Clofarabine added to prephase and consolidation therapy in acute lymphoblastic leukemia in adults.

A prospective randomized trial.

A joint study of the HOVON and the EORTC

PROTOCOL

Principal Investigator	:	A.W. Rijneveld(HOVON)
Principal Investigator EORTC	:	J-P. Marie (EORTC)
Study Coordinators	:	S.M.G.J. Daenen
		B. Labar
		J.J. Cornelissen
Sponsor	:	HOVON
		VU University Medical Center,
		P.O.Box 7057
		1007 MB Amsterdam
Statisticians	:	B. van der Holt (HOVON)
		S. Suciu (EORTC)
Head DSMB	:	R. Pieters
Datamanagement	:	HOVON Data Center
Registration	:	HOVON Data Center
		Erasmus MC Cancer Institute
		Clinical trial Center (HS-423)
		P.O.Box 2040
		3000 CA ROTTERDAM
		The Netherlands
		tel. +31.10.7041560
		fax +31.10.7041028
		https://www.hdc.hovon.nl/top
Serious Adverse Events	:	HOVON Data Center,Fax: +31.10.4239087
EudraCT number	:	2008–005798-36

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Principal Investigator:

Signature of Investigator

1/4/14

Date

Dr. A.W. Rijneveld

Printed Name of Investigator

LOCAL INVESTIGATOR SIGNATURE PAGE

Local site name: _____

Local Investigator:

_____ _____

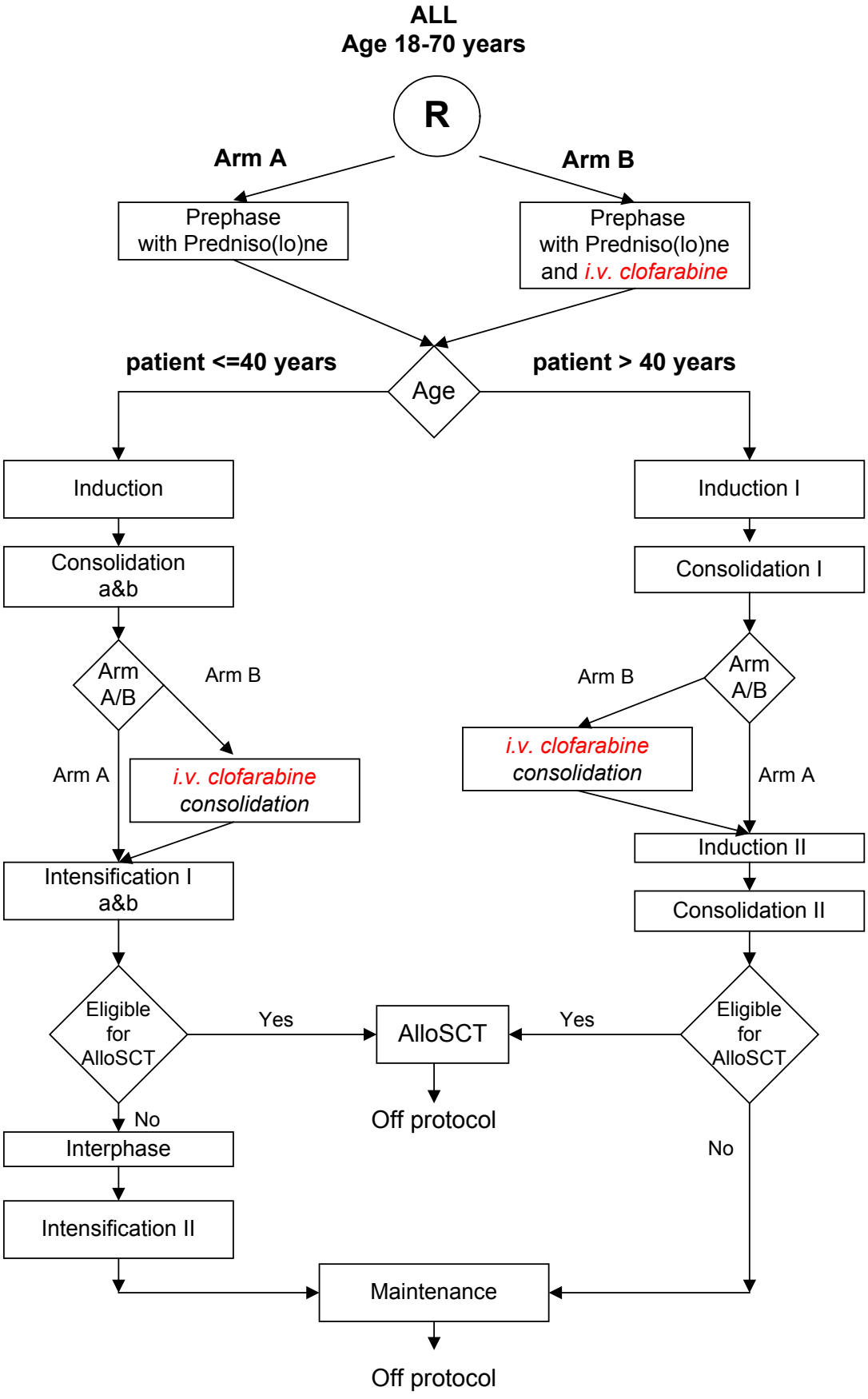
Signature of Investigator Date

_____

Printed Name of Investigator

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

1 Scheme of study



2 Table of contents

Page

3 Synopsis

Study phase	II-III
Study objectives	Phase II of the study: • To determine the feasibility of i.v. clofarabine given prior to standard induction chemotherapy as part of pre-phase Phase III of the study: • To improve event free survival by adding i.v. clofarabine to prephase and consolidation therapy
Patient population	Adult patients, 18 to 70 years of age, with ALL or T-LBL
Study design	Prospective, multicenter, randomized
Duration of treatment	3-4 years
Number of patients	340 (treated with dose level used in phase III)
Adverse events	Adverse events will be documented if observed and serious adverse events will be reported immediately.
Planned start and end of recruitment	Start of recruitment: October 2009 End of recruitment: April 2017
End of trial	At 32 months after registration and randomization of the last enrolled patient, which equals 2 years after the expected date the last patient will start with maintenance therapy. Subsequently all patients will be followed until 10 years after registration.

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Principal investigator HOVON	A.W.Rijneveld	Erasmus MC, Rotterdam
Principal investigator EORTC	J-P. Marie	Hospital St. Antoine, Paris
Study Coordinators	S.M.G.J. Daenen	University Medical Center Groningen
	B. Labar	University Hospital Zagreb
	J.J. Cornelissen	Erasmus MC, Rotterdam
Representative of Sponsor (HOVON)	J.J. Cornelissen, chairman	VU University Medical Center Amsterdam
Registration	HOVON Data Center	Erasmus MC Cancer Institute, Clinical Trial Center P.O.Box 2040 3000 CA ROTTERDAM The Netherlands tel. +31.10.7041560 fax +31.10.7041028 https://www.hdc.hovon.nl/top
Monitoring	HOVON Data Center	Erasmus MC Cancer Institute, Clinical Trial Center
Writing Committee	D. Breems	Hospital Stuivenberg Antwerp (B)
	D. Bron	Institut Jules Bordet, Brussels (B)
	A.W. Dekker	University Medical Center Utrecht (NL)
	P.C. Huijgens	VU University Medical Center Amsterdam (NL)
	B. Löwenberg	Erasmus MC, Rotterdam (NL)
	J. Maertens	University Hospital Gasthuisberg Leuven (B)
	M. van Marwijk Kooy	Isala Clinic – Sophia, Zwolle (NL)
	M.H.J. van Oers	Academic Medical Center, Amsterdam (NL)
	G.J. Ossenkoppele	VU University Medical Center Amsterdam (NL)
	E. Petersen	University Medical Center Utrecht (NL)
	H.C. Schouten	University Hospital Maastricht (NL)
	D. Selleslag	Brugge University Hospital (B)
	G.E.G. Verhoef	University Hospital Gasthuisberg Leuven (B)
	P.W. Wijermans	Haga Hospital – Leyenburg, The Hague (NL)
	R. Willemze	Leiden University Medical Center (NL)
	S. Wittebol	Meander Medical Center, Amersfoort (NL)
	Th. de Witte	UMC St. Radboud, Nijmegen (NL)
Cytogenetics review	H.B. Beverloo	Erasmus MC, Rotterdam (NL)
Cytological and immunophenotype	M. Jongen-Lavrencic	HOVON Hematology Review Committee, Erasmus MC, Rotterdam

review		
MRD diagnostics Netherlands	J.J.M. van Dongen	Erasmus MC, Rotterdam
	Dr. V. de Haas (head)	Dutch Childhood Oncology Group (DCOG), The Hague
	dr. V.H.J. van der Velden	Department of Immunology, Erasmus MC, Rotterdam
	prof. dr. C.E. van der Schoot	Department of Immunohematology Sanquin, Amsterdam
MRD diagnostics Belgium	Dr. M. Bakkus	Department of Hematology, VUB, Brussels
MRD diagnostics France	Dr. H. Cavé	Laboratoire de Biochemie Génétique, Hôpital Robert Debré, Paris
Statisticians	B. van der Holt (HOVON)	Erasmus MC, Rotterdam
	S. Suciu (EORTC)	EORTC Data Center, Brussels
Head DSMB	R. Pieters	Erasmus MC- Sophia, Rotterdam
Datamanagement	HOVON Data Center	Erasmus MC Cancer Institute, Clinical Trial Center
Serious Adverse Events (SAEs) notification	HOVON Data Center	Erasmus MC Cancer Institute, Clinical Trial Center fax +31.10.4239087

4.1 Cytological immunophenotype review

Review of bone marrow aspirate at diagnosis by the HRC is required at diagnosis.
Four unstained blood and 6 unstained bone marrow smears should be sent together with a filled out HRC cytology form, a copy of the report of the immunological marker analysis, a copy of the cytogenetics report and a copy of the molecular diagnosis report to ms. T de Jong, Room Nc-828, HOVON Hematology Review Committee, Erasmus MC ‘s Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands, at the time of registration. Confirmation of diagnosis is not necessary for registration or start of treatment but sending in of smears for review is required.

4.2 Cytogenetic review

Central review will be performed for cytogenetic analysis at diagnosis.
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. A filled out cytogenetic form together with 2 representative karyotypes and a copy of the original cytogenetic report is requested to be sent within 5 weeks to the HOVON Data Center for central review.
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.

4.3 MRD diagnostics

MRD diagnostics will be performed at the DNA level via real-time quantitative PCR (RQ-PCR) analysis of rearranged immunoglobulin (Ig) and T-cell receptor (TCR) genes according to the guidelines of the European Study Group for MRD detection in ALL (ESG-MRD-ALL, 23) (see appendix G). Central intake and processing of diagnosis and follow-up samples will be performed by the laboratory of the country (see overview above). The results per patient will be reported to the study coordinator Dr. A. W. Rijneveld.

When a patient develops a relapse, a bone marrow sample should be send to SKION-DCOG laboratory as well, to investigate the stability of the rearrangements. (more information on MRD diagnostics is given in section 11.2.9 and appendix G).

5 Introduction

5.1 Acute lymphoblastic leukemia, remission induction

Acute lymphoblastic leukemia (ALL) is a malignant disease that originates from B- or T-lymphocyte precursors. 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. Treatment regimens for ALL have evolved empirically into complex schemes that use numerous agents in various doses, combinations, and schedules (1-7). Current induction therapy combinations of vincristine, corticosteroids, an anthracycline, asparaginase and cyclophosphamide result in a complete remission rate of more than 80% in adult patients. In vitro and in vivo sensitivity to corticosteroids in the remission induction (RI) phase seems to predict for prognosis (7). Induction therapy has been further intensified by increasing the dose of cyclophosphamide, cytarabine and methotrexate or by adding a new drug. In childhood ALL, L-asparaginase appears to prolong disease free survival (DFS) when given during consolidation (8,9).

5.1.1 Post-remission therapy

To improve the prognosis of adult patients with ALL the current recommendations are 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 (3,10). Consolidation with high dose Ara-C and/or methotrexate has definitely improved the prognosis of certain subsets of ALL (T ALL, pro-B ALL), but its value remains unproven or is absent in other subsets (2,3). Allogeneic stem cell transplantation (SCT) has only been shown more valuable than autologous SCT or intensive maintenance treatment (MT) in younger patients with poor prognosis ALL, especially Ph+ALL (7,10). Deleting long-term

maintenance treatment is probably detrimental in certain subgroups of ALL (common/pre-B ALL) but randomized trials are not available. The most recent EORTC study (ALL-4) compared long-term intensive maintenance courses with autologous SCT followed by low intensity maintenance; allogeneic SCT was part of the study for those with a HLA identical donor. The complete remission (CR) rate after the remission induction (RI) regimen in the ALL-4 is approximately 70% and after "consolidation" approximately 80%. Differences between the randomized arms are currently not apparent. Survival at 5 years is in the range of 35-40%. The relapse rate during the first 2 years of remission is high. The results from the HOVON studies (HOVON 18 and 37) are comparable to the EORTC-LG and French LALA findings. They also utilized autologous and allogeneic SCT, with a CR rate of 83% after two cycles of induction treatment and an overall survival (OS) for all patients of 34-42% at 5 years, for those following autologous SCT it was 40-57% and after allogeneic SCT 58-66%, respectively (unpublished data). Donor/no donor comparisons showed a trend towards superiority of allogeneic SCT in the EORTC-LG ALL-4 trial and a significant difference with respect to disease-free survival (DFS) and OS in favor of allogeneic SCT in the HOVON trials.

5.1.2 New developments on intensification therapy

Boissel et al. (11) compared the outcome of adolescents (15-20 years) with ALL treated in France in either the pediatric FRALLE 93 or the adult LALA-94 (almost similar to the EORTC ALL-4 protocol) clinical trials. With a median follow up of about 3.5 years, the CR rate (94% vs. 83%), event free survival (EFS) at 5 years (67% vs.41%), and the DFS for the CR patients (72% vs. 49%) were far superior in the pediatric group and multivariate analysis confirmed the independent effect of the treatment trial on outcome. The major differences between the pediatric or adult approach were the actually given dosages of asparaginase, corticosteroids and vinca-alkaloids. Another difference was the strict discipline with which the treatment courses were administered by the pediatricians compared to the flexibility of the internists. Analyses with similar outcome have been reported by comparing ALL patients aged 15 till 21 years treated in Dutch and British children and adult protocols (12,13).
The feasibility of an actualization of the FRALLE 93, called the FRALLE 2000, was recently tested in Paris (and presented at ASH -2007, Haiat S. et al 14) in 28 adult ALL patients (age 16-57 years), of whom 83% reached a CR. Ten patients had undetectable minimal residual disease (MRD) and none of them experienced relapse. Grade 3 - 4 toxicities included a considerable number of severe infections, liver function abnormalities, unexpected peripheral neuropathies and denutrition. These toxicities increased with age. The adherence discipline with respect to time-lines of the treatment schedules was successful in approximately 50% of the limited number of patients studied so far. Similar experience was observed in the HOVON-70 study, which included 54 patients aged 18 to 39 years, of whom 84% reached complete hematological remission. Fourty-six grade 3-4 toxicities were noted, including 26 infections, 19 patients with liver toxicity, 4 thrombotic events, and 4 patients with

severe neuropathy. Five patients succumbed due to treatment-related mortality (TRM), including 2 following allogeneic SCT. The median number of days as from the start of therapy until the next course of chemotherapy for, respectively, consolidation, intensification, interphase, and maintenance, appeared prolonged in less than 50% of patients, while the time-line compared well to the French experience. Overall, it was concluded that results appeared very encouraging, but that side effects necessitated prolongation of the induction/consolidation/intensification phase in a considerable number of patients and that the protocol seemed too intensive for older patients.

In patients over 40, HOVON therefore, applied a novel schedule which had earlier shown good outcome independent of age in a single center experience with a CR rate of 88%, a relapse rate of 25%, and DFS and OS at 5 year of 71% and 60%, respectively (6,15). Typical for this schedule is a so-called pre-induction course with the non-crossreacting drugs cytarabine, etoposide, and methotrexate aiming at a significant tumor load reduction in the early phase of therapy. This was immediately followed by two courses of RI with vincristine, dexamethasone, and doxorubicine and a consolidation course with intermediate dose cytarabine and asparaginase. Thereafter, MT was instituted for a total treatment duration of 30 months. HOVON enrolled 60 patients with a median age of 57 year in this feasibility study (HOVON-71). While results are still immature the CR rate of 80% and DFS at 18 months of 61% are very promising, but this occurred at the expense of higher than expected toxicity, especially in patients over 60. About 15% of patients died during intensive RI therapy, mainly due to infectious complications; in addition, mucositis could be severe (grade 3 – 4), and prolonged cytopenia often necessitated long delays between treatment phases. Although mortality was considered within the expected range for age, several adaptations were implemented in order to maintain this treatment schedule for older patients. Prednisone was therefore to be utilised instead of dexamethasone (which has more profound metabolic effects and at the same time conceals possible complications), longer interruptions between treatment phases were to be accepted, and better antibacterial and antifungal prophylaxis was to be applied.

In conclusion, present treatment in younger adults with ALL is based on pediatric protocols as applied in the HOVON 70 trial and as also documented recently by the Boston group (16). For older adults, i.e. 40 to 70 years of age, HOVON and EORTC decided to use the described HOVON 71 variant schedule as backbone for further investigation. Although the described treatment schemes vary between patient subgroups according to age, the same research question with the same investigational drug can be examined in both subgroups combined, provided stratification for age is applied.

5.1.3 Detection of minimal redisual diseases in ALL patients

The vast majority of ALL patients achieve complete remission according to cytomorphological criteria. However sensitive methods for detection of malignant cells (“minimal residual disease”; MRD) have shown that leukemic cells are detectable at submicroscopic levels in many ALL patients.

Over the last 20 years many different techniques have been used for MRD detection in ALL patients (17,18), but only two of them appeared to be applicable in most ALL patients, reaching a sensitivity of 10^{-3} to 10^{-5}: 4-color flow cytometric immunophenotyping and real-time quantitative PCR (RQ-PCR) analysis of rearranged Ig/TCR genes (18). The application of flow cytometric immunophenotyping is based on the detection of aberrant antigen expression by the leukemic cells (19,20). The PCR technique exploits the occurrence of unique Ig/TCR gene rearrangements, which differ between normal and malignant lymphocytes, but are identical in all cells of the same lymphoid malignancy (21).

This MRD technique is standardized between the European MRD laboratories and the interpretation and reporting of the MRD-PCR result is done according to the ESG-MRD-ALL guidelines (23).

The flow cytometric and Ig/TCR-based MRD techniques have been used in several childhood ALL treatment protocols in order to assess the prognostic value. All prospective MRD studies have shown that MRD diagnostics can identify MRD-based high-risk, medium-risk, and low-risk patient groups, which differ significantly in treatment outcome (EFS 0% - 30% in MRD-high-risk , EFS 80% - >95% in MRD low-risk (24-29). Recently, the first large-scale prospective MRD study in adult ALL has been completed, showing that also in adult MRD three MRD-based risk groups can be identified, although the distribution of patients over the risk groups is different (30,31).

5.2 Clofarabine

5.2.1 Overview of clofarabine

Clofarabine (2-chloro-9-[2’-deoxy-2’-fluoro-b-D-arabinofuranosyl]adenine; Cl-F-ara A; CAFdA) is a rationally designed, second generation purine nucleoside analogue. Clofarabine was designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of both fludarabine (F-ara-A) and cladribine (2-CdA, CdA) both of which are currently approved by various regulatory authorities for treatment of hematologic malignancies. Because clofarabine has a chloro group at the 2-position of adenine, its chemical structure is more closely related to 2-CdA than to F-ara-A. Halogenation at the 2 position of adenine renders this class of compounds resistant to intracellular degradation by the enzyme adenosine deaminase. Substitution of a fluorine at the C-2’-position of the

arabinofuranosyl moiety of clofarabine increases its stability in gastric acid (33,34) and decreases its susceptibility to phosphorolytic cleavage by the bacterial enzyme Escherichia coli purine nucleoside phosphorylase in the gastrointestinal tract (34) both of which may lead to enhanced oral bioavailability. Clofarabine was approved in December 2004 by the United States Food and Drug Administration (US FDA) for the treatment of pediatric patients with relapsed or refractory ALL after at least 2 prior regimens based on the induction of complete responses.

5.2.2 Toxicology and pharmacology

For detailed information on clofarabine toxicology and pharmacology we refer to the clofarabine Investigator's Brochure (35).

5.2.3 Clinical experience with clofarabine i.v.

Phase I trials were initiated in 1999 and the first study was a traditional dose-escalation study where the objective was to establish the Maximum tolerated dose (MTD) in adult patients with solid tumors or hematologic malignancies (36). The starting dose was 15 mg/m^2 administered daily for 5 days and the dose was escalated in patients with hematologic malignancies to 55 mg/m^2, at which point patients experienced dose-limiting toxicities (DLTs) of reversible hepatotoxicity. The MTD was determined to be 40 mg/m^2/day, which is lower than the tolerable daily dose for pediatric patients of 52 mg/m^2/day. In a Phase II study reported by Kantarjian et al (37) 62 adult patients with relapsed or refractory acute leukemia received clofarabine 40 mg/m^2 i.v. once daily for 5 days every 3 to 6 weeks. Twenty patients achieved a CR, 9 achieved a CR with inadequate platelet recovery (CRp), and 1 achieved a partial response (PR) for a total response rate of 48%. The predominant toxicities were reversible liver dysfunction (as indicated by elevated aminotransferases [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] and hyperbilirubinemia), skin rashes, palmar-plantar erythrodysesthesia syndrome, and mucositis. Based upon the single-agent activity observed with clofarabine in several populations of patients with relapsed or refractory acute leukemias, efforts have been undertaken to combine clofarabine with other antileukemic agents. Faderl and colleagues conducted a Phase I/II study in adult patients with first relapse or first salvage of primary refractory AML or ALL (38). Patients were treated with doses of clofarabine of 15-40 mg/m^2/day by 1-hour i.v. infusion daily for days 2 through 6 followed 4 hours later by cytarabine 1 g/m^2/day by 2 hour i.v. infusion daily for days 1 through 5. Cytarabine alone was administered on day 1 at the same dose. Thirty-two patients were treated; 12 in the Phase I portion and 20 in the Phase II portion at 40 mg/m^2/day. Efficacy data are available for 32 patients; of these 7/32 (22%) achieved a CR and an additional 5/32 (16%) achieved a CRp for an overall response rate of 38% (12/32 patients). The most frequently reported drug-related adverse events (AE i.e., those occurring in ≥ 20% of patients) were

nausea, diarrhea, vomiting, dermatitis, flushing, palmar-plantar erythrodysesthesia syndrome, and headache. Changes in post-baseline chemistry parameters were mild to moderate in the majority of patients and were reversible if not attributable to the disease. Bone marrow function was suppressed, resulting in neutropenia, lymphocytopenia, anemia, and thrombocytopenia.

5.2.4 Pediatric Leukemia

In parallel to the adult program, a pediatric program was initiated in 2000. In the Phase I study in pediatric patients with hematologic malignancies, 25 patients were treated in cohorts of escalating doses up to 70 mg/m^2, a dose at which 1 patient had grade 4 hyperbilirubinemia and grade 3 elevated transaminases, and 1 had a grade 3 skin rash; the MTD was determined to be 52 mg/m^2 (39). Of the 13 patients treated at 52 mg/m^2, grade 2 to grade 3 increases in bilirubin and liver transaminases were observed. A total of 5 patients achieved a CR and 3 achieved a PR for an overall response rate of 32%. Thus, the MTD was determined to be 52 mg/m^2 and the recommended Phase II dose. Then phase II trials evaluated clofarabine 52 mg/m^2 administered once daily over a 2-hour i.v. infusion for 5 days every 2 to 6 weeks. In the 61 patients enrolled in the relapsed or refractory ALL study, the overall remission rate (CR+CRp) was 20%; 30% (18/61) of patients showed a response (7 CR, 5 CRp, 6 PR). Responses were noted in 15 of 50 (30%) patients with B- lineage ALL, 2 of 6 (33%) with T-cell ALL. After clofarabine treatment, 10 patients proceeded to transplant (including 8 responders). Six of 10 patients who received a transplant were alive at last follow up (survival range: 30.1+ - 145.1+ wks). Response duration in 6 patients with CR or CRp who did not receive a transplant ranged from 4.3 to 58.6 weeks; 2 patients maintained CR for 47.9 and 58.6 weeks after clofarabine therapy. Median overall survival for the patients who achieved at least a PR was 66.6 weeks compared to 12.9 weeks for all patients (40,41).

5.3 Rationale of the study

Clofarabine is a new active drug for treatment of ALL whose activity for RI is to be explored in combination with standardized therapy in order to improve outcome of this disease which is still lethal in most adult patients. Ultimate proof of efficacy resides in an increase of CR rate, prolongation of CR, and long-term survival. Since CR rate in adult ALL is high already and defining long-term survival in a large clinical trial takes many years, additional outcome parameters are worthwhile. The “quality” of CR can be assessed by quantifying MRD in patients who attain CR. This can be done by flow cytometric immunophenotyping and RQ-PCR analysis of patient-specific Ig /TCR gene rearrangements. Although younger (up to 40 years) patients are treated more intensively nowadays than older patients (older than 40 years of age), the investigational questions concerning clofarabine can be examined in both subgroups as both younger and older patients receive the same type of

maintenance chemotherapy and the same type of pre-phase chemotherapy, preceding induction/consolidation/intensification chemotherapy, although the latter varies according to age subgroup. In the consolidation phase both subgroups, the younger and older patients, also receive the same Clofarabine dose, if randomized for.

6 Study objectives

Phase II part

- To determine the feasibility of adding i.v. clofarabine to standard prephase therapy (followed by induction chemotherapy)

Phase III part

Primary objective:

- To improve EFS in adult ALL patients by the addition of i.v. clofarabine to prephase and consolidation therapy

Secondary objectives:

- To improve the molecular response rate of adult ALL following RI by the addition of i.v. clofarabine to standard prephase and consolidation therapy
- To improve DFS, and OS in adult ALL patients by the addition of i.v. clofarabine to the standard prephase and consolidation therapy
- To document safety and toxicity of adding clofarabine to standard prephase and consolidation therapy in adult ALL
- To assess and compare clinical outcome of patients with and without an HLA-identical sibling in a donor vs no-donor analysis
- To determine the incidence and clinical relevance of thromboembolic (TE) complications during adult ALL treatment with LMWH as antithrombotic prophylaxis
- To determine the influence of ALL (treatment) on different coagulation markers

7 Study design

A prospective, open-label, randomized phase II-III trial in which i.v. clofarabine is added in a randomized way to pre-phase and consolidation chemotherapy. The study starts as a randomized phase II feasibility study and continues as a randomized phase III study upon meeting the criteria for feasibility after 20, 40 and 60 patients randomized. If addition of 20 mg/m^2/day (x5) clofarabine is not feasible, the dose of clofarabine will be reduced to 15 mg/m^2/day (x5). If the dose of 20 mg/m^2/day (x5) is feasible, the dose will be increased to 30 mg/m^2/day (x5). Depending on the advice of the

DSMB, the dose of clofarabine will be decreased or increased. This will be followed by a randomized feasibility phase in, again, about 60 patients.

Accrual will continue until inclusion of 340 patients in approximately 3.5 to 4 years (or 340 plus 20-60 if the initial dose of clofarabine appeared not feasible or if the dose was increased to 30 mg/m^2/day (x5)). The standardized HOVON and EORTC treatment schedules of adult ALL will be the backbone to which clofarabine will be added, i.e. the HOVON-70/FRALLE-2000 schedule for patients up to 40 years of age, and a schedule for patients over 40, based on the above described HOVON-71 (Groningen) approach.

8 Study population

8.1 Eligibility for randomization

8.1.1 Inclusion criteria

- Patients aged 18 to 70 years inclusive
- Primary previously untreated B or T-lineage ALL (excluding ALL with mature B-cell phenotype, but including Philadelphia positive or BCR-ABL positive ALL) or previously untreated T-LBL (pretreatment with prednisolone for 7 days is allowed)
- WHO performance status 0 – 2
- Adequate renal and hepatic function tests as indicated by the following laboratory values:
  - Serum creatinine ≤1.0 mg/dl (≤ 88.7 micromol/L); if serum creatinine >1.0 mg/dl (>88.7 micromol/L), then the glomerular filtration rate (GFR) must be >60 ml/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease equation where the predicted GFR (ml/min/1.73 m^2) = 186 x (Serum Creatinine in mg/dl)$^{-1.154}$ x (age in years)$^{-0.023}$ x (0.742 if patient is female) x (1.212 if patient is black)
    NOTE: if serum creatinine is measured in micromol/L, recalculate it in mg/dl according to the equation: 1 mg/dl = 88.7 micromol/L) and used above mentioned formula.
  - Serum bilirubin ≤ 1.5 × upper limit of normal (ULN)
  - Aspartate transaminase (AST)/alanine transaminase (ALT) ≤ 2.5 × ULN
  - Alkaline phosphatase ≤ 2.5 × ULN
- Negative pregnancy test at inclusion, if applicable
- Written informed consent

8.1.2 Exclusion criteria

- Mature surface Ig positive B-cell leukemia/lymphoma
- Acute undifferentiated leukemia
- Severe cardiovascular disease (arrhythmias requiring chronic treatment, congestive heart failure or symptomatic ischemic heart disease)
- Severe pulmonary dysfunction (CTCAE grade III-IV, see appendix D)
- Severe neurological or psychiatric disease
- History of active malignancy during the past 5 years with the exception of basal carcinoma of the skin or stage 0 cervical carcinoma
- Active, uncontrolled infection
- Patient known to be HIV-positive
- Patient is a lactating woman
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule
- Unwilling or not capable to use effective means of birth control (all men, all premenopausal women under the age of 50 need contraception for two years after the last period, and women older than 50 yrs for at least one year)

9 Treatment

All patients, irrespective of age, will receive pre-phase chemotherapy according to treatment-arm assigned, following randomization.
Patients will receive treatment with standard pre-phase prednisone (Arm A) or prednisone combined with clofarabine (Arm B) as described in paragraph 9.1.
In France, according to regulatory demands, appropriate measures should be taken in men and women to avoid conception during and for at least 6 months following cessation of methotrexate therapy. Both men and women receiving methotrexate should be informed of the potential risk of adverse effects on reproduction.

9.1 Prephase treatment

Supportive care:
Due to the high dose of prednisolone either with or without clofarabine, it is of importance to carefully prevent tumor lysis in prephase period. Therefore it is advocated to prescribe rasburicase to all patients included. Preferably rasburicase is given at a dose of 3 mg per day to be followed by daily monitoring of uric acid, renal function and electrolytes. In case of insufficient decline of uric acid or

increase, rasburicase will be repeated by 3 mg per day, again followed by monitoring of laboratory abnormalities and continuation of rasburicase until resolution of tumor lysis. To prevent transfusion related graft versus host disease, irradiated blood products are necessary for one year after start of treatment with clofarabine. Do not combine gifts of imatinib and/or valaciclovir with clofarabine on the same day. It is recommended to administer sufficient fluids i.v. at days of clofarabine administration to avoid tumor lysis syndrome.

Arm A

Agent	Dose/day	Route	Days
Predniso(lo)ne	60 mg/m^2, divided in 2 daily doses	p.o.	1-7
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	Day 1, or upon peripheral blast clearance

Blood morphology should be performed on day 8 to determine corticosteroid sensitivity (but this will not affect further therapy).

Arm B

Agent	Dose/day	Route	Days
Clofarabine	Assigned dose level	i.v. over 1 hour	1-5
Predniso(lo)ne	60 mg/m^2, divided in 2 daily doses	p.o.	1-7
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	Day 1, or upon peripheral blast clearance

At study start, patients are assigned to a dose level Clofarabine of 20 mg/m^2/day (x5), if randomized for arm B. If this dose is not feasible according to the decision rules as described in section 17, the dose of clofarabine to be randomized for will be reduced to 15 mg/m^2/day (x5). If the dose of 20 mg/m^2/day (x5) is feasible, the dose is increased to 30 mg/m^2/day (x5). Depending on the advice of the DSMB, the dose of clofarabine will be decreased or increased.
Blood morphology should be performed on day 8 to determine corticosteroid sensitivity (but this will not affect further therapy).

9.2 Induction-Consolidation-Intensification

Following pre-phase, patients will proceed with induction chemotherapy at day 8 (with day 1 being the start of prephase therapy), irrespective of hematological toxicity, whereby most patients will start with

induction during neutropenia following preceding clofarabine. G-CSF is indicated in case of neutropenia until recovery of peripheral blood.

Induction-consolidation-intensification chemotherapy will be stratified according to age (> 40 years versus ≤ 40 years (inclusive)).

All younger patients (in Arm A and Arm B) will proceed with induction/consolidation/clofarabine consolidation, if randomized for/intensification/interphase/intensification, according to treatment schedules outlined in section 9.2.1.

All older patients (in Arm A and Arm B) will proceed with age adapted induction/consolidation at day 8, according to treatment schedules outlined in section 9.2.2 (with an additional clofarabine consolidation cycle after consolidation I, if randomized for).

9.2.1 Induction-Consolidation-Intensification for younger patients

9.2.1.1 Remission Induction (RI) (≤ 40 y)

No dose modification due to hematological toxicity should be made during induction treatment. Induction will start at day 8 following prephase therapy. For infection prophylaxis and other supportive care measures see the appropriate section 9.7.

Agent	**Dose/day**	**Route**	**Days**
Predniso(lo)ne	40 mg/m^2, divided in 2 daily doses	p.o.	8-28, then taper to 0 within 7 days
Vincristine	1.5 mg/m^2, maximum 2 mg	i.v.	8, 15, 22, 29
Daunorubicin	40 mg/m^2	i.v. in 60 minutes	15, 22
PEG-L-Asparaginase* (postponed in case of livertoxicity!!, see Appendix E)	1000 IU/m^2	i.v.	8, 21
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	8, 15 in case of CNS localisation also on day 22
G-CSF	According local policy	s.c.	From day ANC < 0.5 until ANC > 1.0 x 10^9/L

* see Appendix E

Early chemosensitivity will be evaluated by bone marrow examination at day 15 (with day 1 being the start of prephase therapy). BM blasts will be documented and estimated as M1 (<5% blasts), M2 (5 – 25% blasts), or M3 (>25% blasts) in order to correlate early chemosensitivity with final outcome later on.
Response (see appendix B) after induction treatment will be evaluated preferably between days 35-42. Patients in hematological CR will continue with consolidation treatment. Patients who did not achieve CR will go off protocol treatment. In these patients intensive reinduction and consolidation by high dose Ara-C and an anthracyclin followed by allogeneic stem cell transplantation is advised.

9.2.1.2 Consolidation (≤ 40 y)

The consolidation will be given in two parts, the first one to be given is indicated 'a', the second one 'b'.
Consolidation treatment starts as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l, providing that the eligibility criteria below are met.

Eligibility criteria for consolidation treatment:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0–2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections

Treatment should be postponed until the eligibility criteria are met.

Consolidation a

Agent	Dose/day	Route	Days
6-Thioguanine	60 mg/m^2	p.o.	1-21
Etoposide	150 mg/m^2	i.v. in 60 minutes	1, 8, 15
ARA-C	60 mg/m^2, divided in 2 daily doses	s.c.	1, 2, 8, 9, 15, 16
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	1, in case of initial CNS localization also on day 15

Consolidation b

The second part of the consolidation treatment starts no sooner than day 29 (with day 1 being the start of consolidation treatment A) and as soon as ANC > 0.5 x 10^9/l and platelets > 50 x 10^9/l.

Agent	Dose/day	Route	Days
Predniso(lo)ne	40 mg/m^2, divided in 2 daily doses	p.o	29-35
Vincristine	1.5 mg/m^2, maximum 2 mg	i.v.	29, 43
6-Mercaptopurine	50 mg/m^2	p.o.	29-49
MTX*	5000 mg/m^2	i.v.	29, 43
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	29, 43 (at hour 24 of high dose MTX i.v.)

* see Appendix F (re-check liver and renal functions before high dose MTX administration)

Response (see appendix B) after consolidation treatment will be evaluated preferably between days 50 and 57. Patients with CR will continue with clofarabine consolidation if randomized for, or with intensification treatment, if not randomized for clofarabine treatment. Patients not in a CR or with a relapse after CR will go off protocol treatment.

9.2.1.3 Clofarabine consolidation (≤ 40 y)

Patients randomized for treatment with clofarabine will start with clofarabine consolidation no sooner than day 58 (with day 1 being start consolidation treatment a), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l, and if the eligibility criteria below are met. Do not combine gifts of imatinib and/or valaciclovir with clofarabine on the same day. It is recommended to administer sufficient fluids i.v. at days of clofarabine administration, because of possible decrease of side effects.

Eligibility criteria for clofarabine consolidation treatment:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0–2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections

Treatment should be postponed until the eligibility criteria are met.

Agent	Dose/day	Route	Days
Clofarabine	assigned dose level (same as in prephase	i.v. over 1 hour	1-5

Response (see appendix B) after clofarabine consolidation treatment will be evaluated preferably between day 22 and start of next cycle of chemotherapy. Patients with CR will continue with Intensification treatment. Patients not in a CR or with a relapse after CR will go off protocol treatment.

9.2.1.4 Intensification I (≤ 40 y)

The first Intensification consists of two consecutive parts, the first one to be given is indicated 'a', the second one 'b'.

For the patients who have received clofarabine consolidation, the intensification treatment starts no sooner than day 22 (with day 1 being start ***clofarabine* consolidation** treatment, which differs from the control arm where the start is related to consolidation treatment a), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l, and if the eligibility criteria below are met.
For the patients who did not receive clofarabine consolidation, the intensification treatment starts no sooner than day 58 (with day 1 being start ***consolidation treatment a***), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l, and if the eligibility criteria below are met.

Eligibility criteria for starting intensification treatment I:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0-2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections

Treatment should be postponed until the eligibility criteria are met.

Intensification I a

Agent	Dose/day	Route	Days
Dexamethasone	10 mg (2dd 5 mg)	p.o.	1-14, then taper to 0 within 7 days
Vindesine Alternative in case of unavailability: Vinblastine	3 mg/m^2, maximum 4 mg 6 mg/m^2 (maximum 10 mg)	i.v. i.v.	1, 8, 15 1, 8, 15
Doxorubicin	25 mg/m^2	i.v. in 60 minutes	1, 8, 15
PEG-L-Asparaginase*	1000 IU/m^2	i.v.	4, 18
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	1, in case of initial CNS localization also on day 15

* see Appendix E

Intensification I b

The second part of the intensification treatment starts no sooner than day 29 (with day 1 being start intensification treatment I a) and as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l.

Agent	Dose/day	Route	Days
6-Thioguanine	60 mg/m^2	p.o.	29-49
Etoposide	150 mg/m^2	i.v. in 60 minutes	29, 36, 43
ARA-C	60 mg/m^2, divided in 2 daily doses	s.c.	29, 30, 36, 37, 43, 44
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	29

Response (see appendix B) after intensification treatment I will be evaluated preferably between days 50 and 57. Patients with CR will continue with interphase treatment. Patients with a relapse after CR will go off protocol treatment.

Patients in CR with a suitable stem cell donor may proceed to allogeneic stem cell transplantation (see section 9.4).

9.2.1.5 Interphase (≤ 40 y)

Interphase treatment starts no sooner than day 29 (with day 1 being start intensification treatment Ib), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l, and if the eligibility criteria below are met.

Eligibility criteria for starting interphase treatment:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0-2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections

Treatment should be postponed until the eligibility criteria are met.

Agent	Dose/day	Route	Days
Predniso(lo)ne	40 mg/m^2, divided in 2 daily doses	p.o.	1-7
Vincristine	1.5 mg/m^2, maximum 2 mg	i.v.	1, 15
6-Mercaptopurine	50 mg/m^2	p.o.	1-21
MTX*	5000 mg/m^2	i.v	1, 15
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4mg	i.t.	1, 15 (at hour 24 of high dose MTX i.v.)
Cranial irradiation** (only in case of CNS localisation at diagnosis, and no alloSCT is planned including myeloablative TBI)	24 Gy total dose	-	Between day 29-43

* see Appendix F (re-check liver and renal functions before high dose MTX administration)

9.2.1.6 Intensification II (≤ 40 y)

Intensification treatment II starts no sooner than day 29, or day 43 in case of CNS-irradiation, (with day 1 being start interphase treatment), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l, and if the eligibility criteria below are met.

Eligibility criteria for starting intensification treatment II:

- Patient without signs of relapse

- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0 -2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections

Treatment should be postponed until the eligibility criteria are met.

Agent	Dose/day	Route	Days
Predniso(lo)ne	40 mg/m^2, divided in 2 daily doses	p.o.	1-14, then taper to 0 within 7 days
Vincristine	1.5 mg/m^2, maximum 2 mg	i.v.	1, 8, 15
Daunorubicin	30 mg/m^2	i.v. in 60 minutes	1, 8, 15
PEG-L-Asparaginase*	1000 IU/m^2	i.v.	4,18
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	1 (not in case of previous cranial irradiation)

*see Appendix E

Response (see appendix B) after intensification II treatment will be evaluated preferably between days 30 and 40. Patients with CR will continue with maintenance treatment. Patients with a relapse after CR will go off protocol treatment.

9.2.2 Induction-consolidation courses in older patients (> 40 years)

Older patients (> 40 years of age) will receive a less intensive schedule of chemotherapy. Patients will continue first with standard induction I to be followed by consolidation I, clofarabine consolidation (if randomized for) induction II, and consolidation II chemotherapy.

9.2.2.1 Remission Induction (RI) I (> 40 y)

Induction starts at day 8 following pre-phase chemotherapy, irrespective of hematological recovery.

Agent	Dose/day	Route	Days
Predniso(lo)ne	40 mg/m^2, divided in 2 daily doses	p.o.	8 – 28 then taper to 0 within 7 days
Vincristine	1 mg	i.v.	8, 15, 22
Doxorubicin	40 mg/m^2	i.v.	15, 22
MTX +dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	8, 15, 22

At day 15 (with day 1 being start of prephase therapy), BM blasts will be documented and estimated as M1 (<5% blasts), M2 (5 – 25% blasts), or M3 (>25% blasts) in order to correlate early chemosensitivity with final outcome later on. Response (see appendix B) after induction treatment will be evaluated preferably between days 28 and 42. Patients in CR will continue with consolidation treatment. Patients who did not achieve CR nor meet the above mentioned criteria will go off protocol treatment. In these patients intensive reinduction and consolidation by high dose Ara-C and an anthracyclin followed by allogeneic stem cell transplantation is advised.

9.2.2.2 Consolidation I (> 40 y)

Eligibility criteria for consolidation treatment:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0–2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections
- Hematological recovery

Treatment should be postponed until the eligibility criteria are met.

Agent	Dose/day	Route	Days
Cytarabine	200 mg/m^2	i.v. over 1 hour	1, 8
Etoposide (VP16)	120 mg/m^2	i.v. over 1 hour	1, 8
Methotrexate (MTX)	500 mg/m^2	i.v. over 2 hours	4, 11
Leucovorin (see Appendix F)	60 mg/m^2 in 4 doses	first dose i.v., rest may be given p.o.	5, 12, continuing until plasma MTX level <0,2 µmol/l
MTX +dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	1

Response (see appendix B) after consolidation treatment I will be evaluated preferably between days 15 and 22. Patients with CR will continue with clofarabine consolidation, if randomized for, if not randomized for clofarabine treatment they will continue with induction II treatment. Patients not in a CR or with a relapse after CR will go off protocol treatment.

9.2.2.3 Clofarabine consolidation (> 40 y)

Patients randomized for treatment with clofarabine will start with clofarabine consolidation treatment no sooner than day 15 (with day 1 being start consolidation treatment I), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l and if the eligibility criteria (as described below) are met. Do not combine gifts of imatinib and/or valaciclovir with clofarabine on the same day. It is recommended to administer sufficient fluids i.v. at days of clofarabine administration, because of possible decrease of side effects.

Eligibility criteria for clofarabine consolidation treatment:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0–2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections

Treatment should be postponed until the eligibility criteria are met.

Agent	Dose/day	Route	Days
Clofarabine	assigned dose level (same as in prephase)	i.v. over 1 hour	1-5

Response (see appendix B) after clofarabine consolidation treatment will be evaluated preferably between day 15 and start of next cycle of chemotherapy. Patients with CR will continue with induction II treatment. Patients not in CR or with a relapse after CR will go off protocol treatment.

9.2.2.4 Remission Induction II (> 40 y)

For the patients who have received clofarabine consolidation, induction II starts no sooner than day 22 (with day 1 being the start of ***clofarabine consolidation***, which differs from the control arm where the start is related to consolidation I), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l and if the eligibility criteria are met.

For the patients who have not received clofarabine consolidation, induction II starts no sooner than day 22 (day 1 being the start of ***Consolidation I*** chemotherapy), as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l and if the eligibility criteria are met.

Eligibility criteria for induction II:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0-2
- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections
- Hematological recovery

Agent	Dose/day	Route	Days
Predniso(lo)ne	40 mg/ m^2, divided in 2 daily doses	p.o.	1 – 21, then taper to 0 within 7 days
Vincristine	1 mg	i.v.	1, 8, 15
Doxorubicin	40 mg/m^2	i.v.	1, 8, 15*
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	15

*Note that in contrast to RI I, RI II has one additional doxorubicin dose at day 1.

Response (see appendix B) after induction treatment will be evaluated preferably between days 22 and 35, at recovery of peripheral blood. Patients with CR will continue with consolidation treatment. Patients with a relapse after CR will go off protocol treatment.

9.2.2.5 Consolidation II (> 40 y)

Consolidation treatment starts as soon as ANC > 1.0 x 10^9/l and platelets > 100 x 10^9/l and if the eligibility criteria below are met.

Eligibility criteria for consolidation treatment:

- Patient in CR
- Absence of severe renal and liver function abnormalities, i.e. creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal
- WHO performance status 0-2

- Absence of severe cardiac, pulmonary, neurologic or metabolic disease
- Absence of uncontrolled infections
- Hematological recovery

Agent	Dose/day	Route	Days
Cytarabine*	1000 mg/m^2 per 12 hours (2 times daily; 4 times total)	i.v. over 1 hour	1, 2
PEG-L-Asparaginase* (postponed in case of livertoxicity!!, see Appendix E)	1000 IU/m^2	i.v.	3*, 18
MTX + dexamethasone (or prednisolone 25 mg)	15 mg 4 mg	i.t.	15
Cranial irradiation (only in case of CNS localisation at diagnosis, and no allo SCT including myeloablative TBI is planned)	24 Gy total dose	-	Between day 29-43

*Asparaginase is to start at least 6 hours after the last dose of cytarabine for synergistic activity (see appendix E).

Response (see appendix B) after consolidation treatment will be evaluated preferably between days 22 and 29. Patients with CR will continue with MT. Patients with a relapse after CR will go off protocol treatment.

9.3 Maintenance chemotherapy of adult ALL

Maintenance chemotherapy is similar for younger and older patients.

Eligibility criteria for maintenance treatment:

- Persistent CR and having completed induction/consolidation/intensification-chemotherapy
- Recovery from the previous chemotherapy
- Absence of active uncontrolled infection
- Absence of severe hepatic, renal, cardiac, neurological, or psychiatric complications

First year of maintenance treatment

Agent	Dose/day	Route	Days
Predniso(lo)ne	1 mg/kg	p.o.	1 – 7
Vincristine	1.4 mg/m^2 (max 2 mg)	i.v.	1
MTX	15 mg/m^2	p.o.	8, 15, 22
6-Mercaptopurine (6-MP)	75 mg/m^2	p.o.	1 – 28

Day 29 = day 1 of next cycle.

Please note that MTX (15 mg) + dexamethasone (4 mg) (or prednisolone 25 mg) i.t.is to be given at day 1 only during the first 4 cycles of maintenance chemotherapy. The maximum number of intrathecal injections for the whole protocol treatment period for prophylaxis is 15 for the younger patients, , maintenance inclusive; for the older patients the maximum number of prophylactic intrathecal injections is 10 (maintenance inclusive).

Dose adjustments during maintenance treatment

Target WBC numbers during maintenance are: ANC 0.8-1.2 x 10^9/l l.

The dose of oral Methotrexate (MTX) and/or 6-mercaptopurine (6-MP) during maintenance will be adjusted according to the following scheme:

	ALT < 5 x ULN	ALT 5 –10 x ULN	ALT > 10 x ULN
ANC < 0.5 x10^9/l	Stop until resolved	Stop until resolved	Stop until resolved
ANC 0.5- 0.8 x10^9/l	Decrease 6-MP and MTX with 33% until resolved	Decrease 6-MP with 33% and stop MTX until resolved	Stop until resolved
ANC 0.8 –1.2 x10^9/l	No modification	Decrease 6-MP with 33% and stop MTX until resolved	Stop until resolved
ANC> 1.2 x10^9/l	No modification	Decrease 6-MP with 33% and stop MTX until resolved	Stop until resolved
ANC> 2 x10^9/l	Increase of 6-MP and MTX by 33%	Decrease 6-MP with 33% and stop MTX until resolved	Stop until resolved

Maintenance chemotherapy as outlined above will be continued for 1 year (12 cycles scheduled).

Second year of maintenance treatment

Thereafter, patients will continue another year with 6MP, to be combined, if possible, with 3 oral MTX administrations per cycle (28 days), i.e. prednisone and vincristine will be stopped after 12 cycles. No i.t. administrations will be given anymore. MT will stop definitely 24 months from start of MT.

Agent	Dose/day	Route	Days
MTX	15 mg/m^2	p.o.	1, 8, 15
6-Mercaptopurine (6-MP)	75 mg/m^2	p.o.	1 – 28

Day 29 = day 1 of next cycle.

Evaluation of hematology and chemistry will be done approximately every month (BM only at clinical or laboratory signs of relapse).

9.4 Allogeneic stem cell transplantation

Standard-risk and high-risk (for definition of high-risk: see paragraph 11.4) patients in CR1 with an HLA-matched sibling donor will proceed to alloSCT after intensification I (younger patients) or after consolidation II (older patients).
High-risk patients in CR after intensification I (younger patients) or after consolidation II (older patients) with a suitable alternative stem cell donor (MUD, cord blood, haploidentical family donor) should also proceed to allogeneic stem cell transplantation, since AlloSCT using a sibling or an alternative donor is considered indicated for all high-risk patients.
HLA typing of patient and siblings should be performed at study entry.
Allogeneic SCT using either a myeloablative or non-myeloablative conditioning regimen will be carried out according to the standard guidelines and general procedures operational in the local allogeneic transplantation centers. However, it is advocated to apply myeloablative alloSCT up to the age of 40, and to offer older patients (> 40 years of age) a non-myeloablative alloSCT, using the Seattle fludarabine-2Gy conditioning regimen, followed by unmanipulated alloSCT.

Patients receiving an allogeneic stem cell transplantation will NOT go off protocol treatment: alloSCT is considered part of protocol and these patients will be followed-up as detailed in section 11.

9.5 Imatinib treatment in Philadelphia positive ALL patients

Philadelphia-positive (Ph+) ALL patients are eligible for this study and can participate. However, due to the unknown interaction of imatinib and clofarabine, imatinib will only be started after pre-phase

chemotherapy as soon as the diagnosis of Ph+ or BCR/ABL positive ALL has been made. These patients are considered high-risk patients.
Preferably, Ph+ patients proceed to allogeneic SCT using either a related or alternative donor, see paragraph 9.4. Patients not qualifying for alloSCT may receive standard maintenance therapy.
Imatinib 600 mg per day orally will be given as from the day of detection of the (9;22) translocation or the BCR/ABL product until relapse, until 6 months after allogeneic stem cell transplantation or until the end of maintenance treatment, whichever comes first. In case of confirmed PCR (BCR/ABL) positivity after complete molecular remission, an alternative tyrosine kinase (TK) inhibitor, preferably dasatinib, is advocated.
Because of the unknown interaction of imatinib and asparaginase, imatinib must be withheld during the periods when PEG-L-Asparaginase is administered, until two weeks after the last dose of PEG-L-Asparaginase.

9.6 Study drug information

9.6.1 Physical and Chemical Characteristics

Clofarabine is a white to off-white solid with a melting point of 228°C to 230°C and a molecular weight of 303.5. The drug substance is very stable in the dry state, and aqueous solutions are stable to heat treatment. Clofarabine is freely soluble in water (1.5 mg/ml) or buffered solutions at room temperature. Clofarabine is not less than 97% pure on a dried basis by high performance liquid chromatography (HPLC) analysis.
Clofarabine is formulated at a concentration of 1 mg/ml. Clofarabine is provided by the manufacturer and supplied in 1 vial size: a 20-mL clear, glass vial with gray stopper and blue flip off seal. The 20-ml vials contain 20 ml (20 mg) of sterile solution. The pH range of the solution is 4.5 to 7.5. The solution is clear and practically colorless, preservative free, and free from foreign matter.

9.6.2 Storage and Handling

Vials containing undiluted clofarabine for injection should be stored at controlled room temperature. The commercial expiry period for Clolar (clofarabine) is 24 months at room temperature. Ongoing stability studies will continue to confirm the appropriate quality of drug product used for clinical trials beyond 24 months. Clofarabine for injection should be diluted with 0.9% sodium chloride injection USP or European Pharmacopeia (EP) normal saline (NS) or 5% dextrose injection (D5W) USP or EP prior to IV infusion. The resulting admixture may be stored at room temperature, but must be used within 24 hours of preparation. Clofarabine will be infused in a 1 hour infusion.

9.6.3 Special considerations for administration of clofarabine

Clofarabine is excreted primarily by the kidneys, therefore, drugs with known renal toxicity should be avoided during the 5 days of clofarabine treatment in each cycle. Additionally, the liver is a known target organ for clofarabine toxicity, therefore, concomitant use of medications known to induce hepatic toxicity should be avoided. Hepatic and renal function should be assessed prior to and during treatment with clofarabine and it is recommended that the patient's fluid status and hepatic and renal function be carefully monitored during the drug administration period. All patients should receive hydration each day of clofarabine treatment, giving careful consideration to the cardiac and renal function of the patient. To the extent possible, use of nephrotoxic (eg, vancomycin, amphotericin B, etc) and hepatotoxic (eg, voriconazole, cyclosporine, etc) agents is to be avoided during clofarabine administration

9.6.4 Clofarabine drug accountability

The local investigator is responsible for ensuring that all study drug received at the site is inventoried and accounted for throughout the study. The dispensing of study drug to the subject, and the return of study drug from the subject (if applicable), must be documented.
Study drug must be handled strictly in accordance with the protocol and the container label and will be stored under appropriate environmental conditions. Contents of the study drug containers must not be combined.
Study drug should be dispensed under the supervision of the investigator, a qualified member of the investigational staff, or by a hospital/clinic pharmacist. Study drug will be supplied only to subjects participating in the study. Returned study drug must not be dispensed again, even to the same subject. Study drug may not be relabeled or reassigned for use by other subjects. The investigator agrees neither to dispense the study drug from, nor store it at, any site other than the study sites agreed upon with the sponsor.

9.7 Supportive care

As the intensified chemotherapy schedules for both younger and older patients are highly immunosuppressive, infectious prophylaxis is of extreme importance. The following is advocated:

1. Viral prophylaxis using valacyclovir 2 x 500 mg daily for the entire protocol.
2. Pneumocysitis carinii prophylaxis by cotrimoxazole according to local rules (e.g. 960 mg 3 times weekly or 480 mg daily) during entire treatment protocol.
3. Prophylaxis for gram-positive and gram-negative bacteria during neutropenia AND mucositis, using i.v. penicillin (6x 10^6 Units/day) and ciprofloxacin (2x500 mg, daily), respectively, or according to institutional standards.

4. Fungal prophylaxis during high-dose prednisone therapy and during neutropenia. Fluconazol can suffice because it is effective against *Candida albicans*, but based on previous experience (HOVON 71) voriconazol or posaconazol is preferable during preface, remission induction I, and consolidation I in elderly patients.
5. The use of broad-spectrum antibiotics and antifungal drugs with activity against Aspergillus is advocated in case of fever and hypotension during prednisone therapy in neutropenic patients.
6. G-CSF should be given to patients with neutrophils < 0.5 x 10^9/l until recovery. (G-CSF is mandatory in RI for younger patients (see paragraph 9.2.1.1)

Parental nutrition is indicated during induction and consolidation in case of > 10% weight loss.

9.7.1 Thrombosis prophylaxis

Treatment of ALL is frequently complicated by (venous) thromboembolic (TE) complications. Incidence rates in recent ALL-HOVON studies were 15%, 12% and 12% (HO37, HO70 and HO71 respectively). To reduce the TE risk during chemotherapy treatment in this study, thrombosis prophylaxis will be used. Different interventions are widely used to reduce TE during ALL, e.g. suppletion of fresh frozen plasma (FFP), antithrombin (AT), or the prophylactic use of heparin or low-molecular-weight heparin (LMWH). FFP nor AT suppletion significantly reduced TE incidence in previous studies. However, a significant thrombosis reduction was seen in children with prophylactic LMWH, without bleeding events (46).

In this study, antithrombotic prophylaxis will be administered in the form of LMWH, specifically nadroparin (Fraxiparin®). Nadroparin must be administered subcutaneously in a **high prophylactic dose of 5700 IE anti-Xa / 24 hours (= 0.6 mL)**, irrespective of patient weight. This dosage has proven to be safe and effective in previous ALL studies, without significant bleeding. **No FFP or AT** supplementation should be given as antithrombotic intervention.
Nadroparin is started on the first day of treatment (prephase Arm A and B) and is continued until 14 days after the last administration of PEG-L-asparaginase in the first remission induction cycle (± day 35) for patients <= 40 y or until start consolidation for patients > 40 y, irrespective of clofarabine randomization result . This duration is based on the pharmacokinetic effects of PEG-L-asparaginase; the end of its maximum therapeutic and thrombotic effect occur about 14 days after administration (47).
TE complications are particularly observed during the first remission induction cycle and studies have shown that re-exposure to asparaginase is safe in later treatment cycles (48). Therefore, nadroparin will **only** be administered in the prephase and induction cycles in both age categories and not in later treatment cycles containing PEG-L-asparaginase.

LMWH should be stopped 24 hours before and after diagnostic / therapeutic lumbar punctions.
Patients with renal insufficiency (GFR < 30 ml/min) should be given an adjusted dose (3800 anti-Xa / 24 hours (= 0.4 mL). Patients with heparin allergy or a history of heparin-induced thrombocytopenia (HIT) should not receive nadroparin, but danaparoid 1500 AXa-E / 24 hours (= 1.2 mL) subcutaneously or fondaparinux 2.5 mg / 24 hours (= 0.5 mL).
The known and potential risks of LMWH are bleeding, (local) allergic reactions and a minor chance of HIT. Bleeding and allergic reactions due to LMWH should be documented on the CRF. Bleeding is categorized as minimal, minor or major hemorrhage, according to the modified Thrombolysis In Myocardial Infarction (TIMI) study group criteria. In case of major hemorrhage, thrombosis prophylaxis should be withheld for 24 hours and reinstated as soon as hemorrhaging has stopped.
Platelet levels should be monitored. Transfusions are advised if platelet levels drop below $20x10^9$/L (also left to discretion of local physician).

9.7.2 Thrombophilic mutations

All patients in Dutch participating centers will be screened for the factor V Leiden and factor II mutation upon inclusion.
Blood samples should be collected, centrifuged and stored at -80°C at treatment site until shipment to the AMC, Amsterdam, for central analysis.

9.7.3 Diagnosis of thromboembolic (TE) complications

Patients are monitored for clinical symptoms of TE complications during all HOVON 100 protocol treatment cycles and a 3-month follow-up period. No screening will be done for asymptomatic thrombosis. Patients with a central venous catheter (CVC) thrombosis (at the catheter tip), without any symptoms, are considered asymptomatic. Symptomatic TE events must be objectively confirmed using appropriate radiologic imaging procedures:

- central nervous system thrombosis: MRI or CT-angio of the head;
- upper extremity thrombosis: ultrasonography and venography if negative;
- lower extremity thrombosis: ultrasonography (Doppler);
- pulmonary emboli: CT angiography.

10 End of protocol treatment

- ♦ Inability to attain CR at the end of RI course
- ♦ Relapse of ALL at any time after attainment of CR

- Life-threatening and uncontrollable complications (infectious or metabolic) which prohibited further intensive chemotherapy
- Death of the patient whatever cause
- Withdrawal of informed consent by the patient
- Physician's opinion that termination of protocol treatment is in the best interest of the patient
- Major protocol violation
- Completion of protocol therapy

11 Required clinical evaluations

11.1 Time of clinical evaluations

- At entry: The investigations before start of treatment should be no older than 14 days prior to randomization unless otherwise noted in the following part
- During induction, consolidation, intensification, allo-SCT, interphase and maintenance therapy
- During follow up

11.2 Required investigations

Required investigations at entry, during treatment and during follow up.

	At entry	Pre-phase	Induction (younger) Induction I (older)	Consolidation (younger) Consolidation I (older) Clofarabine Consolidation	Interphase (younger) Induction II (older)	Intensification I&II (younger) Consolidation II (older) Allo-SCT (all)	Maintenance	FU
Medical history	X	X	X	X	X	X	X	X
Physical examination	X	X	X	X	X	X	X	X
Hematology	X	X[1)]	X[2)]	X[2)]	X[2)]	X[2)]	X	X
PB immunophen.	X		X	X	X	X	X[3)]	X[3)]
PB PCR BCR/ABL	X		X[4)]	X[4)]	X[4)]	X[4)]	X[4)]	X[4)]
PB storage	X							
Blood chemistry	X	X	X[5)]	X[5)]	X[5)]	X[5)]	X	
Asparaginase levels			X[8]			X[8]		

Bone marrow aspirate								
Morphology	X		X[6)]	X	X	X	X[3)]	X[3)]
BM immunophen.	X		X	X	X	X	X[3)]	X[3)]
Cytogenetics	X		X[7)]	X[7)]				
MRD PCR TCR/BCR gene rearrangements	X		X	X	X	X	X[3)]	X[3)]
BM PCR BCR/ABL	X		X[4)]	X[4)]	X[4)]	X[4)]	X[4)]	X[4)]
BM storage	X		X			X		
Specific investigations								
Chest X-ray	X							
ECG	X							
CSF examination	X	X	X	X	X	X	o.i.	o.i.
EMD examination	o.i.	o.i.	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.	o.i.	o.i.
Microbiological tests	X	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.	o.i.
HLA typing	X							
Grading GVHD[9)]						X		X
Central lab for coagulation side study[10] • Mutational analysis • Thrombophilic mutations • Coagulation markers	X X X[10]	X[10]	X[10]	X[10]	X[10]	X[10]		

o.i. on indication

1) Morphology will be performed on day 8 of prephase to determine corticosteroid sensitivity (see 11.2.10)

2) Complete blood count (CBC) and platelets during induction treatment twice weekly, during consolidation, interphase and intensification I & II every week.

3) at start of maintenance and thereafter only on clinical and laboratory signs of relapse

4) if applicable (see 11.2.9)

5) Blood chemistry tests during induction treatment twice weekly, during consolidation, interphase and intensification I & II every week.

6) At day 15 (with day 1 being the start of prephase therapy) BM blasts will be documented and estimated as M1 (<5% blasts), M2 (5 – 25% blasts), or M3 (>25% blasts) in order to assess early chemosensitivity with final outcome; BM of between day 35 and 42 (with day 1 being the start of prephase therapy) will be evaluated for response.

7) At achievement of morphologic CR if aberrant karyotype at diagnosis

8) For monitoring asparaginase activity levels, blood samples must be taken at days 8, 15, 21 and 35 of induction and days 4, 11, 18 and 32 of intensification Ia and II for young patients (not applicable for older patients >40 yrs). In case of treatment delays, collection of blood samples should be performed 7 days after

PEG-L-asparaginase administration and directly before administration of the next PEG-L-asparaginase dose (see appendix E).

9) See appendix H for grading of GVHD

10) For coagulation side study: see side studies lab manual

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
- Previous thrombotic events and current anticoagulant use

11.2.2 Physical examination

Standard physical examination, with special attention for:

- Height (only at entry)
- Body weight
- 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
- Platelets
- WBC
- WBC differential
- Immunophenotyping (see paragraph 11.2.7)
- Molecular analysis (see paragraph 11.2.9)

Morphology at diagnosis is to be done within 3 days prior to randomization.

In maintenance hematology (Hb, WBC, platelets) once a month is advised.

11.2.4 Blood chemistry

- Creatinine

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

At entry and at least twice weekly during asparaginase administration:

- Amylase
- Lipase

Blood chemistry at diagnosis is to be done within 3 days prior to randomization.

In maintenance blood chemistry (liver and kidney function) once a month is advised.

11.2.5 Bone marrow aspirate

- Morphology
- Immunophenotyping (see paragraph 11.2.7)
- Cytogenetic analysis (see paragraph 11.2.8)
- Molecular analysis (see paragraph 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, EBV, PCR)
- Microbiological tests
- HLA typing of patient and family (at diagnosis or as soon as possible thereafter)

11.2.7 Immunophenotyping

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

Review by the HRC will be performed (see paragraph 4.1)

11.2.8 Cytogenetic analysis

Conventional cytogenetic analysis should be performed in all patients at diagnosis 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 and FISH of diagnosis (see paragraph 4.2).

11.2.9 Molecular analysis

Blood and bone marrow cells at diagnosis will be investigated for Ig/TCR gene re-arrangements and for the presence of BCR/ABL fusion transcripts. In case of BCR/ABL positivity, follow up investigations for BCR/ABL should be performed at all bone marrow examinations mentioned in this protocol. In addition, MRD diagnostics will be performed 5 times during treatment and follow-up as indicated on the flow-sheet. These MRD-PCR investigations will be performed by the Dutch Childhood Oncology Group (DCOG) as outlined in appendix G for logistics.

Blood and bone marrow cells will also be stored for further analysis for leukemia-specific breakpoint fusion regions, gene expression profiling or SNP-analysis.
Diagnostic and follow-up samples should be sent to DCOG.

11.2.10 Blood blast clearance at day 8

Corticosteroid (+/-clofarabine) sensitivity will be determined on day 8 of the pre-phase.
Sensitive: leukemic blasts < 1 x 10^9/l in the blood
Resistant: leukemic blasts > 1 x 10^9/l in the blood

11.2.11 Chemosensitivity at day 15

Chemotherapy-sensitivity will be determined at day 15 counted from start prephase by morphological bone marrow examination:
Sensitive (M1): <5% blasts,
Intermediate insensitive (M2): 5-25% blasts,
Chemotherapy/Steroid-insensitive (M3): > 25% blasts.

11.3 Response evaluation

Response will be assessed after the induction, consolidation and intensification courses by evaluation of the bone marrow aspirate according to the definitions described in appendix B. After interphase treatment and after alloSCT (after approx. 3 months), during maintenance treatment and later on, 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. Definitions for CNS involvement, i.e. meningeal leukemia (ML) are described in appendix A. For T-LBL response wil be assessed by CT scan on day 15 after start prephase and after repopulation at the end of induction. Subsequently, by CT scan at each evaluation point (instead of bone marrow investigation).

11.4 Risk assessment

Several parameters which correlate with response rate and response duration in adult ALL have been identified and should be documented in this study but only few, that have unequivocally been demonstrated in multiple studies, will affect the treatment, see below. Factors correlating with poor prognosis may include:

- WBC >30x10^9/l (especially in B-lineage ALL)
- WBC >100x10^9/l (especially in T-lineage ALL)
- unfavorable karyotype, i.e. t(9;22), t(4;11) and other 11q23 abnormalities, and hypodiploidy
- pro-B cell ALL
- increasing age
- LDH >4x ULN
- t(1;19), +8, and complex structural and numerical chromosomal abnormalities
- meningeal involvement at diagnosis
- hepatomegaly/splenomegaly

In addition, response to therapy is a major determinant of outcome:

- level of minimal residual disease (MRD) after remission induction (I) and consolidation (I).

In this study high-risk patients are defined by:

- unfavorable karyotype, i.e. t(9;22) , t(4;11) and other 11q23 abnormalities, and hypodiploidy
- complex abnormalities (≥ 5), excluding hyperdiploidy
- no CR after prephase and first induction therapy
- high WBC (WBC > 30 x 10^9/l in B-ALL, WBC > 100 x 10^9/l in T-ALL).

These high-risk patients qualify for related or unrelated donor alloSCT as described in paragraph 9.4.

11.5 Coagulation side study

A side study to investigate the activity of the plasma coagulation system during ALL (treatment) will be performed in Dutch participating centers only. Markers of coagulation activation will be determined upon inclusion and during chemotherapy treatment cycles, especially when PEG-L-asparaginase is administered. More details can be found in the side studies lab manual.

12 Toxicity assessment

12.1 Chemotherapeutic and other agents

All chemotherapeutic agents used in this protocol cause prolonged pancytopenia during 3-6 weeks and can induce septic or hemorrhagic complications.
Congestive heart failure is a major complication of anthracyclines, frequently observed after high cumulative doses. The doses used are considerably lower than those associated with congestive heart failure.
Non-hematological drug toxicities include:
Doxorubicin/daunorubicine: hair loss, mucositis, cardiomyopathy, nausea, vomiting, colitis, infertility.
Cytarabine (Ara-C) : anorexia, nausea, vomiting, hepatic dysfunction, skin rash, fever.
Etoposide: nausea, vomiting, mucositis, hepatic dysfunction, neurotoxicity, skin rash.
PEG-L-Asparaginase: allergy, pancreatitis, hypofibrinogenemia, hepatic dysfunction, thrombosis.
Vincristine, vindesine: peripheral neuropathy, obstipation.
Methothrexate: nausea, vomiting, mucositis, hepatic dysfunction.
G-CSF (granulocyte colony-stimulating factor): fever, diarrhoea, abdominal pain, vomiting, skin rash, headache, bone pain and injection site reactions have been reported following the use of G-CSF.

12.2 Clofarabine

Drug-related AEs observed in at least 10% of adult patients treated with clofarabine in previous clinical trials include myelosuppression, nausea, vomiting, infections, fatigue, headache, diarrhea, rigors, dermatitis, anorexia, febrile neutropenia, myalgia, asthenia, petechiae, transient elevated liver enzymes, stomatitis, mucositis, pyrexia, flushing, constipation, edema, dehydration, nervousness, stomach pain, insomnia, depression, dry skin, back pain, and decreased weight.
Adverse events reported in <10% of adult patients include tumor lysis syndrome, capillary leak syndrome, palmar plantar erythrodysesthesia, pancreatitis, seizures, irregular heart beat, edema, pericardial effusion, multi-organ failure, and death. (see reference 35)

12.3 Toxicity assessment

Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 3.0 (see appendix D).

13 Reporting serious adverse events and SUSARS

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, unintended and clinically related 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.

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.

Suspected unexpected serious adverse reaction (SUSAR)

All **suspected** Adverse Reactions which occur in the trial and that are both **unexpected** and **serious**. Suspected 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. 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).

13.2 Reporting of (serious) adverse events

Adverse event

All AEs of CTCAE grade 2 or higher, with the exception of alopecia, nausea/vomiting, hematological toxicities and progression of the disease under study, have to be reported on the Adverse Events CRF. ***Moreover, all thromboembolic (TE) events MUST be reported, also in case of CTCAE grade 1, on the TE event report form.***

Adverse events will be reported from the first study-related procedure until 30 days following the last protocol treatment (after AlloSCT until date Off protocol) or until the start of subsequent systemic therapy for the disease under study, if earlier.

Adverse events occurring after 30 days should also be reported if considered related to study drug.

Grade 3 or 4 adverse events considered related to study drug must be followed until recovery or until 6 months after the last protocol treatment, whichever comes first.

All other adverse events must be followed until recovery or until 30 days after the last protocol treatment, whichever comes first.

Serious adverse events

Serious Adverse Events (SAEs) will be reported from the first study-related procedure until 30 days following the last protocol treatment or until the start of subsequent systemic therapy for the disease under study, if earlier.

Serious adverse events occurring after 30 days should also be reported if considered to be at least possibly related to the investigational drug by the investigator.

All SAEs must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the event**, except:

- progression of the disease under study; complications as a result of disease progression remain reportable Serious Adverse Events

and 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 source document and the CRF). Prolonged hospitalization for a complication considered to be at least possibly related to the study drug remains a reportable serious adverse event.

All details should be documented on the Serious Adverse Event 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.

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
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 principal investigator and the product manufacturer (for clofarabine: the Pharmacovigilance group of Genzyme/Sanofi).
The HOVON Data Center will assess all SAE reports for SUSAR criteria.
The HOVON Data Center will ensure that a six-monthly line listing of all reported SAE's is provided to the Ethics Committee(s) if this is required by national laws or regulations or by the procedures of the Ethics Committee.
Suspected unexpected serious adverse reactions (SUSARs) will be reported by HOVON Data Center to the investigators, to the Ethics Committees that approved the study and to applicable Health Authorities within required timelines. The manufacturer will receive a copy.

Genzyme/Sanofi will also be informed about any complaints on the product clofarabine by phone immediately, but in any event within 1 business day, after becoming aware of the complaint as described in detail in the pharmacist's information for this study.

Detailed information with the contact information to report SAE's and product complaints to Genzyme/Sanofi will be available in a separate document at the HOVON Data Center.

13.4 Pregnancies

Pregnancies of a female subject or the female partner of a male subject, occurring while the subject is on protocol treatment or within 30 days following the last dose of any drug from the protocol treatment schedule, should be reported to the sponsor. Pregnancies must be reported to the HOVON Data Center by fax within 24 hours after the event was known to the investigator, using the pregnancy report form provided.

The investigator will follow the female subject until completion of the pregnancy, and must notify the sponsor of the outcome of the pregnancy within 5 days or as specified below. The investigator will provide this information as a follow-up to the initial pregnancy report. If the outcome of the pregnancy meets the criteria for classification as a SAE (i.e., spontaneous or therapeutic abortion, stillbirth, neonatal death, or congenital anomaly - including that in an aborted fetus), the investigator should follow the procedures for reporting SAEs. In the case of a live "normal" birth, the sponsor should be informed as soon as the information is available. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the investigator suspects is related to the *in utero* exposure to the investigational medicinal product(s) should also be reported.

The investigator is encouraged to provide outcome information of the pregnancy of the female partner of a male subject, if this information is available to the investigator and the female partner gives her permission.

14 Endpoints

14.1 Phase II of the study

14.1.1 Primary endpoint

- Feasibility (i.e. defined as less than 10% increase in DLT (e.g. from 20% to 30%) and 5% increase in TRM in arm B compared to arm A (see 17.2.1)

14.2 Phase III of the study

14.2.1 Primary endpoint

- Event free survival (i.e. time from registration until no CR on protocol treatment, relapse or death in 1st CR, whichever comes first)

14.2.2 Secondary endpoints

- MRD level following induction chemotherapy
- MRD level prior start of maintenance
- Blood blast clearance (corticosteroid sensitivity)(day 8 of prephase)
- Chemosensitivity (day 15 counted from start prephase therapy)
- Hematological response
- Disease free survival (hematologically; i.e. time from CR until relapse or death, whichever comes first)
- Disease free survival (molecularly); i.e. time from molecular CR until molecular relapse or death (whichever comes first)
- Overall survival, measured from the time of registration. Patients still alive or lost to follow up are censored at the date they were last known to be alive.
- Adverse events
- Relapse as from start of maintenance
- Incidence of objectively diagnosed symptomatic thromboembolic events
- Influence of ALL (treatment) on plasma coagulation markers

15 Registration and Randomization

15.1 Regulatory Documentation

The following documents must be provided to the HOVON Data Center before shipment of the study drug to the investigational site and before enrollment of the first patient.
By the principal investigator or study coordinator for all sites within their country:

- ♦ name and address of the (central) Ethical Committee including a current list of the members and their function;
- ♦ any other documentation required by local regulations.

15.2 Randomization

Eligible patients should be randomized before start of treatment. Patients need to be registered at the HOVON Data Center, Erasmus MC Cancer Institute, Clinical Trial Center via the Internet via TOP (Trial Online Process; https://www.hdc.hovon.nl/top) or by phone call: +31.10.7041560 or fax +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET. A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Patient's initials or code
- Sex
- Date of birth
- Date written informed consent
- Eligibility criteria

All eligibility criteria will be checked with a checklist. Patients will be randomized, stratified by age (18-40 vs 41-70 years), precursor- B-ALL versus T-ALL immunophenotype and center with a minimization procedure, ensuring balance within each stratum and overall balance.
Each patient will be given a unique patient study number. Patient study number and result of randomization will be given immediately by TOP or phone and confirmed by fax or email.

16 Data collection

16.1 CRF's

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 name code (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.

16.2 Reporting DLT information

To monitor the incidence of dose limiting toxicity (DLT) in the feasibility part of the trial, see paragraph 17.2.1, a separate CRF (DLT-form) will be used. This DLT-form must be filled out for every patient until feasibility has been established, independent of randomization result. The form should be dated, signed by the responsible investigator and returned to the HOVON Data Center by fax within 24 hours after DLT-occurrence, or after 38 days after pre-phase treatment if no DLT occurred. An automatic reminder will be sent to the local investigator 38 days after randomization and 2-weekly thereafter if

necessary until DLT information is available. As of November 2013 sending in DLT forms is not required anymore.

16.3 Reporting TE information

Every symptomatic, objectively confirmed thromboembolic event must be centrally reported to the HOVON Data Center on a separate CRF (TE event report form (17)). This TE event report form must be filled out for all TE events occurring throughout the entire treatment phase and 3-month follow-up period. The TE event form should be filled out completely, dated and signed by the responsible investigator and returned to the HOVON Data Center.

17 Statistical considerations

The aim of this study is to assess in patients with newly diagnosed ALL whether the addition of clofarabine to prephase treatment is feasible and improves clinical outcome.

17.1 Patient numbers and power considerations

The primary goal of the trial is to evaluate whether the addition of i.v. clofarabine to induction treatment will improve event-free survival (EFS), due to a higher complete response (CR) rate, and improved disease-free survival (DFS) from CR.
In the previous HOVON ALL-trials (HOVON-18, -37, -70 and -71) CR rates were about 85%. Long-term EFS is only available for HOVON-18 and -37, and are about 40% and 35% at 2 and 5 years, respectively.

Trial	Age [yr]	# patients	CR (%)	EFS_{1y} (95%CI)	EFS_{2y} (95%CI)	EFS_{5y} (95% CI)
HO18	18-60	193	82	50 (43-57)	36 (29-42)	30 (24-37)
HO37	18-60	240	88	57 (51-63)	43 (37-49)	38 (32-44)
HO70	18-39	47*	85	77 (60-87)	$EFS_{18\ months}$ = 67% (46-81)	
HO71	40-70	56*	86	55 (36-71)	$EFS_{18\ months}$ = 51% (32-67)	

* Data of HO70 and HO71 are not yet complete. Table based on data available as of June 27, 2008 (47 of 54 patients in HO70, and 56/60 in HO71)

In order to detect with 80% power (two-sided significance level α = 0.05; 1:1 randomization) an improvement of EFS with hazard ratio (HR) = 0.65 - which corresponds to an improvement of the CR rate from 85% to 90%, and 2-year EFS from 40% to 55% -, 174 events have to be observed. This requires 316 patients to be accrued in 3.5 to 4 years – with an expected accrual of about 90 patients

per year – and 2 years of follow up after the last registered patient. In order to overcome possible dropout, 340 patients will be registered.

17.2 Statistical analysis

All analyses will be according to the intention to treat principle, i.e. patients will be analyzed according to the treatment arms they were assigned to. However, patients initially randomized but considered ineligible afterwards based on information that should have been available before randomization, will be excluded from all analyses.

17.2.1 Feasibility analysis

In order to safeguard patients against treatment with too toxic schemes, feasibility will be monitored carefully in the first series of patients, with special consideration of dose limiting toxicity (DLT).
DLT is defined as:

- any non-hematological toxicity CTCAE grade ≥ 4, or
- death

occurring within 38 days after start of prephase and before the start of the cycle after induction . In the HOVON-70 and HOVON-71, 26% and 32% DLT were observed, respectively (see table below), but in the HOVON-100 modified schemes will be applied which are expected to be less toxic.

Trial	Age [yr]	# patients	Death (%)	CTCAE ≥ 4 (%)	Total (%)
HO70	18-39	47	4	21	26
HO71	40-70	56	7	26	32

It is expected that no more than 25% DLT (including no more than 5% treatment-related mortality [TRM]) will be observed in arm A (without clofarabine) following pre-phase and first induction chemotherapy. As a guideline for the data and safety monitoring board (DSMB), for arm B (with clofarabine) to be considered feasible, there should be no more than 10% increase in DLT (e.g. from 20% to 30%) and 5% increase in TRM in arm B compared to arm A, neither in all patients, nor in the younger and older patients separately. In order to monitor the feasibility, DLTs have to be reported within 24 hours and investigators will receive a questionnaire for patients who are still at risk (first questionnaire 38 days after randomization, and 2-weekly thereafter until DLT data are available). Feasibility reports will be generated after DLT information of the first 10, 20 and 30 registered patients in arm B is available. The reports will contain a tabulation of the number of patients recruited, the number of evaluable patients, the number of DLTs and a specification of the DLTs and their outcome, and the incidence and intensity of the other reported adverse events. The results will be shown for all

patients together, and also for patients aged 18-40 years and 41-70 years separately, and will be split by treatment arm. This will be done separately for each investigated dose level.
The reports will be sent to the data safety monitoring board (DSMB) for evaluation. The DSMB, headed by Professor Dr. R. Pieters, Erasmus MC- Sophia, is free in her public recommendations to the study coordinators and the confidential recommendations to the study statistician.
[Note: inclusion of patients will NOT be temporarily discontinued while waiting for the DLT information]

17.2.2 Efficacy analysis

As a result of the interim analyses, the DSMB will propose a dose level of clofarabine to be used in the phase III part of the trial, i.e. 15, 20 or 30 mg/m^2/day (x5). Only those patients who could have been randomized to that specific dose level will be included in the efficacy analyses, irrespective if they were randomized during the phase II or the phase III part. Patients randomized when either of the two other dose levels was open for inclusion, will therefore be excluded from the efficacy analysis. Main endpoint for the comparison of the two treatment arms will be EFS from registration as defined in chapter 14. Formal test for the difference in EFS between the two treatment arms will be done with a multivariate Cox regression analysis with adjustment for the stratification factors age (18-40 vs 41-70 years) and immunophenotype (B-cell vs T-cell). We will also perform a non-modelling based stratified logrank for difference in EFS between the two treatment arms, but this analysis should be regarded as a secondary analysis.

Secondary efficacy endpoints are response rate (hematological, as well as molecular), DFS from CR, OS from registration, and DFS and OS from allogeneic transplantation and from start maintenance.

The proportion of patients with a CR and CMR will be determined per treatment arm with a 95% confidence interval (CI), and compared using logistic regression with adjustment for age group.

In addition, the best molecular response will be determined for patients 18-40 and 41-70 years separately, and by treatment arm.

Actuarial probabilities of EFS, DFS and OS at appropriate time points including 95% confidence intervals (Cis) will be calculated using the actuarial method of Kaplan and Meier. Kaplan-Meier survival curves will be constructed, and compared with a logrank test. For illustrative purpose only, also a Kaplan-Meier curve will be generated for EFS, with EFS for patients without a CR on protocol set at day 1.

A preliminary efficacy analysis on molecular response (CMR) on day 15 and day 36 after randomization is planned shortly after end of recruitment, as soon as these data are available. No conclusions will be drawn based on this analysis.

17.2.3 Toxicity analysis

The analyses of treatment toxicity will be done primarily by tabulation of the incidence of adverse effects with CTCAE grade 2 or more (appendix D) and thromboembolic events of all grades, by treatment and cycle.

17.2.4 Additional analyses

Additional analyses will at least involve the impact of early CMR with respect to EFS and OS. Cox regression can be used for this purpose. These analyses should also be regarded as exploratory, and therefore only as hypothesis-generating.

A detailed statistical analysis plan (SAP) will be made for the final analysis. It will be discussed with the study coordinators and can only affect the exploratory analyses, but not the primary (confirmatory) analysis on which the sample size is based.

17.3 Interim analysis

At least three formal interim analyses are planned, as described in paragraph 17.2.1.

17.4 Data and Safety monitoring board

A data and safety monitoring board will be installed before start of the study, consisting of two international clinical hematologists with a broad background in ALL therapeutics as well as an independent statistician. The DSMB will give recommendations about dose, dose reductions, continuation at a dose level or stopping because of inefficacy on the basis of interim reports at specific timepoints in the study as specified in the statistical section above. These confidential interim reports are prepared by the study statistician. On the basis of an interim analysis report the DSMB will give a recommendation to the study coordinators. The dose reduction/escalation and stopping rules described above serve as a guideline for the DSMB. However, the DSMB is free in her recommendations and may take external information into account in her recommendations. In case stopping or dose reduction would be required by the decision rules we return to a lower dose level, if not possible the study will be put on hold until the DSMB has given her recommendations and a final decision has been made. In all other cases the study remains open and the DSMB will advise on the

dose level of clofarabine for the forthcoming patients (20 or 30 mg/m^2/ day (x5)). . The study coordinators make the final decision.

18 Ethics

18.1 Accredited ethics committee or Institutional review board

The study protocol and any substantial amendment will be approved by an accredited Ethics Committee or Institutional Review Board. The principal investigator will inform the subjects 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 subject's health. The investigator will take care that all subjects are kept informed.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU directive for Good Clinical Practice (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study at the study site.

18.3 Patient information and consent

Written Informed consent of patients is required before randomization. The procedure and the risks and the opinions for therapy will be explained to the patient.

19 Trial insurance

The HOVON insurance program covers all patients from participating centers in the Netherlands according to Dutch law (WMO). The WMO insurance statement can be viewed on the HOVON Web site www.hovon.nl.

20 Publication policy

The final publication of the trial results will be written by the Principal Investigator and 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 other co-authors the manuscript will be sent to a peer reviewed scientific journal.

Authors of the manuscript will include the study coordinator(s), investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion), the statistician(s) and the HDC datamanager 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 Principal Investigator and Study Coordinator(s). This is applicable to any individual patient 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)

AE	Adverse Event
ALL	Acute lymphoblastic leukemia
AlloSCT	Allogeneic stem cell transplantation
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANC	Absolute Neutrophil Count
AST	Aspartatae aminotransferase
AT	Antithrombin (III)
BM	Bone Marrow
CI	Confidence interval
CKTO	Commissie voor Klinisch Toegepast Onderzoek'
CMR	Complete molecular response
CMV	Cyto megalo virus
CNS	Central nervous system
CR	Complete Remission
CRF	Case Report Form
CRP	C-Reactive Protein
CSF	Cerebral spine fluid
CTC	Common Toxicity Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CV	Curriculum vitae
D5W	5% dextrose injection
DCOG	Dutch childhood oncology group
DFS	Disease free survival
DLT	Dose limiting toxicity
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
EMD	Extra Medullary Disease
EFS	Event free survival
EORTC	European Organization for Research and Treatment of Cancer
ESG-MRD-ALL	European Study Group for MRD diagnostics in ALL
FCM	Flow cytometry

FFP	Fresh Frozen Plasma
FISH	Fluorescence In Situ Hybridisation
γ-GT	Gamma glutamyl transpeptidase
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor
Gy	Gray
HDC	HOVON Data Center
HIT	Heparin-induced thrombocytopenia
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte histocompatibility Antigen
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group
HR	Hazard ratio
HRC	Hematocytology Review Committee
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
Ig	Immunoglobulin
i.m.	Intramuscular
i.t.	Intrathecal
IU	International Units
i.v.	Intravenous
KCl	Potassium chloride
LDH	Lactate Dehydrogenase
LMWH	Low molecular weight heparin
METC	Medical Ethical Review Committee
ML	Meningeal leukemia
6-MP	6 mercaptopurine
MRD	Minimal residual disease
MT	Maintenance treatment
MTD	Maximum tolerated dose
MTX	Methotrexate
MUD	Matched unrelated donor
NCI	National Cancer Institute
NR	No response
NS	normal saline
OS	Overall Survival
PCR	Polymerase chain reaction

PB	Peripheral Blood
PEG	Pegylated
Ph+	Philadelphia-positive
PO	Per Os
PR	Partial Response
RI	Remission Induction
RQ-PCR	Real-time quantitative PCR
RT	Reverse transcriptase
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SC	Subcutaneous
SCT	Stem cell transplantation
SGOT	Serum glutamic oxaloacetatic transaminase
SGPT	Serum glutamic pyruvic transaminase
SKION	Stichting Kinderoncologie Nederland
SUSAR	Suspected unexpected serious adverse reactions
TBI	Total body irridiation
TCR	T-cell receptor
TE	Thromboembolic
TK	Tyrosine kinase
T-LBL	T-cell lymphoblastic lymphoma
TRM	Treatment related mortality
ULN	Upper Limit of Normal
US FDA	United States Food and Drug Administration
WBC	White blood cell
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

22 References

1. Stryckmans, P, De Witte, T, Marie, JP, et al. Therapy of adult ALL: overview of 2 successive EORTC studies: (ALL-2; ALL-3). The EORTC Leukemia Cooperative Study Group. Leukemia 1992; 6:199.
2. Hoelzer, D, Gokbuget, N. New approaches to acute lymphoblastic leukemia in adults: where do we go?. Semin Onco 2000; 27:540-559.
3. Hoelzer D, Gökbuget N, Ottmann O. Clinical management of adult ALL. In: Acute lymphoblastic leukemia. Hematology, American Society of Hematology Education Program Book 2002;162-168.
4. Parentesis JP. Why is age such an important independent prognostic factor in acute lymphoblastic leukemia ? Leukemia. 1997;11: S4-S7.
5. Dekker AW et al. Postremission Treatment with Autologous Stem Cell Transplantation (Auto-SCT) or Allogeneic Stem Cell Transplantation (allo-SCT) in Adults with Acute Lymphoblastic Leukemia (ALL). A phase II Clinical Trial (HOVON 18 ALL). Blood 2001;98:859a.
6. Daenen S, van Imhoff GW, van den Berg E, et al. Improved outcome of adult acute lymphoblastic leukaemia by moderately intensified chemotherapy which includes a “pre-induction” course for rapid tumour reduction: preliminary results on 66 patients. Brit J Haematol 1998;100:273-282.
7. Annino, L, Vegna, ML, Camera, A, et al. Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow-up of the GIMEMA ALL 0288 randomized study. Blood 2002; 99:863.
8. Rubnitz JE, Pui C-H. Recent advances in the treatment and understanding of childhood acute lymphoblastic leukaemia. Cancer Treat Rev 2003;29:31-44.
9. Pui C, H, Campana, D, Evans W, E. Childhood acute lymphoblastic leukaemia--current status and future perspectives. Lancet Oncol 2001;2:597.
10. Groupe Française de Cytogénétique Hématologigue. Cytogenetic abnormalities in adult acute lymphoblastic leukemia: correlations with hematologic findings and outcome. A collaborative study of the Groupe Française de Cytogénétique Hématologigue. Blood 1996; 87:3135-3142.
11. Boissel N, Auclerc MF, Lheritier V, et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRAILLE-93 and LALA-94 trials. J Clin Oncol 2003;21:774-780.
12. de Bont JM, van der Holt B, Dekker AW, van der Does-van den Berg A, Sonneveld P, Pieters R. Significant difference in outcome for adolescents with acute lymphoblastic leukemia treated on pediatric vs adult protocols in the Netherlands. Leukemia 2004;18:2032-5.

13. Chessells JM, Hall E, Prentice HG, Durrant J, Bailey CC, Richards. The impact of age on outcome in lymphoblastic leukaemia; MRC UKALL X and XA compared: a report from the MRC Paediatric and Adult Working Parties. Leukemia. 1998; 12: 463-473.
14. Haiat S, Vekhoff A, Marzac C, et al. Improved outcome of adult acute lymphoblastic leukemia treated with a pediatric protocol: results of a pilot study. Blood 2007;110:2822.
15. Daenen S, van Imhoff GW, de Wolf JT, Vellenga E, van den Berg-de Ruiter E, Kluin-Nelemans HC. A “pre-induction course” with non-cross-reacting cytostatic drugs for rapid tumour load reduction improves outcome in adult acute lymphoblastic leukaemia. Br J Haematol 2007, 138, 275-7;
16. DeAngelo DJ, Dahlberg S, Silverman LB, et al. A mulricenter phase II study using a dose intensified pediatric regimen in adults with untreated acute lymphoblastic leukemia. Blood 2007;110:587.
17. Van Dongen, JJM, Breit TM, Adriaansen HJ, Beishuizen A, Hooijkaas H. Detection of minimal residual disease in acute leukemia by immunological marker analysis and polymerase chain reaction. Leukemia 1992;6:47-59.
18. Szczepanski T, Orfão A, Van der Velden VHJ, San Miguel JF, Van Dongen JJM. Minimal residual disease in leukaemia patients. Lancet Oncol 2001;2:409-417.
19. Lúcio P, Parreira A, Van den Beemd MWM, Van Lochem EG, Van Wering ER, Baars E, Porwit-MacDonald A, Bjorklund E, Gaipa G, Biondi A, Orfao A, Janossy G, Van Dongen JJM, San Miguel JF. Flow cytometric analysis of normal B-cell differentiation: A frame of reference for the detection of minimal residual disease in precursor-B-ALL. Leukemia 1999;13:419-427.
20. Porwit-MacDonald A, Bjorklund E, Lucio P, Van Lochem EG, Mazur J, Parreira A, Van den Beemd MW, Van Wering ER, Baars E, Gaipa G, Biondi A, Ciudad J, Van Dongen JJM, San Miguel JF, Orfao A. BIOMED-1 concerted action report: flow cytometric characterization of CD7+ cell subsets in normal bone marrow as a basis for the diagnosis and follow-up of T cell acute lymphoblastic leukemia (T-ALL). Leukemia 2000;14:816-825.
21. Pongers-Willemse MJ, Seriu T, Stolz F, d'Aniello E, Gameiro P, Pisa P, Gonzalez M, Bartram CR, Panzer-Grümayer ER, Biondi A, San Miguel JF, Van Dongen JJM. Primers and protocols for standardized detection of minimal residual disease in acute lymphoblastic leukemia using immunoglobulin and T cell receptor gene rearrangements and TAL1 deletions as PCR targets: Report of the BIOMED-1 CONCERTED ACTION: Investigation of minimal residual disease in acute leukemia. Leukemia 1999;13:110-118.
22. Van Dongen JJM, Szczepanski T, Van der Velden VHJ. Minimal residual disease. In: Kaspers GJL, Coiffier B, Heinrich MC, Estey E, eds. Innovative Leukemia and Lymphoma Therapy 2008. New York: Informa Healthcare 2008;45-84 (ISBN-10: 0-8493-5083-2; ISNB-13: 987-0-8493-5083-2).

23. Van der Velden VHJ, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER, Flohr T, Sutton R, Cave H, Madsen HO, Cayuela JM, Trka J, Eckert C, Foroni L, Zur Stadt U, Beldjord K, Raff T, Van der Schoot CE, Van Dongen JJM. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia 2007;21:604-611.
24. Van Dongen JJM, Seriu T, Panzer-Grümayer ER, Biondi A, Pongers-Willemse MJ, Corral L, Stolz F, Schrappe M, Masera G, Kamps WA, Gadner H, Van Wering ER, Ludwig W-D, Basso G, De Bruijn MAC, Cazzaniga G, Hettinger K, Van der Does-van den Berg A, Hop WCJ, Riehm H, Bartram CR. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 1998; 352:1731-1738.
25. Cavé H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, Bakkus M, Thielemans K, Grandchamp B, Vilmer E. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. N Engl J Med 1998;339:591-598.
26. Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, Rubnitz JE, Rivera GK, Sandlund JT, Pui CH, Campana D. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet 1998;351:550-554.
27. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM, Behm FG, Raimondi SC, Sandlund JT, Rivera GK, Rubnitz JE, Ribeiro RC, Pui CH, Campana D. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 2000;96:2691-2696.
28. Nyvold C, Madsen HO, Ryder LP, Seyfarth J, Svejgaard A, Clausen N, Wesenberg F, Jonsson OG, Forestier E, Schmiegelow K; Nordic Society for Pediatric Hematology and Oncology.Precise quantification of minimal residual disease at day 29 allows identification of children with acute lymphoblastic leukemia and an excellent outcome. Blood 2002;99:1253-1258.
29. Willemse MJ, Seriu T, Hettinger K, d'Aniello E, Hop WC, Panzer-Grumayer ER, Biondi A, Schrappe M, Kamps WA, Masera G, Gadner H, Riehm H, Bartram CR, Van Dongen JJM. Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood 2002;99:4386-4393.
30. Brüggemann M, Raff T, Flohr T, et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukaemia. Blood 2006;1107:1116-1123
31. Raff T, Fökbuget N, Lüschen S, et al. Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 and 07/03 trials. Blood 2007;109:910-915.

32. DeAngelo D, Dahlberg S, Silverman L,et al. A multicenter phase II study using a dose intensified pediatric regimen in adults with untreated acute lymphoblastic Leukemia. Blood 2007;110:Abstract 587 © 2007 American Society of Hematology
33. Reichelova V, Liliemark J, Albertioni F. Liquid chromatographic study of acid stability of 2-chloro-2'-arabino-fluoro-2'deoxyadenosine, 2-chloro-2'-deoxyadenosine and related analogues. J Pharm Biomed Anal 1995;13:711-714.
34. Carson DA, Wasson DB, Esparza LM, Carrera CJ, Kipps TJ, Cottam HB. Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2'arabino-fluoro-2'-deoxyadenosine. Proc Natl Acad Sci USA 1992;89:2970-2974.
35. Clofarabine Investigator's Brochure. 7th ed. San Antonio, TX: Genzyme Corp. Final report dated 23 June 2008.
36. Kantarjian HM, Gandhi V, Kozuch P, et al. Phase I clinical and pharmacology study of clofarabine in patients with solid and hematologic cancers. J Clin Oncol 2003;21:1167-1173.
37. Kantarjian H, Gandhi V, Cortes J, et al. Phase 2 clinical and pharmacologic study of clofarabine in patients with refractory or relapsed acute leukemia. Blood 2003;102:2379-2386.
38. Faderl S, Gandhi V, O'Brien S, et al. Results of a phase 1-2 study of clofarabine in combination with cytarabine (ara-C) in relapsed and refractory acute leukemias. Blood 2005;105:940-947.
39. Jeha S, Gandhi V, Chan KW, et al. clofarabine, a novel nucleoside analog, is active in pediatric patients with advanced leukemia. Blood 2003;103:784-789.
40. Jeha S, Gaynon P, Razzouk B, et al., Phase II study of clofarabine in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. J Clin Oncol 2006;24(12):1917-1923.
41. Jeha S, Razzouk B, Gaynon P, Rytting M, Kadota R, Rheingold R, Luchtman-Jones L, Arceci R, Steinherz P, clofarabine in Children with Refractory/Relapsed Acute Leukemia: Results of 2 Phase II Open Label Studies. Blood 2006; 108: Abstract 4460.
42. Clofarabine Investigator's Brochure. 6th ed. San Antonio, TX: Genzyme Corp. Final report dated 15 March 2007.
43. Filipovich AH, Weisdorf D, Pavletic S et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Group Report. Biology of Blood and Marrow Transplantation 2005;11:945-956.
44. Glucksberg H, Storb R, Fefer A et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18:295-304.

45. Przepiorka D, Weisdorf D, Martin P et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825-828.
46. Elhasid R, Lanir N, Sharon R et al. Prophylactic therapy with enoxaparin during L-asparaginase treatment in children with acute lymphoblastic leukemia. Blood Coagul.Fibrinolysis 2001;12:367-370.
47. Panetta JC, Gajjar A, Hijiya N et al. Comparison of native E. coli and PEG asparaginase pharmacokinetics and pharmacodynamics in pediatric acute lymphoblastic leukemia. Clin.Pharmacol.Ther. 2009;86:651-658.
48. Qureshi A, Mitchell C, Richards S, Vora A, Goulden N. Asparaginase-related venous thrombosis in UKALL 2003 - re-exposure to asparaginase is feasible and safe. Br.J.Haematol. 2010;149:410-413.
49. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J.Clin.Oncol. 2007;25:579-86.

A. Criteria for ALL diagnosis

Morphologic criteria for ALL:

- >20% blasts in a representative bone marrow aspirate or otherwise in a bone marrow biopsy
- 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	cytCD79+, CD19+, HLA-DR+, TdT+, CD10+
	Pre-B ALL	cytCD79+, CD19+, HLA-DR+, TdT+/-, CD10+/-, cytIgμ+
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+
	Extra	CyTCRβ, TCRγδ, TCRαβ, SmCD3

Indicated are the minimal requirements for subtyping; additional markers are advisable, like mentioned in the table: CyTCRβ, CyTCRγδ, SmCD3.

CNS leukemia, 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 CSF with increased protein level, in absence of blasts on cytological examination.

B. Response criteria for ALL and T-LBL

Response criteria ALL

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.

OR

- 5-10% blast cells by morphology in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy, and <1% leukemic cells by immunophenotyping in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy, and meeting all other criteria for CR.

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

- 5-10% blast cells by morphology in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy
- ≥1% leukemic cells by immunophenotyping in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy.
- Absence of extramedullary leukemia.

OR

- 10-25% malignant cells by morphology in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy, and 0-25% malignant cells by immunophenotypical analysis in a representative* bone marrow aspirate or otherwise in a bone marrow biopsy, and absence of extramedullary leukemia.

No response (NR):

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

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.
- 5-10% leukemic cells by morphology and more than 1% leukemic cells by immunophenotyping in a representative* bone marrow aspirate or bone marrow biopsy
- More than 10% leukemic cells by morphology
- Appearance or reappearance of extramedullary leukemia, proven by biopsy or cytology.

* representative bone marrow aspirate defined as >20 % cellularity

Response criteria T-LBL

Evaluation of response will be done according to the International Workshop to Standardize Response Criteria for Non-Hodgkin's Lymphoma (Cheson et al., 2007 [49]) in case of T-LBL. N.B. PET scan is not validated for T-LBL and should not be performed!

Complete Response (CR) requires complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms, and normalisation of biochemical abnormalities (e.g. LDH) definitely assignable to NHL. Furthermore, all lymph nodes and nodal masses must have regressed to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their long axis and > 1.0 cm in their short axis before treatment must have decreased to ≤ 1 cm in their short axis after treatment.
The spleen and liver, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. However, no normal size can be specified because of the difficulties in accurately evaluating splenic and hepatic size. Similarly, other organs considered to be enlarged before therapy due to involvement by lymphoma, must have decreased in size.
Partial response (PR) requires 50% decrease in the dominant nodal masses.
No response (NR) is defined as less than a PR.
Relapse: appearance of any new lesion or increase by (≥ 50%) in the size of previously involved sites.

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
http://www.hovon.nl (under Trials > General information about studies)

E. Administration of Asparaginase

PEG-L-asparaginase stands for pegylated L-asparaginase. Asparaginase stands for E. coli asparaginase, unless Erwinia asparaginase (Erwinase) is mentioned.

- Different preparations of asparaginase are available, in this study we will prefer PEG-L-asparaginase. In a recent CALGB trial in adult ALL, it was demonstrated that effective asparagine depletion with pegylated asparaginase is feasible as part of an intensive multiagent therapeutic regimen in adult acute lymphoblasic leukemia and appears associated with improved outcome and less toxicity (Wetzler M, Sanford BL. Kurtzzberg J et al, Blood. 2007;109:4164-4167) Therefore, PEG-L-asparaginase is preferred and will be provided for free by the manufacturer sigma-tau. All patients receive a starting dose of 1000 IU/m^2 PEG-L-asparaginase i.v.Keep antihistamines, corticosteroids and adrenaline ready during each asparaginase administration
- Observe the patient for at least 1 hour after asparaginase administration
- Dose adjustments should be made according to the following table:

Event	Dose adjustment
Pancreatitis	withhold asparaginase until resolved
Amylase > 2x ULN	withhold asparaginase until resolved
Liver toxicity (bilirubin and/or transaminases ≥ 5 x ULN)	withhold asparaginase until resolved
Clinical allergic reaction or silent inactivation (when this is observed by measurement of asparaginase activity levels, Dr. A. Rijneveld will inform site as soon as possible)	change to Erwinia asparaginase one gift of PEG-L-asparaginase is to be replaced by 6 gifts Erwinia asparaginase at starting dose 15.000 IU/m^2 in 1 hour i.v. 3 times a week for two weeks
Thrombosis	report on TE reporting form; consideration to stop asparaginase left to discretion of local physician

Therapeutic drug monitoring program (for Dutch and Belgian sites only)
In pediatric ALL it was shown that the dose-schedule used resulted in asparaginase levels that were significantly higher than the necessary level of 50-250 U/ml to achieve therapeutic asparagine depletion or levels that were not detectable due to inactivation by allergy or silent inactivation. Inactivation was found in ca. 40% of patients (treated with non-pegylated asparaginase). The subsequent replacement by Erwinia asparginase resulted in adequate levels in

almost all patients. Therefore, a therapeutic drug monitoring program of PEG-L-asparaginase will be introduced to individualize dosing schedules.
Monitoring of PEG-L-asparaginase and antibody levels at day (from start of prephase) 8*, 15, 21* and 35 of induction and day 4*, 11, 18* and 32 of intensification Ia and II in young patients of ≤ 40 years (in case of treatment delay, collection of blood samples should be performed 7 days after PEG-L-asparaginase administration and directly before the next administration of PEG-L-asparaginase (marked with *). In older patients of > 40 years no drug monitoring will be done, since only two infusions of PEG-L-asparaginase are given in total. Therefore, there is no additional benefit for this group.

Timepoints for PEG-L-asparaginase drug monitoring in patients ≤ 40 years

Remission induction
Day 8* (from prephase)
Day 15
Day 21*
Day 35

Intensification Ia and II
Day 4*
Day 11
Day 18*
Day 32

At the given timepoints 3 ml whole blood is collected and processed for serum by centrifugation. Serum will be harvested and sent to a central lab (Erasmus MC-Sophia Children's Hospital, Rotterdam), where asparaginase activity and anti-asparaginase antibodies will be assessed. All details can be found in the side studies lab manual.

Clinical allergy or silent inactivation of PEG-L-asparaginase:
Silent inactivation of PEG-L-asparaginase is defined as asparaginase serum level < 100 IU/l at day 7 or < 20 IU/l at day 14 after administration of PEG-L-asparaginase in a patient without clinical allergy.
In case of **clinical allergy or silent inactivation** of PEG-L-asparaginase substitution of 1 dose PEG-L-asparaginase by 6 doses Erwinia asparaginase (starting dose 15,000 IU/m^2 in 1 hour i.v., 3 times a week for 2 weeks). When the switch has been made, the drug monitoring will continue before every administration of Erwinase, during 2 weeks (thus, **6 time points** in total).

Targeted asparaginase level and advice
The targeted trough asparaginase level is 50 - 250 IU/l. In case of clinical allergy or silent inactivation, PEG-L-aspagarinase should be substituted by Erwinase.The advice concerning asparaginase switch will be communicated to the local investigator by Dr. A.W. Rijneveld (a.rijneveld@erasmusmc.nl). Erwinase should then be given during Intensification Ia and II based on the asparaginase levels measured in the remission-induction cycle in patients ≤ 40 years.

F. Administration of high dose MTX

- do not use H2 blockers with high dose MTX, because of nephrotoxicity
- stop cotrimoxazol from 3 days before until 5 days after the high dose MTX
- check for possible interactions with azoles, quinolones, macrolides, NSAIDs, thiazide diuretics and aminosides
- Start when neutrophils > 0.5 x 10^9/l, platelets > 50 x 10^9/l, creatinine ≤ 1.5 x upper limit of normal and bilirubin/transaminases ≤ 3 x upper limit of normal

Dosage

5000 mg/m^2 in continuous i.v. infusion for 24 hours with:

- 1/10 of the dose = 500 mg/m^2 in 60 minutes (diluted in glucose 5%), and
- 9/10 of the dose = 4,500 mg/m^2 in 23 hours (diluted in glucose 5%)

The intrathecal MTX therapy will be given at hour 24 (H24) of the high dose MTX infusion.

Hyperhydration and alkalinisation

Timepoint	Action
H -1	infuse 6 ml/kg (1 mEq/kg) sodium bicarbonate 1.4% in 30 minutes
H0 until H72	hydration (i.v. or p.o.): 2000 ml/m^2/d with 1/3 of sodium bicarbonate 1.4% and 2/3 of glucose 5% (+ 2 g/l KCl)

- Make sure that the pH of the urine is > 7 before start of the MTX infusion
- Determine plasma levels of methotrexate at H36, H48, H72, H96 and beyond if necessary; if MTX level is >2x10^{-7} M (> 0.2 micromol/L) folinic acid rescue must be applied

Folinic acid rescue

Folinic acid rescue is given from H36 (from the start of the MTX infusion) until MTX blood levels reach ≤ 2 x 10^{-7} mol/l (≤ 0.2 micromol/L). Folinic acid will be administered once every 6 hours according to the following table:

MTX level $x10^{-7}$ M (mol/L)	MTX level micromol/L	H36	H48	H72	H96	> H96
> 100	> 10	45 mg p.o. x 2	50 mg/m^2 i.v. x 4	200 mg/m^2 i.v. x 4	200 mg/m^2 i.v. x 4	200 mg/m^2 i.v. x 4
> 50	> 5	45 mg p.o. x 2	45 mg p.o. x 4	100 mg/m^2 i.v. x 4	200 mg/m^2 i.v. x 4	200 mg/m^2 i.v. x 4
> 10	> 1	45 mg p.o. x 2	45 mg p.o. x 4	50 mg/m^2 i.v. x 4	100 mg/m^2 i.v. x 4	200 mg/m^2 i.v. x 4
> 5	> 0.5	45 mg p.o. x 2	45 mg p.o. x 4	45 mg p.o. x 4	50 mg/m^2 i.v. x 4	100 mg/m^2 i.v. x 4
> 2	> 0.2	45 mg p.o. x 2	45 mg p.o. x 4	45 mg p.o. x 4	45 mg p.o. x 4	50 mg/m^2 i.v. x 4
≤ 2	≤ 0.2	no rescue	no rescue	no rescue	no rescue	no rescue

G. MRD diagnostics

1. MRD diagnostics via PCR analysis of rearranged Ig/TCR genes

Rearrangements of variable (V), diversity (D) and joining (J) gene segments in the Ig and TCR genes occurs early during B-and T-cell differentiation and aims at creating a broad repertoire of antigen-specific receptors, i.e. the Ig and TCR molecules.This diversity in Ig/TCR genes is in part based on the potential of many different combinations of the available V, D, and J gene segments and particularly on the unique composition of the coupling sites of the gene segments, the so-called junctional regions. These junctional regions are characterized by the random deletion and insertion of nucleotides during the rearrangement process, which results in unique segments that can serve as clone-specific markers. Consequently, the junctional regions of rearranged Ig/TCR genes can be regarded as unique “DNA fingerprints” for each lymphoid malignancy (including ALL) and therefore as PCR target for MRD diagnostics.

The Ig/TCR based MRD technique requires that for each individual ALL patient, the Ig/TCR gene rearrangement pattern of the leukemia cells has to be determined, the junctional regions have to be sequenced, junctional regions-specific primers have to be designed for TaqMan-based RQ-PCR analysis, and sensitivity testing has to be performed for each set of TaqMan probe/primers (6). Based on the results of the sensitivity testing, at least two MRD-PCR targets are selected, which reach a sensitivity of $\leq 10^{-4}$, including at least one target with a quantitative range of $\leq 10^{-4}$ and a second target with a quantitative range of $\leq 5 \times 10^{-4}$ (7).

Analysis of the obtained RQ-PCR data and interpretation of these results into MRD levels will be performed according to the guidelines of the European Study Group on MRD detection in ALL (ESG-MRD-ALL) (7), a European Consortium of 35 Ig/TCR-MRD laboratories.

2. PCR-based MRD diagnostics for ALL patients

Since MRD diagnostics has recently been proven to have prognostic value for children but also adult ALL as well (21,22), it was decided to include MRD diagnostics in the HOVON 100 ALL/EORTC protocol in order to evaluate treatment response during the first 6 to 12 months. The obtained MRD information will be used as “second endpoint” in the evaluation of the treatment protocol and will consequently be correlated with relapse and survival. Because MRD diagnostics will be performed at diagnosis and 4 consecutive follow-up time points in

the HOVON 100 ALL/EORTC protocol, the MRD results will provide insight into the cumulative effects of clofarabine and chemotherapy.

3. Logistics of MRD diagnostics

MRD diagnostics for the HOVON 100 ALL/EORTC treatment protocol will be performed by Ig/TCR-MRD laboratories of the country. The HOVON data center will inform the laboratories about inclusion of a new patient.

The Netherlands

In the Netherlands, the diagnosis and follow-up samples (bone marrow in heparin) will be sent to the DCOG laboratory in The Hague (head: Dr. V. de Haas), where the cell samples are processed. The DNA samples will be divided between two Dutch Ig/TCR-MRD laboratories, i.e. the department of Immunology, Erasmus MC, Rotterdam and the department of Immunohematology, Sanquin, Amsterdam.
Bone marrow samples should be sent in the blue envelope, provided by SKION-DCOG laboratory (tel: 070-3674545). This blue envelope contains an instruction form and also a few patient details need to be filled in. This blue envelope including the instruction form should be send by courier to The Hague. For detailed information, see MRD logistics document.

Belgium

In Belgium, the diagnosis and corresponding follow-up samples (bone marrow in EDTA) will be sent to the Ig/TCR-MRD laboratory in BrusselsDr. M. Bakkus (Marleen)
For detailed information, see MRD logistics document.

France

In France, the patient samples will be analyzed for MRD levels by the Ig/TCR-MRD laboratories and send to:

Dr. H. Cavé (Hélène)
Lab. de Biochimie Génétique
Hopital Robert Debré
48, Boulevard Sérurier
75019 PARIS
France

Tel. 33-1-4003.5711
Fax. 33-1-4003.2277
E-mail helene.cave@rdb.aphp.fr

or

Dr. E. Clappier (Emmanuelle)
Lab. de Biochimie Génétique
Hopital Robert Debré
48, Boulevard Sérurier
75019 PARIS
France

Tel. 33-1-4003.4065
Fax. 33-1-4003.2277
E-mail emmanuelle.clappier@rdb.aphp.fr

4. Technical and laboratory aspects of MRD diagnostics

The diagnosis samples will be used for assessment of the complete Ig/TCR gene rearrangement status: *IGH* (VH-JH and DH-JH), *IGK* (Vκ-Jk, Vκ-Kde, and intronRSS-Kde), *TCRD* (Dδ2-Dδ3, Vδ2-Dδ3, Dδ2-Jδ1, Vδ-Jδ), *TCRG, TCRB* (Vβ-Jβ and Dβ-Jβ), and Vδ2-Jα (8,9,23-25). Germline TaqMan probe/primer sets are available for all indicated Ig/TCR gene rearrangements (26-31).

Junctional region-specific primers will be designed for three selected MRD-PCR targets (if available) and tested for sensitivity in TaqMan-based RQ-PCR assays. All follow-up samples of the patient will be analyzed collectively within 8 weeks after arrival of the last follow-up sample. The combined MRD results will be reported to the study coordinator (A.W. Rijneveld).

If a patient relapses, the Ig/TCR gene rearrangement pattern of the diagnosis and relapse samples will be compared to investigate the stability of the rearrangements. So at time of a relapse another BM sample should be send to the laboratory of the country as well!

Literature references

1. Van Dongen JJM, Breit TM, Adriaansen HJ, Beishuizen A, Hooijkaas H. Detection of minimal residual disease in acute leukemia by immunological marker analysis and polymerase chain reaction. Leukemia 1992;6:47-59.
2. Szczepanski T, Orfão A, Van der Velden VHJ, San Miguel JF, Van Dongen JJM. Minimal residual disease in leukaemia patients. Lancet Oncol 2001;2:409-417.
3. Van Dongen JJM, Szczepanski T, Van der Velden VHJ. Minimal residual disease. In: Kaspers GJL, Coiffier B, Heinrich MC, Estey E, eds. Innovative Leukemia and Lymphoma Therapy 2008. New York: Informa Healthcare; 2008:45-84 (ISBN-10: 0-8493-5083-2; ISNB-13: 987-0-8493-5083-2).
4. Szczepanski T, Willemse MJ, Brinkhof B, Van Wering ER, Van der Burg M, Van Dongen JJM. Comparative analysis of Ig and TCR gene rearrangements at diagnosis and at relapse of childhood precursor-B-ALL provides improved strategies for selection of stable PCR targets for monitoring of minimal residual disease. Blood 2002;99:2315-2323.
5. Szczepanski T, Van der Velden VHJ, Raff T, Jacobs DCH, Van Wering ER, Brüggemann M, Kneba M, Van Dongen JJM. Comparative analysis of T-cell receptor gene rearrangements at diagnosis and relapse of T-cell acute lymphoblastic leukemia (T-ALL) shows high stability of clonal markers for monitoring of minimal residual disease and reveals the occurrence of second T-ALL. Leukemia 2003;17:2149-2156.
6. Szczepanski T, Flohr T, Van der Velden VH, Bartram CR, Van Dongen JJM. Molecular monitoring of residual disease using antigen receptor genes in childhood acute lymphoblastic leukaemia. Best Pract Res Clin Haematol 2002;15:37-57.
7. Van der Velden VHJ, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER, Flohr T, Sutton R, Cave H, Madsen HO, Cayuela JM, Trka J, Eckert C, Foroni L, Zur Stadt U, Beldjord K, Raff T, Van der Schoot CE, Van Dongen JJM. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia 2007;21:604-611.

8. Van der Velden VH, Szczepanski T, Wijkhuijs JM, Hart PG, Hoogeveen PG, Hop WC, van Wering ER, van Dongen JJ. Age-related patterns of immunoglobulin and T-cell receptor gene rearrangements in precursor-B-ALL: implications for detection of minimal residual disease. Leukemia 2003;17:1834-1844.
9. Szczepanski T, Van der Velden VHJ, Hoogeveen PG, De Bie M, Jacobs DC, Van Wering ER, Van Dongen JJM. Vδ2-Jα gene rearrangements are frequent in precursor-B-acute lymphoblastic leukemia but rare in normal lymphoid cells. Blood 2004;103:3798-3804.
10. Flohr T, Schrauder A, Cazzaniga G, Panzer-Grümayer R, Van der Velden VHJ, Fischer S, Stanulla M, Basso G, Niggli FK, Schäfer BW, Sutton R, Koehler R, Zimmermann M, Valsecchi MG, Gadner H, Masera G, Schrappe M, Van Dongen JJM, Biondi A, Bartram CR; International BFM Study Group (I-BFM-SG). Minimal residual disease-directed risk stratification using real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements in the international multicenter trial AIEOP-BFM ALL 2000 for childhood acute lymphoblastic leukemia. Leukemia 2008;22:771-782.
11. Szczepanski T, Langerak AW, Wolvers-Tettero ILM, Ossenkoppele GJ, Verhoef G, Stul M, Petersen EJ, De Bruijn MAC, Van 't Veer MB, Van Dongen JJM. Immunoglobulin and T cell receptor gene rearrangement patterns in acute lymphoblastic leukemia are less mature in adults than in children: Implications for selection of PCR targets for detection of minimal residual disease. Leukemia 1998;12:1081-1088.
12. Van Dongen JJM, Seriu T, Panzer-Grümayer ER, Biondi A, Pongers-Willemse MJ, Corral L, Stolz F, Schrappe M, Masera G, Kamps WA, Gadner H, Van Wering ER, Ludwig W-D, Basso G, De Bruijn MAC, Cazzaniga G, Hettinger K, Van der Does-van den Berg A, Hop WCJ, Riehm H, Bartram CR. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 1998;352:1731-1738.
13. Cavé H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, Bakkus M, Thielemans K, Grandchamp B, Vilmer E. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. N Engl J Med 1998;339:591-598.
14. Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, Rubnitz JE, Rivera GK, Sandlund JT, Pui CH, Campana D. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet 1998;351:550-554.
15. Nyvold C, Madsen HO, Ryder LP, Seyfarth J, Svejgaard A, Clausen N, Wesenberg F, Jonsson OG, Forestier E, Schmiegelow K; Nordic Society for Pediatric Hematology and Oncology. Precise quantification of minimal residual disease at day 29 allows identification of children with acute lymphoblastic leukemia and an excellent outcome. Blood 2002;99:1253-1258.
16. Willemse MJ, Seriu T, Hettinger K, d'Aniello E, Hop WC, Panzer-Grumayer ER, Biondi A, Schrappe M, Kamps WA, Masera G, Gadner H, Riehm H, Bartram CR, Van Dongen JJM. Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood 2002;99:4386-4393.
17. Eckert C, Biondi A, Seeger K, Cazzaniga G, Hartmann R, Beyermann B, Pogodda M, Proba J, Henze G. Prognostic value of minimal residual disease in relapsed childhood acute lymphoblastic leukaemia. Lancet 2001;358:1239-1241.
18. Coustan-Smith E, Gajjar A, Hijiya N, Razzouk BI, Ribeiro RC, Rivera GK, Rubnitz JE, Sandlund JT, Andreansky M, Hancock ML, Pui CH, Campana D. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia after first relapse. Leukemia 2004;18:499-504.
19. Knechtli CJ, Goulden NJ, Hancock JP, Grandage VL, Harris EL, Garland RJ, Jones CG, Rowbottom AW, Hunt LP, Green AF, Clarke E, Lankester AW, Cornish JM, Pamphilon DH, Steward CG, Oakhill A. Minimal residual disease status before allogeneic bone marrow transplantation is an important determinant of successful outcome for children and adolescents with acute lymphoblastic leukemia. Blood 1998;92:4072-4079.
20. Krejci O, van der Velden VH, Bader P, Kreyenberg H, Goulden N, Hancock J, Schilham MW, Lankester A, Révész T, Klingebiel T, van Dongen JJ. Level of minimal residual disease prior to haematopoietic stem cell transplantation predicts prognosis in paediatric patients with acute lymphoblastic leukaemia: a report of the Pre-BMT MRD Study Group. Bone Marrow Transplant 2003;32:849-851.
21. Brüggemann M, Raff T, Flohr T, Gökbuget N, Nakao M, Droese J, Lüschen S, Pott C, Ritgen M, Scheuring U, Horst HA, Thiel E, Hoelzer D, Bartram CR, Kneba M; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood 2006;107:1116-1123.

22. Raff T, Gökbuget N, Lüschen S, Reutzel R, Ritgen M, Irmer S, Böttcher S, Horst HA, Kneba M, Hoelzer D, Brüggemann M; GMALL Study Group. Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 trials. Blood 2007;109:910-915.
23. Beishuizen A, De Bruijn MAC, Pongers-Willemse MJ, Verhoeven M-AJ, Van Wering ER, Hählen K, Breit TM, De Bruin-Versteeg S, Hooijkaas H, Van Dongen JJM. Heterogeneity in junctional regions of immunoglobulin kappa deleting element rearrangements in B cell leukemias: A new molecular target for detection of minimal residual disease. Leukemia 1997;11:2200-2207.
24. Pongers-Willemse MJ, Seriu T, Stolz F, d'Aniello E, Gameiro P, Pisa P, Gonzalez M, Bartram CR, Panzer-Grümayer ER, Biondi A, San Miguel JF, Van Dongen JJM. Primers and protocols for standardized detection of minimal residual disease in acute lymphoblastic leukemia using immunoglobulin and T cell receptor gene rearrangements and *TAL1* deletions as PCR targets: Report of the BIOMED-1 CONCERTED ACTION: Investigation of minimal residual disease in acute leukemia. Leukemia 1999;13:110-118.
25. Szczepanski T, Pongers-Willemse MJ, Langerak AW, Harts WA, Wijkhuijs AJM, Van Wering ER, Van Dongen JJM. Ig heavy chain gene rearrangements in T-cell acute lymphoblastic leukemia exhibit predominant DH6-19 and DH7-27 gene usage, can result in complete V-D-J rearrangements, and are rare in T-cell receptor alpha beta lineage. Blood 1999;93:4079-4085.
26. Verhagen OJ, Willemse MJ, Breunis WB, Wijkhuijs AJ, Jacobs DC, Joosten SA, Van Wering ER, Van Dongen JJM, Van der Schoot CE. Application of germline IGH probes in real-time quantitative PCR for the detection of minimal residual disease in acute lymphoblastic leukemia. Leukemia 2000;14:1426-1435.
27. Van der Velden VH, Willemse MJ, Van der Schoot CE, Hahlen K, Van Wering ER, Van Dongen JJM. Immunoglobulin kappa deleting element rearrangements in precursor-B acute lymphoblastic leukemia are stable targets for detection of minimal residual disease by real-time quantitative PCR. Leukemia 2002;16:928-936.
28. Van der Velden VHJ, Wijkhuijs JM, Jacobs DCH, Van Wering ER, Van Dongen JJM. T cell receptor gamma gene rearrangements as targets for detection of minimal residual disease in acute lymphoblastic leukemia by real-time quantitative PCR analysis. Leukemia 2002;16:1372-1380.
29. Szczepanski T, van der Velden VHJ, van Dongen JJM. Real-time quantitative (RQ)-PCR for the detection of minimal residual disease in childhood acute lymphoblastic leukemia. Haematologica 2002;87S1:183-191.
30. Bruggemann M, Van der Velden VH, Raff T, Droese J, Ritgen M, Pott C, Wijkhuijs AJ, Gokbuget N, Hoelzer D, Van Wering ER, Van Dongen JJM, Kneba M. Rearranged T-cell receptor beta genes represent powerful targets for quantification of minimal residual disease in childhood and adult T-cell acute lymphoblastic leukemia. Leukemia 2004;18:709-719.
31. Van der Velden VHJ, De Bie M, Van Wering ER, Van Dongen JJM. Immunoglobulin light chain gene rearrangements in precursor-B-acute lymphoblastic leukemia: characteristics and applicability for the detection of minimal residual disease. Haematologica 2006;91:679-682.

H. Diagnosis, staging and grading of GVHD

Diagnosis

Acute and chronic GVHD is defined according to the proposal of the recent National Institutes of Health (NIH) Consensus Conference, which recognizes 2 categories of GVHD [36]:

(1) **acute GVHD** (absence of features consistent with chronic GVHD), comprising:

(a) classic acute GVHD (before day 100), and,

(b) persistent, recurrent, or late acute GVHD (after day 100, often upon withdrawal of immunosuppression);

(2) **chronic GVHD**, comprising:

(a) classic chronic GVHD (no signs of acute GVHD), and,

(b) an overlap syndrome, in which features of both acute and chronic GVHD are present.

B.1 Staging and grading of acute GVHD

For staging and grading the Glucksberg classification updated according to Przepiorka et al [37;38] is used:

Stage	Skin Rash	Liver Total bilirubin (μmol/L)	Intestinal tract Diarrhea (ml/day)
1	<25%	34-50	500 –1000 or persistent nausea without diarrhea*
2	25-50%	50-102	1000-1500
3	generalized erythroderma	102-255	>1500
4	bullae	>255	severe pain/ileus
Grade			
I	Skin: stage 1-2 and Liver: stage 0 and Gut: stage 0;		
II	Skin: stage 3 or Liver: stage 1 or Gut: stage 1;		
III	Skin: stage 3 or Liver: stage 2-3 or Gut: stage 2-4;		
IV	Skin or Liver: stage 4;		

*persistent nausea with histologic evidence of GVHD in the stomach or duodenum

B.2 Signs and symptoms of chronic GVHD according to the National Institutes of Health (NIH) Consensus Conference

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
Skin	Poikiloderma Lichen planus-like features Sclerotic features Morphea-like features Lichen sclerosus-like features	Depigmentation	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritus
Nails		Dystrophy Longitudinal ridging, splitting, or brittle features Onycholysis Pterygium unguis Nail loss (usually symmetric; affects most nails)†		
Scalp and body hair		New onset of scarring or nonscarring scalp alopecia (after recovery from chemoradiotherapy) Scaling, papulosquamous lesions	**Thinning scalp hair, typically patchy, coarse, or dull (not explained by endocrine or other causes)** Premature gray hair	
Mouth	Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseudomembranes Ulcers		Gingivitis Mucositis Erythema Pain
Eyes		New onset dry, gritty, or painful eyes# Cicatricial conjunctivitis Keratoconjunctivitis sicca# Confluent areas of punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus-like features Vaginal scarring or stenosis	Erosions Fissures Ulcers		

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GVHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features	Common (Seen with Both Acute and Chronic GVHD)
GI tract	Esophageal web Strictures or stenosis in the upper to mid third of the esophagus		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss Failure to thrive (infants and children)
Liver				Total bilirubin, alkaline phosphatase >2 × upper limit of normal ALT or AST >2 × upper limit of normal
Lung	Bronchiolitis obliterans diagnosed with lung biopsy	Bronchiolitis obliterans diagnosed with PFTs and radiology#		BOOP
Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis or polymyositis#	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hypergammaglobulinemia Autoantibodies (AIHA and ITP)	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality or cardiomyopathy	

* Can be acknowledged as part of the chronic GVHD symptomatology if the diagnosis is confirmed

 # Diagnosis of chronic GVHD requires biopsy or radiology confirmation (or Schirmer test for eyes).

Seattle classification for limited and extensive chronic GVHD

Limited

Either or both:

1. Localized skin involvement
2. Hepatic dysfunction due to chronic GvHD

Extensive

Either

1. Generalized skin involvement, or
2. Localized skin involvement and/or hepatic dysfunction due to chronic GvHD, plus:

(a) Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, or

(b) Involvement of eye (Schirmer's test with <5 mm wetting), or

(c) Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or

(d) Involvement of any other target organ