acivitiy and toxicity. Experience with dosages up to 100 mg resulted in severe and prolonged immunosuppresion with a high incidence of opportunistic infections (in the setting of allogeneic stem cell transplantation) (25).

5.5 Minimal residual diseases and relapse of ALL

Most of the patients with ALL will experience a recurrence and die of leukemia. Relapse is thought to result from residual leukemic cells that remain following achievement of "complete" remission, but are below the limits of detection using conventional morphologic assessment. However, sensitive techniques are now available to detect subclinical levels of residual leukemia, termed minimal residual disease (MRD). A variety of techniques have been studied for the detection of

residual disease, including cytogenetics, cell culture systems, fluorescence in situ hybridization (FISH), Southern blotting, immunophenotyping, and polymerase chain reaction (PCR) techniques. Immunophenotyping techniques using multicolor-gated flow cytometry are based on aberrant expression of antigens by the leukemic cell population. Sequential MRD monitoring using multiparameter flow cytometry has been shown to be a valuable predictor of relapse in a study of 128 children with ALL (26). In one study, the cumulative rate of relapse for those negative for MRD by flow cytometry was 10 percent, whereas it was 23, 43, and 72 percent for those with MRD of <0.1, 0.1 to <1.0, and ≥1.0 percent, respectively (27). Amplification of a DNA or cDNA sequence unique to the leukemic clone using the polymerase chain reaction (PCR) technique permits identification of one malignant cell among 10^4 to 10^6 normal cells. Two targets can be used for MRD in ALL patients, namely immunoglobulin (Ig) or T-cell receptor (TCR) gene rearrangements, or leukemia-specific chromosomal rearrangements (28-31).

5.6 Rationale

Since more than 80 percent of newly diagnosed adults with ALL nowadays enter CR, it has become difficult to demonstrate incremental improvements in initial response rates in a statistically significant way. The intensification of induction or adding a new antileukemic drug is currently directed to produce a longer remission duration. However the majority of patients will relapse within one or two years and eventually die from the disease. The use of monoclonal antibodies is a new treatment modality that has been shown very effective in the treatment of B cell Non-Hodgkin's lymphoma.

Our randomized phase II study intends to analyze the effects of the monoclonal antibody against CD52, alemtuzumab, in two dosages in the induction of a second or third remission of patients with a relapsed ALL. A dose of thirty milligrams of alemtuzumab has been selected on the basis of the experience in patients with CLL. Since the biology of ALL is different (more aggressive, rapidly cell proliferating, disease) a higher dose of alemtuzumab may be necessary to induce leukemic cell kill in a more rapid way. Since 100 mg per day has been shown in the transplantation setting a dose with too many infectious consequences, we selected a two times higher dose (60 mg) than the CLL-dose for comparison.
Alemtuzumab will be given on a daily basis during the first week of treatment in order to have enough drug available to interact with as many as possible initial ALL cells in the blood, the bone marrow and other organs; after diminition of the tumor size the disappearance of the drug in the blood will be slower and an every other day schedule has been considered sufficient. The efficacy of alemtuzumab will be also assessed by measuring minimal residual disease after therapy with

cytogenetics, immunophenotyping and (at a later stage) PCR techniques for Ig and TCR gene rearrangement.

6 Study objective

The objectives of this randomized phase II study are:

1) To assess the efficacy of alemtuzumab to destroy ALL cells using two dosages of the study drug
2) To investigate the tolerability and safety of alemtuzumab using two dosages of the study drug.

7 Study design

Details of all treatments (dose and schedule) are given in paragraph 9.
This is a multicenter randomized phase II study. Relapsed ALL patients under the age of 71 years will be registered and randomized to receive prednisone and methotrexate in the pre-phase and thereafter two remission induction courses of alemtuzumab.
In addition to the more classical evaluation methods (morphology, immunophenotyping), the leukemic mass will be studied afterwards from stored blood and bone marrow samples by using the PCR technique for Ig and TCR gene rearrangement as well as for leukemia-specific breakpoint fusion regions at diagnosis and after achievement of first CR.

8 Study population

8.1 Eligibility for registration

All eligible patients have to be registered before start of treatment (see 16).

8.1.1 Inclusion criteria

- Age 18 - 70 years inclusive
- First or second relapse of precursor B-ALL or T-ALL (including Philadelphia chromosome or BCR-ABL positive ALL)
- Duration of last complete remission at least 6 months
- WHO performance status 0, 1, or 2
- Negative pregnancy test at inclusion if applicable
- Written informed consent

8.1.2 Exclusion criteria

- Mature B-cell ALL, i.e. Burkitt leukemia/lymphoma
- Acute undifferentiated leukemia (AUL)
- Treatment with alemtuzumab at any time prior to registration
- Intolerance of exogenous protein administration
- Central nervous system (CNS) leukemia (appendix A)
- Severe cardiovascular disease (arrhythmias requiring chronic treatment, congestive heart failure or symptomatic ischemic heart disease)
- Severe pulmonary dysfunction (CTCAE grade III-IV)
- Severe neurological or psychiatric disease
- Significant hepatic dysfunction (serum bilirubin or transaminases ≥ 3 times normal level)
- Significant renal dysfunction (serum creatinin ≥ 3 times normal level)
- Patients with active, uncontrolled infections
- Patients with uncontrolled asthma or allergy, requiring oral steroid treatment at the time of registration
- Patients known to be HIV-positive
- Patient is a lactating woman
- Any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule

9 Treatments

9.1 Pre-phase

Agent	Dose/day	Route	Days
Prednisone	60 mg/m^2, divided in 2 daily doses	p.o.	1-7
MTX	15 mg	i.t.	on day 1 or at the time that most blast cells have disappeared from the blood

Blood morphology should be performed on day 8 to determine corticosteroid sensitivity

9.2 Remission Induction cycle I

Study arm A

Agent	Dose/day	Route	Days
Alemtuzumab	3 mg	i.v.	8
Alemtuzumab	10 mg	i.v.	9
Alemtuzumab	30 mg	i.v.	10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 23, 26, 28, 30, 33, 35, 37, 40, 42, 44

Study Arm B

Agent	Dose/day	Route	Days
Alemtuzumab	3 mg	i.v.	8
Alemtuzumab	10 mg	i.v.	9
Alemtuzumab	60 mg	i.v.	10, 11, 12, 13, 14, 15, 16, 17, 19, 21, 23, 26, 28, 30, 33, 35, 37, 40, 42, 44

See paragraph 9.5.2 for special management of alemtuzumab administration.

Response (see appendix B) will be evaluated on day 18 (or the day following 8 days of the study dose (30 mg or 60 mg)). Patients with progressive disease as measured after 8 days of the study dose (or at any time between that time point and the last alemtuzumab administration) will go off protocol treatment.
After induction cycle I response will be evaluated again as soon as WBC > 1 x 10^9/l and platelets > 100 x 10^9/l, or in case of delayed hematopoietic recovery at least within two weeks of the last alemtuzumab administration. Patients in complete remission (CR) will continue with remission induction cycle II, while all other patients will go off protocol treatment.

9.3 Remission Induction cycle II

This cycle consists of 4 infusions of alemtuzumab given on weekly basis. The first infusion will be given within 15 days after the last alemtuzumab administration of Remission Induction cycle I.

Study arm A

Agent	Dose/day	Route	Days
Alemtuzumab	30 mg	i.v.	1, 8, 15, 22

Study Arm B

Agent	Dose/day	Route	Days
Alemtuzumab	60 mg	i.v.	1, 8, 15, 22

See paragraph 9.5.2 for special management of alemtuzumab administration.

After the last alemtuzumab infusion response will be assessed as soon as WBC > 1 x 10^9/l and platelets > 100 x 10^9/l, and in case of delayed hematopoietic recovery at last on day 28 after the last alemtuzumab administration.
After clinical evaluation and response assessment patients will go off protocol treatment. Further treatment depends on the stage of disease, see also paragraph 9.6.

9.4 Dose modifications of alemtuzumab

In most patients dose escalation to 30 or 60 mg can be accomplished in 3 days. However, if acute severe adverse events, especially hypotension, severe chills, fever >40° C, shortness of breath, bronchospasm or rashes, (treatment: see 9.5.2) occur at either the 3 or 10 mg dose levels, then those doses should be repeated daily (for a maximum of an additional 3 days) until they are well tolerated prior to further dose escalation. If the 3 or 10 mg levels are not tolerated after this adjustment of the schedule and the assigned study dose cannot be reached, the patient will go off protocol treatment.
If later on therapy is discontinued due to a serious infection or unacceptable toxicity, alemtuzumab at the assigned dose may be re-instituted after the infection or toxicity has been resolved, provided that the delay has not exceeded 21 days.

9.5 Special management orders during treatment

Attempts should be made prior and during treatment to control any medical problems, such as infection, metabolic complications and bleeding.

- Tumor lysis syndrome (even possible during prednisone pre-phase) should be prevented by allopurinol, sodium bicarbonate, hyperhydration and furosemide.
- Electrolyte abnormalities should be monitored and corrected if indicated.
- Irradiated erythrocyte and/or platelet transfusions are recommended to be given according to the local guidelines.
- During periods with high dose corticosteroids: “prophylactic” blood cultures are strongly recommended.

- Patients with fever should be treated with empiric broad-spectrum antibiotics.
- Antibiotics should be given as prescribed by the in vitro sensitivity tests, whenever a pathogen has been isolated.
- Protective environment and selective intestinal tract decontamination are recommended for prophylaxis of infection during remission induction therapy.
- Monitoring of cytomegalovirus infections is advised.
- Because of increased risk for pneumocystis carinii pneumonia, fungus or herpes infection prophylaxis with cotrimoxazole, itraconazole and valaciclovir during the first 12 months is mandatory.

9.5.1 Product information of alemtuzumab

Alemtuzumab is a genetically modified humanized monoclonal antibody (IgG1-kappa) and is provided as a concentrate for solution for infusion. Each ampoule contains 30 mg alemtuzumab. The excipients of MabCampath are: disodium edetate, polysorbate 80 and phosphate buffered saline consisting of potassium chloride, potassium dihydrogen phosphate, sodium chloride, dibasic sodium and water for injection.

Packaging, labelling and storage: Store at 2 to 8°C (36 to 46°F). Do not freeze. Discard vial if it has been frozen. Do not use beyond expiration date. Protect from direct sunlight. Unused diluted solutions should be discarded after 8 hours.

9.5.2 Administration of alemtuzumab

The dose of alemtuzumab will be diluted in 100 ml of 0.9% normal saline and administered over 2 hours. Vital signs (blood pressure and pulse) will be measured every 15 minutes for the first 2 hours for the first and second dose and will then be monitored as clinically indicated.

Premedication (30 minutes before infusion) with paracetamol (1000 mg p.o), clemastine (2 mg i.v.) or chlorpheniramine (10 mg i.v.) and predniso(lo)ne 40 mg/m^2 i.v. is mandatory until the study dose is reached. Afterwards the prednisone may be deleted from the premedication regimen.

If acute severe adverse events (especially hypotension, severe chills, fever >40° C, shortness of breath, bronchospasm or rashes) occur treatment with intravenous pethidine, support of circulating volume, oxygen and retardation of the infusion rate (up to 4 – 8 hours) is recommended.

9.6 Advised treatment off protocol

Note: This phase is not an official part of this protocol and contains only an advise for treatment.

For patients still in complete remission after completion of protocol treatment it is advised to continue with consolidation therapy and (if possible) stem cell transplantation.
In patients who did not achieve CR, treatment with high dose Ara-C and mitoxantrone followed by (autologous or) allogeneic stem cell transplantation is advised.

Consolidation therapy may consist of a single course of HAM. The HAM protocol has to start within one month after the last alemtuzumab administration, preferably as soon as the leukocytes and thrombocytes have been recovered.

Agent	Dose/day	Route	Days
Ara-C	3 g/m^2, 3-hr infusion every 12 hrs	i.v.	1, 2, 3, 4
Mitoxantrone	10 mg/m^2, 30 min infusion	i.v.	3, 4, 5
MTX	15 mg	i.t.	1

In case a suitable stem cell donor is available, an allogeneic stem cell transplantation should be performed as soon as possible.

10 End of protocol treatment

Reasons for going off protocol treatment are:

1 Normal completion of protocol treatment
2 Progressive disease
3 No CR after induction cycle I
4 Relapse (bone marrow or extra-medullary)
5 Excessive extra-medullary drug toxicity
6 Death whatever the cause
7 No compliance of the patient (especially refusal to continue treatment)
8 Major protocol violation*
9 Delay or interruption of treatment, exceeding the limits (see paragraph 9)

* Major protocol violation defined as:
other ALL treatment given than as described in paragraph 9, or not meeting eligibility criteria for inclusion as described in paragraph 8.1.

11 Required clinical evaluations

11.1 Time of clinical evaluations

- At entry: baseline, at most 4 weeks before start of protocol treatment
- After pre-phase: determine corticosteroid sensitivity on day 8
- After 8 study doses of induction cycle I: clinical evaluation and response assessment on day 18 of induction cycle I or after 8 doses of alemtuzumab 30 mg (arm A) or 60 mg (arm B)
- After induction cycles I and II: clinical evaluation and response assessment as soon as WBC > 1 x 10^9/l and platelets > 100 x 10^9/l, but in case of delayed hematopoietic recovery at last 14 days (induction cycle I) or 28 days (induction cycle II) after the last alemtuzumab administration
- Follow up: clinical evaluation and response assessment every 6 months

11.2 Required investigations

	At entry	After Pre-phase	After 8 study doses	After Induction cycle I	After Induction cycle II	FU
Medical history	x	x	x	x	x	x
Physical examination	x	x	x	x	x	x
Hematology	x	x	x	x	x	x
PB immunophenotyping	x			x		x[1)]
PB PCR BCR/ABL	x			x		
PB storage	x		x[4)]	x[4)]	x[4)]	
Blood chemistry	x	x	x	x	x	
Bone marrow aspirate						
Morphology	x		x	x	x	x[1)]
BM immunophenotyping	x			x[4)]	x[4)]	x[1)]
Cytogenetics	x[3)]			x[4)]	x[4)]	
BM PCR BCR/ABL	x			x[4)]	x[4)]	x[1)]
BM storage	x		x[4)]	x[4)]	x[4)]	
Specific investigations						
Chest x-ray	x					
ECG	x					
CSF examination	x			x		o.i.
EMD examination	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.
Virological tests	x	o.i.	o.i.	o.i.	o.i.	o.i.
Microbiological tests	x	o.i.	o.i.	o.i.	o.i.	o.i.

o.i. on indication

1) only on clinical and laboratory signs of relapse

2) at achievement of morphologic CR if aberrant karyotype at diagnosis

3) if data from initial diagnosis are not available

4) only at first achievement of complete remission

11.2.1 Medical history

Standard medical history, with special attention for:

- WHO performance status
- Adverse events
- Infections
- Bleeding
- Symptoms for CNS involvement
- Concomitant therapy

Only at entry:

- Prior and present other diseases
- Antecedent hematological or oncological diseases
- Previous chemotherapy or radiotherapy

11.2.2 Physical examination

Standard physical examination, with special attention for:

- Height (only at entry)
- Body weight
- Surface area
- Blood pressure
- Pulse
- Temperature (daily during induction treatment)
- Bleeding tendency
- Lymph node enlargement
- Liver and spleen size
- Any sign of possible extramedullary disease

11.2.3 Hematology

- Hemoglobin
- Hematocrit
- Reticulocytes
- Platelets
- WBC
- WBC differential
- Immunophenotyping (see 11.2.7)
- Molecular analysis (see 11.2.9)

11.2.4 Blood chemistry

- Urea
- Creatinin
- Total bilirubin
- AST (SGOT)
- ALT (SGPT)
- Alkaline phosphatase
- Gamma GT
- LDH
- Total proteins
- Albumin
- CRP
- Glucose
- Calcium
- Phosphate
- Sodium
- Potassium
- Uric acid

11.2.5 Bone marrow aspirate

- Morphology
- Immunophenotyping (see 11.2.7)
- Cytogenetic analysis (see 11.2.8)

- Molecular analysis (see 11.2.9)

11.2.6 Specific investigations

- X-Thorax
- ECG
- Cerebral spinal fluid examination
- Pathology of other suspected extramedullary disease
- Virological tests (including CMV)
- Microbiological tests

11.2.7 Immunophenotyping

Immunophenotyping of blood and bone marrow, including CD52, by flowcytometry will be performed at diagnosis of relapse in all patients. In virtually all cases the malignant lymphoblasts will express a phenotype that can be used for detection of minimal residual disease after treatment. Every time the bone marrow is examined, flowcytometry should be performed to quantitate the level of residual lymphoblast by a technique that is able to detect at least one malignant cell among 1,000 bone marrow cells.

Review by the HRC will be performed at diagnosis of relapse only (see 4.1).

11.2.8 Cytogenetic analysis

Conventional cytogenetic analysis on relapse material should be performed in patients from whom no cytogenetic data of initial diagnosis are available and in case of an aberrant karyotype repeated at the first achievement of morphologic CR. For Philadelphia chromosome positivity the detection of BCR/ABL will be required.

Central review will be performed for cytogenetic analysis as described in paragraph 4.2.

11.2.9 Molecular analysis

Blood and bone marrow cells should be investigated for BCR/ABL at entry in this study. In case of BCR/ABL positivity follow up investigations for BCR/ABL should be performed at all bone marrow examinations mentioned in this protocol.

Blood cells (30 ml of peripheral blood (in EDTA) for viable cryopreservation of 5 –10 ampoules with 10 x 10^6 nucleated blood cells each) and bone marrow cells (1 – 2 ml of bone marrow (preferable in citrate) for viable cryopreservation of 5 – 10 ampoules with 10 x 10^6 nucleated bone marrow cells each) should be stored in liquid nitrogen at entry of this study and after first achievement of a complete remission for further analysis in the future of minimal residual disease by using a PCR technique for Ig and TCR gene rearrangement as well as for leukemia-specific breakpoint fusion regions.

Diagnostic and follow-up samples should be sent to the local laboratory. If necessary this local laboratory will forward the samples to a central laboratory as agreed within the Network for Molecular Diagnostics.

11.2.10 Corticosteroid sensitivity

Corticosteroid sensitivity will be determined on day 8 of the pre-phase.
Definition of corticosteroid-sensitivity: leukemic blasts < 1 x 10^9/l in the blood.

11.3 Response assessment

Response will be assessed after 8 days of the study dose. After remission induction cycles I and II, response will be assessed as soon as WBC > 1 x 10^9/l and platelets > 100 x 10^9/l, but within two weeks (induction cycle I) and/or 4 weeks (induction cycle II) of the last alemtuzumab administration, by evaluation of the bone marrow aspirate and blood according to the definitions described in appendix B.
During follow-up bone marrow examination will only be performed when relapse is suspected based on unexpected abnormalities of blood cell counts, appearance of circulating blasts, or clinical abnormalities originating in the CNS.

12 Toxicities

Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events, CTCAE version 3.0, published December 12, 2003 (Appendix D).

12.1 Alemtuzumab

The most frequent adverse events are acute infusion related toxicity including rigors, fever, nausea and vomiting, hypotension, fatigue, rash, urticaria, dyspnoea, headache, pruritus and

diarrhea. The majority of these adverse events are mild to moderate in severity. According to the data in CLL patients the infusion related events decrease in incidence during the treatment, most striking after the first week of therapy. The most serious toxicity of alemtuzumab is infections. The reported opportunistic infections of grade 3 or 4 during the phase II studies are given in table 1. Most frequent infections reported are herpes simplex and pneumonia caused by *Pneumocystis carinii,* cytomegalovirus, *Aspergillus* and Herpes zoster. Mucormycosis infection is also reported but is uncommon.

Table 1. Infections grade 3 or 4 reported during the phase II trials

Infection-site	Infections	
Pathogen	No	% of patients
Pneumonia	23	15.4
Bacterial	8	5.4
PCP	5	3.4
Fungal	4	2.7
Interstitial	2	1.3
Other	5	3.4
Sepsis/bacteremia	15	10.1
Line infection	8	5.4
Sepsis	7	4.7
Herpes infection	7	4.7
CMV	4	2.7
Herpes simplex	1	0.7
Herpes zoster	2	1.3

Hematological toxicity is common but not too relevant for this study. Transient decrease in hemoglobin was observed during therapy. All patients developed lymphopenia. The total number of T-cells was intensively reduced. By the end of therapy T-cell recovery started to occur, regardless of the total cumulative dose of alemtuzumab given.
In phase II studies adverse events contributing to discontinuation of treatment were reported in 19-29% of CLL patients.

13 Reporting serious adverse events

13.1 Definitions

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse reaction (AR)

Adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence that at any dose results in:

- death
- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization
- significant / persistent disability
- a congenital anomaly / birth defect
- any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above)

Note that ANY death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Unexpected SAE

Unexpected Serious Adverse Events are those SAE's of which the nature or severity is not consistent with information in the relevant source documents. For a medicinal product not yet approved for marketing in a country, a company's Investigator's Brochure will serve as a source document in that country.

Suspected unexpected serious adverse reaction (SUSAR)

All suspected ARs which occur in the trial and that are both unexpected and serious.

Protocol treatment period

The protocol treatment period is defined as the period from the first study-related procedure until 30 days following the last dose of protocol treatment or until the start of another systemic anti-cancer treatment off protocol, if earlier.

13.2 Reporting of (serious) adverse events

Adverse event

AEs will be reported on the CRF. All adverse events of Grade 2 or higher, with the exception of progression of disease, occurring during the protocol treatment period will be reported. Adverse events occurring after that period should also be reported if considered related to protocol treatment.

SAE and Unexpected serious adverse event

All SAEs occurring during the protocol treatment period must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the event**, except hospitalizations for:

- a standard procedure for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- the administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- a procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- a procedure that is planned (i.e., planned prior to starting of treatment on study; must be documented in the CRF). Prolonged hospitalization for a complication considered to be at least possibly related to the protocol treatment remains a reportable serious adverse event.

All details should be documented on the **Serious Adverse Event and Death Report**. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report

within a further 2 working days and sent to the HOVON Data Center. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.

At any time after the protocol treatment period, *unexpected* Serious Adverse Events that are considered to be at least suspected to be related to protocol treatment must also be reported to the HOVON Data Center using the same procedure, **within 24 hours after the SAE was known to the investigator**.

The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the serious adverse event form. The assessment of causality is made by the investigator using the following:

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship to the protocol treatment (also include pre-existing conditions)
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

13.3 Processing of serious adverse event reports

The HOVON Data Center will forward all reports within 24 hours of receipt to the study coordinator and the study central data manager. The report of an SAE will be the signal for the central data manager to ask the investigator or the responsible local data manager to complete

and send as soon as possible all relevant CRF's for the involved patient with details of treatment and outcome.

14 Endpoints

14.1 Primary endpoints

- Percentage of patients that reach a CR on induction cycle I in each arm.
- Percentage of patients with severe toxicity on induction cycle I in each arm.

14.2 Secondary endpoints

- Toxicity profile related to each treatment step and intervals between treatment steps.
- Event-free survival (i.e. time from registration until no CR on protocol, relapse or death, whichever comes first); Event-free survival for patients without a CR is set at one day.
- Disease-free survival (i.e. time from achievement of CR to date of relapse or death from any cause, whichever occurs first).
- Overall survival measured from time of registration.

15 Data collection

Data will be collected on Case Report Forms (CRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- inclusion and exclusion criteria
- baseline status of patient including medical history and stage of disease
- timing and dosage of protocol treatment
- adverse events
- parameters for response evaluation
- any other parameters necessary to evaluate the study endpoints
- survival status of patient
- reason for end of protocol treatment

Each CRF page will be identified by a pre-printed trial number, and a unique combination of patient study number (assigned at registration), hospital and patient namecode (as documented at registration) to be filled out before completing the form.

The CRF will be completed on site by the local investigator or an authorized staff member. Each page must be dated and signed by the local investigator upon completion. All CRF entries must be based on source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.
Copies of the CRF will be kept on site. The original CRF pages must be sent to the HOVON Data Center at the requested time points. How and when to send in forms is described in detail in the CRF header and the CRF instructions.
All data from the CRF will be entered into the study database by the HOVON Data Center.

16 Registration

Eligible patients should be registered and randomized before start of treatment. Patients can be registered at the HOVON Data Center of the Erasmus MC - Daniel den Hoed by phone call: +31.10.4391568 or fax +31.10.4391028 Monday through Friday, from 09:00 to 17:00, or via the Internet through TOP (Trial Online Process; https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:

1. Protocol number
2. Institution name
3. Name of caller/responsible investigator
4. Patient's initials or code
5. Patient's hospital record number (optional)
6. Sex
7. Date of birth
8. First or second relapse
9. Duration of last remission
10. Eligibility criteria

All eligibility criteria will be checked with a checklist.
Each patient will be given a unique patient study number. Patients will be randomized, stratified by institution, age (18-44 vs 45-59 vs 60-70), 1st relapse vs 2nd relapse, and duration of last remission (< 12 months vs ≥ 12 months from achievement of last CR) with a minimization procedure, ensuring balance within each stratum and overall balance. Patient study number and result of randomization will be given immediately by TOP or phone and confirmed by fax or e-mail.

17 Statistical considerations

The aim of this study is to assess in adults of 18 - 70 years in 1st or 2nd relapse ALL, the response to and feasibility of alemtuzumab.

17.1 Sample size

For each treatment arm, the optimal Bryant-Day 2-stage design (ref: J. Bryant and R. Day. Incorporating toxicity considerations into the design of two-stage phase II clinical trials. *Biometrics*. 2000: 51(4):1372-1383) will be applied with a modification. This design shields patients from an ineffective or toxic treatment by requiring early termination of the trial if after induction cycle I either the CR rate is poor or the severe toxicity rate is high. In this trial, severe toxicity has been defined as any side effect or infection of CTCAE grade 4 during induction cycle I. The following parameters and decision rules are used:

- P_{CR0} is the largest CR probability which, if true, implies that the therapeutic activity is too low and does not warrant further investigation of the regimen. In the present trial, P_{CR0} has been taken as 20%.
- P_{CR1} is the lowest CR probability which, if true, implies that the therapeutic activity is sufficiently high and does warrant further investigation of the regimen. In the present trial, P_{CR1} has been taken as 40%.
- P_{tox0} is the smallest severe toxicity occurrence probability which, if true, implies that the therapeutic toxicity is unacceptable and does not warrant further investigation of the regimen. In the present trial, P_{tox0} has been taken as 40%.
- P_{tox1} is the largest severe toxicity occurrence probability which, if true, implies that the therapeutic toxicity is acceptable and does warrant further investigation of the regimen. In the present trial, P_{tox1} has been taken as 20%.

Statistical errors will be:

- α_{CR} is the accepted probability of recommending for further investigation a regimen with a true CR rate equal to or lower than P_{CR0}. In the present trial, α_{CR} has been taken as 0.10.
- α_{tox} is the accepted probability of recommending for further investigation a regimen with a true severe toxicity rate equal to or higher than P_{tox0}. In the present trial, α_{tox} has been taken as 0.10.

- β is the accepted probability of rejecting from further trials a regimen with a true CR rate at least equal to P_{CR1} and a true toxicity rate equal to or lower than P_{tox1}. In the present trial, β has been taken as 0.10.

These design parameters imply that in each treatment arm a maximum of 54 patients who started with induction cycle I will be assessed after induction cycle I for CR rate and severe toxicity, with an interim analysis after the first 24 patients, or as soon as 9 severe toxicities have been reported in the first 24 registered patients, whichever comes first:

- if after the first 24 patients in a treatment arm $\leq$ 5 CR's (20.8%) are observed, or if $\geq$ 9 severe toxicities (37.5%) are observed, that arm will be closed to further patient entry with the conclusion that the arm is not enough active or is too toxic, and should not be further investigated. Otherwise patient entry will be extended to 54 patients. However, when 24 patients have been randomized to each treatment arm, a maximum of 4 more patients may be randomized to each treatment arm before the randomization will be put on hold while awaiting the data for and results of the interim analysis.
- If after 54 patients in a treatment arm $\leq$ 14 CR's (14/54 = 25.9%, 95% CI = 15.0-39.7%) are observed, or if $\geq$ 18 severe toxicities (18/54 = 33.3%, 95% CI = 21.1-47.5%) are observed, the conclusion will be that the arm is not enough active or is too toxic, and should not be further investigated.
- Otherwise, the trial will conclude that the arm is active and feasible, and warrants further investigation in this patient population.

Since this design applies per treatment arm and does not take into account that the dose in arm B is higher than the dose in arm A, which implies that the true probability of severe toxicity in arm B is not smaller than in arm A and also that the true CR rate in arm B is not smaller than arm A, unless the latter could be explained by a high toxicity rate in arm B, the above stopping rules will be modified in the following way:

- if the observed toxicity rate in arm B is lower than in arm A, the average toxicity rate of both treatment arms will be used in the stopping/decision rules in both arms.
- If the observed CR rate in arm B is lower than in arm A, and this cannot be explained by a high toxicity rate in arm B, the average CR rate of both treatment arms will be used in the stopping/decision rules in both arms.

Before the interim analysis, CR and toxicity data on induction cycle I will be actively requested for on a monthly basis. For this purpose a short questionnaire will be generated, which should be completed as soon as cycle I has been evaluated, or as soon as it has been decided that cycle I will not be administered at all.

In order to overcome dropouts due to ineligibility, 120 patients will be randomized. With an expected accrual rate of 60 patients per year, entry will be completed in about 2-2.5 years, depending on the speed of initiation in each country.

17.2 Analyses

All randomized patients, eligible and who started the induction treatment will be included in the analysis, according to the intention to treat.

Those patients who are not in CR within 2 weeks after induction cycle I will be considered as treatment failures.

The estimated CR rate after induction cycles 1 and 2 along with the 95% CI interval will be presented for each treatment arm.

The estimated severe toxicity rate (i.e. any side effect or infection of CTCAE grade 4) along with the 95% CI interval will be presented for each treatment arm.

The actuarial curves for EFS, DFS and OS will be computed using the Kaplan-Meier method and 95% CIs will be constructed.

Actuarial probabilities of relapse after CR or death in CR with corresponding standard errors will be calculated using the competing risk method.

In addition the analysis of toxicity will be done tabulation of the incidence of side effects and infections with CTCAE grade 2 or more (appendix D).

17.3 Interim analysis

One formal interim analysis per treatment arm is planned as described above.

17.4 Data and safety monitoring board

A data and safety monitoring board will not be installed.

18 Ethics

18.1 Independent ethics committee or Institutional review board

The study protocol and any amendment that is not solely of an administrative nature will be approved by an Independent Ethics Committee or Institutional Review Board.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (Edinburgh, Scotland, 2000) and the ICH-GCP Guidelines of 17 January 1997. The local investigator is responsible for ensuring that the study will be conducted in accordance with the protocol, the ethical principles of the Declaration of Helsinki, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

18.3 Patient information and consent

Written Informed consent of patients is required before registration. The procedure and the risks and the opinions on treatment for ALL will be explained to the patient.

19 Trial insurance

HOVON will ensure that insurance is in place for all participating sites.

HOVON will provide risk insurance to cover all patients from participating sites in the Netherlands according to Dutch law (WMO).
In case of an intergroup study, risk insurance of patients from centers participating within another cooperative group will be provided by that group, according to all applicable laws and regulations. Individual participating centers from outside the Netherlands have to arrange risk insurance of their own patients according to all applicable laws and regulations.

20 Publication policy

The final publication of the trial results will be written by the Study Coordinator(s) on the basis of the statistical analysis performed at the HOVON Data Center. A draft manuscript will be submitted to the Data Center and all co-authors for review. After revision by the Data Center, the other co-authors, the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinator(s), the lead investigators of the major groups (in case of intergroup studies), investigators who have included more than 5% of the evaluable patients in the trial (by order of number of patients included), the statistician(s) and the HOVON data manager in charge of the trial, and others who have made significant scientific contributions.

Interim publications or presentations of the study may include demographic data, overall results and prognostic factor analyses, but no comparisons between randomized treatment arms may be made publicly available before the recruitment is discontinued.

Any publication, abstract or presentation based on patients included in this study must be approved by the study coordinator(s). This is applicable to any individual patient registered/randomized in the trial, or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study end-points unless the final results of the trial have already been published.

21 Glossary of abbreviations

(in alphabetical order)

ADCC	Antibody-dependent cell mediated cytotoxicity
AE	Adverse Event
ALL	Acute Lymphoblastic Leukaemia
ALT	Alanine Amino Transferase
Ara-C	Cytarabine, cytosine arabinoside
AST	Aspartate Amino Transferase
BM	Bone Marrow
CI	Confidence interval
CKTO	Commissie voor Klinisch Toegepast Onderzoek
CLL	Chronic Lymphocytic Leukemia
CMV	Cytomegalovirus
CNS	Central nervous system
CR	Complete Remission
CRF	Case Report Form
CRP	C-reactive protein
CSF	Cerebral spinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
DFS	Disease free Survival
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	Event Free Survival
EMD	Extra medullary disease
EORTC	European Organization for Research and Treatment of Cancer
FISH	Fluorescence in situ hybridization
FU	Follow up
GCP	Good Clinical Practice
GIMEMA	Italian Group for Hemato-Oncology
HAM	Ara-C, Mitoxantrone, MTX
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte histocompatibility antigen
HOVON	Dutch/Belgian Hemato-Oncology Cooperative Group
HRC	Hematocytology Review Committee

ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IT	Intrathecal
IV	Intravenous
LALA	French ALL Group
LDH	Lactate Dehydrogenase
METC	Medical Ethical review committee
ML	Meningeal leukemia
MPO	Myeloperoxidase
MRD	Minimal residual disease
MTX	Methotrexate
NCI	National Cancer Institute
NHL	Non-Hodgkin Lymphoma
NR	No response
OS	Overall Survival
PB	Peripheral Blood
PCP	Pneumocystis carinii-pneumonia
PCR	Polymerase chain reaction
PEG-asparaginase	Polyethyleenglycol asparaginase
PR	Partial Response
SAE	Serious Adverse Event
SCT	Stem cell transplantation
TCR	T-cell receptor
WBC	White Blood Count
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

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A. Diagnostic criteria for ALL

Morphologic criteria for ALL:

- >20% blasts in a representative bone marrow aspirate or otherwise in a bone marrow biopsy

AND

- Myeloperoxidase (MPO) or Sudan Black positivity of the blasts < 3% (cytochemistry)

Classification of ALL according to immunological phenotype:

B-cell lineage		
	Pro-B ALL	cytCD79^{+}, CD19^{+}, HLA-DR^{+}, TdT^{+}, CD10^{-}
	Common ALL	cyt CD79^{+}, CD19^{+}, HLA-DR^{+}, TdT^{+}, CD10^{+}
	Pre-B ALL	cytCD79^{+}, CD19^{+}, HLA-DR^{+}, TdT$^{+/-}$, CD10$^{+/-}$, cytμ^{+}
T-cell lineage		
	Prothymocyte ALL	cytCD3^{+}, CD2^{-}, CD7$^{+/(-)}$, HLA-DR^{+}, TdT^{+}
	Immature thymocyte	cytCD3^{+}, CD2^{+}, CD7^{+}, TdT^{+}, CD5^{+}, HLA-DR^{-}
	Common thymocyte	cytCD3^{+}, CD2^{+}, CD7^{+}, CD1^{+}, CD4^{+}, CD8^{+}, TdT^{+}
	Mature thymocyte	cytCD3^{+}, CD2^{+}, CD7^{+}, CD1^{-}, CD4^{+}, or CD8^{+}, TdT^{+}

Indicated are the minimal requirements for subtyping; additional markers are advisable.

Meningeal leukemia (ML), if present, will be classified as follows:

- definite: clinical neurological signs of CNS involvement (mainly cerebral palsy) and/or > 5 blasts/ml CSF on cytological examination;
- probable: 1-5 blasts/ml CSF (24);
- dubious: pleiocytosis of CFS with increased protein level, in absence of blasts on cytological examination.

B. Response criteria

Complete response (CR) requires *all* of the following:

- <5% leukemic cells by morphology in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy.
- Peripheral blood without leukemic cells by morphology, in case of doubt to be confirmed by immunophenotyping.
- Absence of extramedullary leukemia.

Partial response (PR) requires *all* of the following:

- 5-25% malignant cells by morphology in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy.
- 0-25% malignant cells by immunophenotypical analysis in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy.
- Absence of extramedullary leukemia.

No response (NR):

- Not meeting the criteria for CR, PR, PD or relapse from CR.

Progressive disease (PD) requires one of the following:

- Increase of the number of leukemic cells in the peripheral blood of more that 50% (with a minimum of 1 x 10^9/l) compared to baseline values
- Increase of more than 50% of blast cells (with a minimum of 1 x 10^9/l) after initial (complete or partial) clearance of blast cells from the blood
- reappearance of blast cells (with a minimum of 1 x 10^9/l) after initial (complete or partial) clearance of blast cells from the blood

Relapse from CR requires at least one of the following:

- Reappearance of leukemic cells by morphology in the blood.
- Reappearance of leukemic cells by immunophenotyping in the blood.
- More than 5% leukemic cells by morphology and more than 1% leukemic cells by immunophenotyping in a representative* bone marrow aspirate or bone marrow biopsy.
- Appearance or reappearance of extramedullary leukemia, proven by biopsy or cytology.

* representative bone marrow aspirate defined as >20 % cellularity

C. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

D. Common Terminology Criteria for Adverse Events

The grading of toxicity and adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 3.0, published December 12, 2003. A complete document (72 pages) may be downloaded from the following sites:

http://ctep.cancer.gov/reporting/ctc.html
http://www.hovon.nl (under Studies > Documents)

A hardcopy may be obtained from the HOVON Data Center on request.