Randomized study with a run-in feasibility phase to assess the added value of Clofarabine in combination with standard remission-induction chemotherapy in patients aged 18-65 years with previously untreated acute myeloid leukemia (AML) or myelodysplasia (MDS) (RAEB with IPSS ≥ 1.5)

A multicenter phase III trial

PROTOCOL

Principal Investigator:		B. Löwenberg, G. Ossenkoppele
Study Coordinators:		B. Löwenberg (NL) G. Ossenkoppele (NL) J. Maertens (B) Th Pabst (CH)
Sponsor	:	HOVON VU University Medical Center (VUmc) P.O.Box 7057 1007 MB Amsterdam
Study drug	:	Clofarabine supplied by Genzyme Inc, Boston, MA, USA
Statistician	:	C. van Montfort
Datamanagement	:	HOVON Data Center
Registration	:	HOVON Data Center Erasmus University Medical Center - Daniel den Hoed P.O.Box 5201 3008 AE ROTTERDAM The Netherlands tel. +31.10.7041560 fax +31.10.7041028 https://www.hdc.hovon.nl/top
SAE notification	:	**HOVON Data Center** Fax +31.10.4239091
EudraCT number	:	2009-011613-24
Final version	:	April 23, 2009
Revised version	:	November 01, 2011
Date of activation	:	January 25, 2010
Approved	:	January 25, 2010

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Principal Investigator:

3 NOV 2011

Signature of Investigator Date

Prof. B. Löwenberg

Printed Name of Investigator

LOCAL INVESTIGATOR SIGNATURE PAGE

Local site name: _____

Local Investigator:

_____ _____

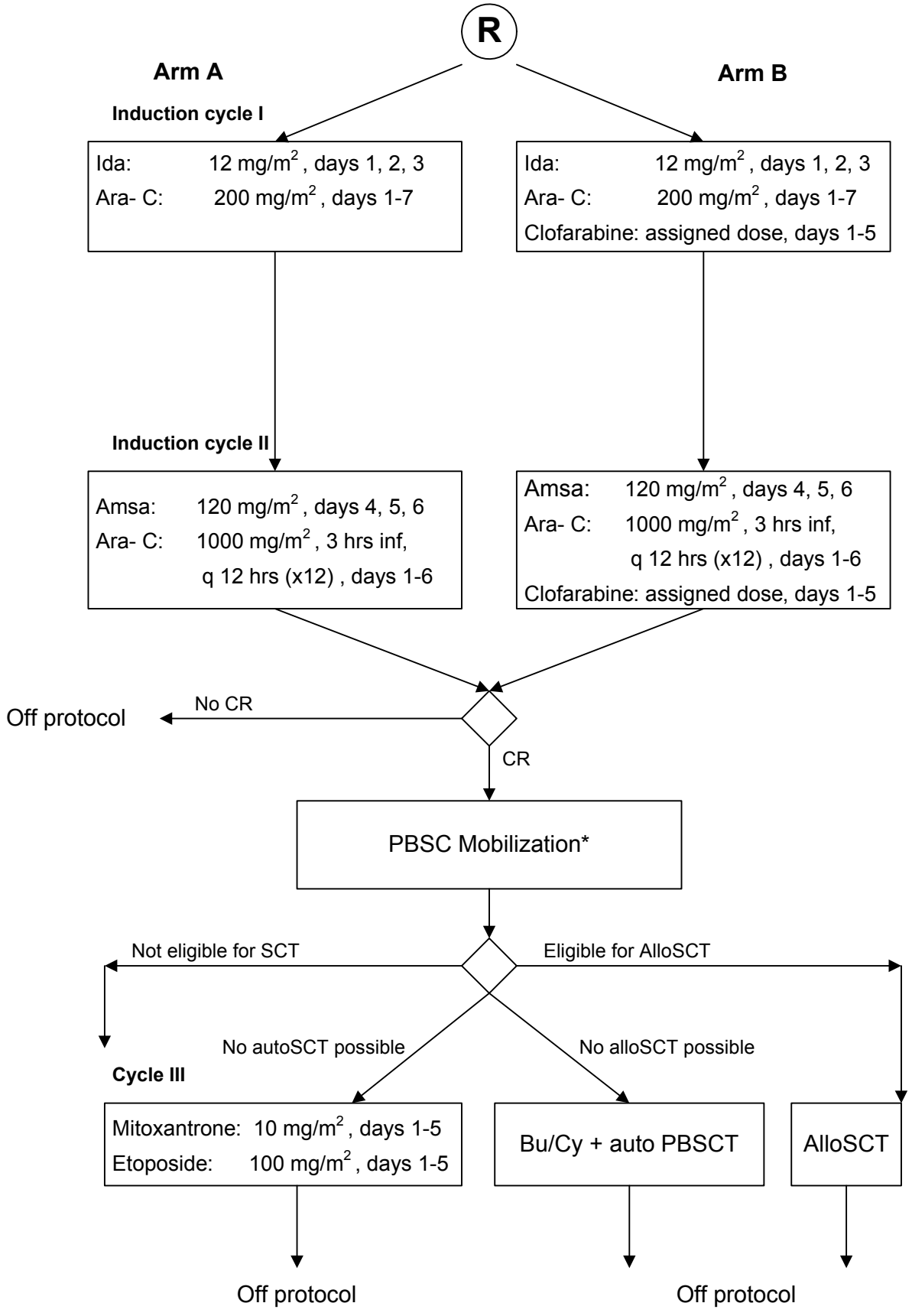
Signature of Investigator Date

_____

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



* unless to proceed to AlloSCT

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3 Synopsis

Study phase: Phase III

Study objectives: Primary objectives:

Part A:

- To determine the feasibility of Clofarabine when given at three possible dose levels together with standard induction cycles I and II in patients with AML/ RAEB with IPSS≥1.5 in a prospective comparison to standard induction cycles I and II without Clofarabine.

Part B:

- To evaluate the effect of Clofarabine at the selected feasible dose level when combined with remission induction chemotherapy cycles I and II as regards clinical outcome ("event-free survival') in comparison to remission induction cycles I and II with no addition of Clofarabine in a phase III study.

Secondary objectives:

Part A:

- To investigate the clinical efficacy of Clofarabine in combination with remission induction chemotherapy cycles I and II with regard to complete remission rate at different dose levels of Clofarabine.

Part B:

- To investigate the clinical efficacy of Clofarabine with regard to the complete remission rate, disease free survival (DFS), risk of relapse and overall survival (OS) when combined with remission induction chemotherapy cycles I and II in all patients.
- To investigate the clinical efficacy of Clofarabine when combined with remission induction chemotherapy cycles I and II in molecularly and cytogenetically distinguishable subsets with regard to the complete remission rate, disease free survival (DFS), risk of relapse and overall survival (OS).
- To investigate the tolerance and toxicity of Clofarabine in combination with remission induction chemotherapy cycles I and II.
- To assess the effect of Clofarabine on peripheral CD34 cell numbers for autologous peripheral blood transplantation.
- To determine the prognostic value of molecular markers and gene expression profiles of the leukemia assessed at diagnosis.
- To evaluate the treatment effects according minimal residual disease (MRD) measurements following therapy by standardized sampling of

	marrow/blood. ♦ To evaluate the outcome of allogeneic sibling or unrelated donor SCT and autologous SCT in cytogenetically and molecularly defined and prognostic subgroups of patients.
Patient population	Patients with previously untreated AML (except acute promyelocytic leukemia) or MDS RAEB with IPSS ≥ 1.5, age 18-65 years.
Study design	Part A: Comparative, randomized feasibility study of remission induction chemotherapy combined with Clofarabine at three possible dose levels 10, 15, 20 mg/m^2 given intravenously for 5 days. Part B: Multicenter, phase III study at the selected feasible dose level of Clofarabine in a prospective randomized approach between Clofarabine combined with two induction cycles of chemotherapy versus the same chemotherapy with no addition of Clofarabine.
Duration of treatment	Expected duration of 2 induction cycles inclusive evaluation is approximately 3 months. Consolidation treatment will take an additional 1-3 months. All patients will be followed until 10 years after randomization.
Number of patients	Part A: 120, Part B: 800
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: September 2009 End of recruitment: September 2013
End of trial	At 7 months after registration and randomization of the finally enrolled patient

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Principal Investigator	B. Löwenberg	Erasmus University Medical Center Rotterdam
Study Coordinators	G. Ossenkoppele (NL)	VU University Medical Center Amsterdam
	J. Maertens (B)	University Hospital Leuven (B)
	Th. Pabst (CH)	University Hospital Bern (CH)
Representative of Sponsor (HOVON)	P.C. Huijgens, chairman	VU University Medical Center Amsterdam
Registration	HOVON Data Center	Erasmus University Medical Center – Daniel den Hoed P.O.Box 5201 3008 AE ROTTERDAM The Netherlands tel. +31.10.7041560 fax +31.10.7041028 https://www.hdc.hovon.nl/top
Monitoring	HOVON Data Center	Erasmus University Medical Center – Daniel den Hoed
Writing Committee	B. Biemond	Academic Medical Center, Amsterdam (NL)
	D. Breems	Hospital Stuivenberg, Antwerp (B)
	J. Cornelissen	Erasmus University Medical Center Rotterdam (NL)
	S. Daenen	University Medical Center Groningen (NL)
	A. Ferrant	University Hospital Bruxelles (B)
	M. Fey	University Hospital Bern (CH)
	B. Gjertsen	Bergen (N)
	A. Gratwohl	University Hospital Basel (CH)
	C. Graux	Mont Godinne, Yvoir (B)
	G.E. de Greef	Erasmus University Medical Center Rotterdam (NL)
	P.C. Huijgens	VU university Medical Center Amsterdam (NL)
	G. Huls	University Medical Center Groningen (NL)
	M. Jongen-Lavrencic	Erasmus University Medical Center Rotterdam (NL)
	A.A. van de Loosdrecht	VU Medical Center Amsterdam (NL)
	B. Löwenberg	Erasmus University Medical Center Rotterdam (NL)
	J. Maertens	University Hospital Leuven (B)
	G. Ossenkoppele	VU University Medical Center Amsterdam (NL)
	Th. Pabst	University Hospital Bern (CH)
	R. Schaafsma	Medisch Spectrum Twente, Enschede (NL)
	H.C. Schouten	Universtiy Hospital Maastricht
	P. Sonneveld	Erasmus University Medical Center Rotterdam (NL)
	J. Kuball	University Medical Center Utrecht (NL)

	E. Vellenga	University Hospital Groningen (NL)
	L. Verdonck	Isala Hospital Zwolle (NL)
	G. Verhoef	University Hospital Leuven (B)
	P. Wijermans	Haga Hospital, The Hague (NL)
	S. Wittebol	Meander Hospital Amersfoort (NL)
	J.M.J.M. Zijlmans	Erasmus University Medical Center Rotterdam (NL)
Cytogenetics review	B. Beverloo	Erasmus University Medical Center Rotterdam
	E. van den Berg-de Ruiter	University Medical Center, Groningen
Cytological and immunophenotype review	M. Jongen-Lavrencic	Erasmus University Medical Center Rotterdam
GEP and molecular Diagnositics coordinator	P. Valk	Erasmus University Medical Center Rotterdam
MRD coordinator	G.J. Schuurhuis	VU University Medical Center Amsterdam
Study Drug	Clofarabine	
Provided by	Genzyme Inc.	Boston, MA, USA
Labeling and distribution by	Penn Pharmaceuticals	UK
Statistician	C. van Montfort	Erasmus University Medical Center Rotterdam
Datamanagement	HOVON Data Center	Erasmus University Medical Center – Daniel den Hoed
Serious Adverse Events (SAEs) notification	HOVON Data Center	Erasmus University Medical Center – Daniel den Hoed Fax: +31.10.4239091

4.1 Cytological and immunophenotype review

Review of bone marrow aspirate at diagnosis by the Hematocytology Review Committee (HRC) is required.

Four unstained blood and 6 unstained bone marrow smears should be sent together with a filled out cytology form and a copy of the report of the immunological marker analysis to

Dr. Mojca Jongen-Lavrencic
Hematocytology Review Committee
Erasmus MC - Daniel den Hoed
Groene Hilledijk 301
3075 EA Rotterdam

The Netherlands.

Confirmation of diagnosis is not necessary for randomization and 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 Cytogenetic and FISH form together with a copy of the original FISH report is also requested to be sent with the cytogenetic data for central review.

5 Introduction

5.1 Acute Myeloid Leukemia (AML)

Acute Myeloid Leukemia (AML) is a bone marrow malignancy of progenitor cells of the myeloid cell lineage (1). AML is classified according to the World Health Organization (WHO) classification together with myelodysplastic syndromes (MDS) which resemble AML (2,5). The myelodysplastic syndromes are a heterogeneous group of hematopoietic disorders among which the refractory anemia with excess of blasts (RAEB) with high (≥1.5) IPSS is one of the most prognostically unfavourable subtypes frequently evolving to AML (3, 4,5).

5.1.1 Chemotherapy in AML

The traditional approach to the patient with AML/high risk MDS has been based on treatment with a combination of an anthracyclin (daunorubicin (DNR) or idarubicin) with cytarabine (cytosine arabinoside or Ara-C). The dose of Ara-C has varied from standard dose of 100 mg/m^2 q day up to intensified dose of 3 g/m^2 during induction treatment (1).
However, ultimately 40-50% of younger patients and 80% of elderly patients with AML do not achieve long-standing remissions and succumb from primary treatment refractory disease or more frequently from relapse. It has become clear that subsets of AML defined by cytogenetic or molecular markers carry different prognostic characteristics. More information on this is given in sections 5.5, 5.6, 5.7 and appendix D.
From accumulated data it is thus clear that current modalities of chemotherapy are not sufficiently effective and that additional treatments are needed. An important approach to improving the response

rates and response durations in AML is to introduce new agents with a unique mechanism of action. The treatment of AML is moving forward by integrating new drugs with different mechanisms of action in the overall treatment plan.
Clofarabine appears an active anti-AML drug. The application of clofarabine integrated in a backbone of standard remission induction chemotherapy appears interesting and such a schedule awaits to be evaluated in the front line context of AML treatment of adults equal or less than 65 years of age.
In addition, it was approved by EMEA for the same indication in May 2006.

5.2 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 and decreases its susceptibility to phosphorolytic cleavage by the bacterial enzyme Escherichia coli purine nucleoside phosphorylase in the gastrointestinal tract both of which may lead to enhanced oral bioavailability (26, 27). 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.

5.2.1 Mechanism of Action of Clofarabine

The precise mechanism of action of clofarabine on dividing and non-dividing cells is unknown. Like other nucleoside analogues (cytarabine, cladribine, and fludarabine), clofarabine must be converted within cells to the 5'-triphosphate form by deoxycytidine kinase (dCK) and mono- and di-phosphokinases to be active. Clofarabine is more efficient as a substrate for purified recombinant dCK, exceeding cladribine and the natural substrate, deoxycytidine (28). Evidence suggests that the primary cytotoxic effect of clofarabine is due to its inhibition of DNA synthesis and repair. The triphosphate form of clofarabine is an inhibitor of both DNA polymerase α and ε and ribonucleotide reductase (29). These inhibitory effects lead to depletion of intracellular deoxynucleotide triphosphate pools and inhibition of elongation of DNA strands during synthesis and DNA repair (30). With respect to inhibition of ribonucleotide reductase, clofarabine and cladribine are superior to fludarabine (28). With respect to inhibition of DNA polymerase α, clofarabine and fludarabine are similar and both are

superior to cladribine (28). Thus, in comparison to cladribine and fludarabine, clofarabine more completely inhibits both ribonucleotide reductase and DNA polymerase α, versus one or the other. Unlike fludarabine, clofarabine is active in vitro in non-dividing cells and in cells with a low proliferation rate. Clofarabine can induce the apoptotic pathway as part of its cytotoxic effect on cells (30). Clofarabine has been shown to disrupt the integrity of mitochondria in primary chronic lymphocytic leukemia (CLL) cells. The damage leads to release of pro-apoptotic mitochondrial factors (31). These effects are postulated to induce apoptosis in indolent, non-dividing CLL cells. This result was not seen with fludarabine and may explain, at least in part, the enhanced cytotoxicity of clofarabine (31), although the physiologic and clinical implications of these observations remain uncertain and under continued investigation.

For detailed information on clofarabine toxicology and pharmacology we refer to the clofarabine Investigator's Brochure (32).

5.2.2 Clinical Experience of Clofarabine in AML

5.2.2.1 Salvage Single Agent Clofarabine Therapy in Adult AML

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 (33). The starting dose was 15 mg/m^2 IV administered daily for 5 days based on an animal study in which this dose was safe with no observable toxicities. However, 2 of the first few patients on study experienced myelosuppression and required dose de-escalations before the MTD of 2 mg/m^2 was identified in patients with solid tumors. Dose escalation in patients with hematologic malignancies increased to 55 mg/m^2, at which point patients experienced dose-limiting toxicities (DLTs) of reversible hepatotoxicity, and the MTD was determined to be 40 mg/m^2/day. Among the 32 patients diagnosed with acute leukemia, 2 patients achieved a complete response (CR) and 3 achieved CR without platelet recovery (CRp) for an overall response rate of 16%. Clofarabine pharmacokinetics were dose proportional across all the doses studied, but intracellular clofarabine triphosphate (which had large interpatient variability) began to show saturation at doses greater than about 20 mg/m^2/day.

In a Phase II study reported by Kantarjian et al (34), 62 adult patients with relapsed or refractory acute leukemia received clofarabine 40 mg/m^2 IV once daily for 5 days every 3 to 6 weeks. Twenty (20) patients achieved a CR, 9 achieved a CRp, and 1 achieved a partial response 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, and mucositis. No correlation was observed between plasma clofarabine concentrations and intracellular clofarabine triphosphate

concentrations, although it was observed that responders showed an accumulation of intracellular clofarabine triphosphate on Day 2 compared to nonresponders.
In contrast, a multi-center Phase II trial (CLO-221) initiated in 2002 in adult patients with relapsed or refractory acute myelogenous leukemia received clofarabine 40 mg/m^2 once daily for 5 days every 28 days for 2 cycles with subsequent cycles being dosed with 30 mg/m^2 (35). Only 1/40 (3%) patients achieved a CR of 20.4 weeks duration. Nausea, vomiting, headache, diarrhea, anorexia, dermatitis, and stomatitis were the most frequently drug-related reported AEs. Drug-related renal toxicities were reported for 10% of the patients; however, these patients either had a concurrent clinical condition associated with renal toxicity or at least 1 concomitant medication known to increase the potential for renal toxicity. Pharmacokinetic data available for 33% of the patients indicate clofarabine had a high tissue distribution with minimal accumulation and rapid elimination (primarily as unchanged drug) in the urine. These seemingly discrepant results must be considered in the context of the patient populations treated. In the CLO-221 experience approximately 25% of patients were refractory to prior therapy, whereas no subgroup of patients with primary refractory disease was reported in the prior experience reported by Kantarjian and colleagues. In addition, the CLO-221 population included 14/29 patients with a duration of first remission $<$6 months and 8/29 patients with a duration of first remission between 6 – 12 months.

5.2.2.2 Single Agent Clofarabine in Previously Untreated Adult AML

In a Phase II study (UWCM-001) conducted by Burnett et al, clofarabine was evaluated as first line treatment in 30 older patients (median age 72, range 61-82 years) with AML who were considered unfit for intensive chemotherapy (36). Patients received clofarabine 30 mg/m^2 for 5 days, which could be repeated for up to 4 cycles at a minimum of 28 days between cycles. Overall response rate was 56% (43% CR, 13% CRp). Toxicities greater than grade 3 included increases in ALAT and bilirubin, hand foot syndrome, skin rash, and nausea and vomiting. There were 4 early deaths.
Burnett et al (37) also evaluated clofarabine in 66 previously untreated AML patients, aged $>$65 years who were considered unsuitable for standard intensive chemotherapy based on age, comorbidity, or performance status. This open-label, multi-center, non-randomized, Phase II study is known as BIOV-121. Patients received clofarabine at 30 mg/m^2/day for 5 consecutive days repeated every 28-42 days, with a trial amendment to reduce to 20 mg/m^2 for subsequent cycles. Overall response rate (ORR) was 44% (21% CR, 23% CR with incomplete blood count recovery (Cri)) for the whole population: 47% ORR in patients with high risk or intermediate cytogenetics, 50% ORR in de novo AML, and 31% ORR in secondary AML. Overall survival (OS) at 21 months was 25%, with 32% OS for patients with CR or PR. Median time to neutrophil recovery (1.0×10^9/L) was 24 days (16-30 days), and median platelet recovery was 38 days (25-46 days). The most common drug related serious adverse events were neutropenic sepsis (19.7%), renal failure (10.6%), renal insufficiency (7.6%), sepsis (6.1%), febrile neutropenia, atrial fibrillation, diarrhea, vomiting and rash (all 3%).

Thirty-nine percent of patients (26 out of 66) experienced renal adverse events of any grade. Fifty-six percent of patients with reduced estimated glomerular filtration rate (GFR) (<60 ml/min/1.73 m^2 at study entry) reported renal adverse events, and only 29% of normal renal function patients reported a renal adverse event. Most patients who developed renal impairment or acute renal failure had co morbid conditions, sepsis and/or used nephrotoxic drugs during the study. Fourteen patients (21%) died within 30 days of clofarabine administration with 6 of these 14 deaths thought to be possibly related to clofarabine therapy (37).

Burnett et al (38) also presented information on a subset of untreated adult AML patients derived from 2 separate studies (BIOV-121 and UWCM-001). This group consisted of 26 elderly AML patients with confirmed adverse cytogenetics who received 30 mg/m^2 of clofarabine daily for 5 days with a median follow-up of 10 months (range 5-17 months). This subset was compared to similar groups of elderly patients derived from other studies who received either non-intensive therapy (hydroxycarbamide or low dose 20 mg cytarabine, schedule not provided) or a daunorubicin/cytarabine-based intensive chemotherapy. Twelve out of 26 clofarabine patients (46%) achieved a CR or CRi which was comparable to the 42% remission rate observed after intensive chemotherapy. No complete responses were observed in patients in either of the low intensity treatment groups (cytarabine or hydroxycarbamide). The 1-year survival rate for clofarabine-treated patients was 21%, which was significantly better than the survival rates noted in the low intensity groups and comparable to the 23% survival rate noted in the intensive chemotherapy group.

In a Phase II, single-arm, open-label study conducted by Erba et al (39), the safety and efficacy of single agent clofarabine was evaluated in previously untreated older adult patients with AML who were unlikely to benefit from standard induction chemotherapy. The primary endpoint of the study was overall remission rate (ORR = CR + CRp). The study, known as CLASSIC II (CLO-243), enrolled 116 patients who were 60 years of age or older (median age 71 years, range 60-88) with Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2 and with at least one adverse prognostic factor (70 years of age or older, antecedent hematologic disorder (AHD), ECOG PS of 2, or intermediate or unfavorable karyotype). Patients received 30 mg/m^2/day of clofarabine as a 1-hour intravenous (IV) infusion on days 1-5 for induction and 20 mg/m^2/day on days 1-5 for re-induction or consolidation therapy (maximum of 6 total cycles). Subsequent cycles were begun after a minimum of 28 days from the start of the previous cycle.

A preliminary analysis of efficacy and safety data (investigator assessment as of 11 April 2008) for the 115 patients evaluated was presented at the 2008 ASCO Annual Meeting. The ORR was 45% (40% CR, 5% CRp), ORR by adverse prognostic factor is summarized in Table 1. Thirty-five patients achieved remission after cycle 1 and 17 patients achieved remission after cycle 2. Treatment failure occurred in 53% of patients (n=61).

Table 1: Overall Response Rate by Adverse Prognostic Factor

Prognostic Factor	**Patients in Subset**	**ORR (CR+CRp), n, (%)**
Age ≥70 years	70	28 (40%)
ECOG PS 2	26	10 (38%)
AHD	42	21 (50%)
Unfavorable karyotype	56	24 (43%)
Intermediate karyotype	46	24 (52%)
Number of Risk Factors		
2	50	24 (48%)
3	38	16 (42%)

The median time to hematopoietic recovery in responders for cycle 1 for neutrophils (ANC) ($\geq 1\times10^9$/L) and platelets ($\geq 10\times10^9$/L) were 31 and 28 days respectively.
Additionally the authors reported that patients received a median of 2 cycles of clofarabine (range 1-6). Of the sixty-nine patients whom were initiated a second cycle, 58% (n=40) were given clofarabine as re-induction and the remaining 29 patients as consolidation. The median duration between the first 2 cycles was 41 days.
The 30-day all-cause mortality was 9.6% (n=11). Three deaths were considered drug-related by the investigator (1 respiratory failure secondary to pneumonia, 1 gram negative sepsis and 1 acute respiratory distress). Clofarabine related adverse events, as determined by the investigator, were reported in a majority of patients (94%). Infection and febrile neutropenia were noted in 58% and 49% of patients respectively regardless of causality to clofarabine. The most common adverse events reported in ≥20% of patients were nausea (45%), febrile neutropenia (34%) vomiting (31%), diarrhea (26%) and rash (22%). The incidence of Grade 4 neutropenia and thrombocytopenia based on lab shift tables was 81% and 89%, respectively.

5.2.2.3 Salvage Combination Therapy with Clofarabine in Adult AML

A Phase I-II study evaluated clofarabine plus intermediate dose cytarabine in 32 adult patients aged 18 years to 84 years (median 59 years) with relapsed acute leukemia (25 AML, 2 ALL), high-risk MDS (n=4), and blast phase chronic myelogenous leukemia (CML; n=1) (40). Clofarabine 40 mg/m^2/day was given as a 1-hour intravenous (IV) infusion for 5 days on days 2-6 followed 4 hours later by cytarabine 1 g/m^2/day as a 2 hour IV infusion for 5 days on days 1-5. Doses were reduced by 25% in subsequent cycles for grade 2 extramedullary toxicities and by 50% for grade 3 or higher extramedullary toxicities including life-threatening infections. Seven AML patients (28%) achieved a

CR and 3 AML patients (12%) achieved a CRp, for an overall response rate of 40%. The response rate in 23 AML and high-risk MDS patients, who were either primary refractory or had a first remission duration <1 year, was 35% (22% CR and 13% CRp). Adverse events included myelosuppression, infection, transient liver test abnormalities, nausea/vomiting, diarrhea, skin rashes, mucositis and palmoplantar erythrodysesthesias. Median time to platelet and neutrophil recovery in complete responders was 42 days and 33 days, respectively.

Faderl et al (41) conducted a dose-finding Phase 1 study in 44 adult patients with relapsed or refractory AML and high-grade MDS that compared 2 study arms using clofarabine in combination with idarubicin (CI group, n=23) and clofarabine/idarubicin plus cytarabine (CIA group, n=21). The CI group (median age 58 years; range 24-71 years), with 9 primary refractory and 15 abnormal cytogenetics patients completed five different dose levels. At the first dosing level, clofarabine 22.5 mg/m^2 for 5 days and idarubicin at 12 mg/m^2 for 3 days (n=6), 3 patients experienced grade 3 or higher toxicities (diarrhea, rash, and mucositis) necessitating a dose de-escalation. The subsequent dose levels were: clofarabine 15 mg/m^2 for 5 days plus idarubicin 8 mg/m^2 for 3 days (6 patients), clofarabine 18 mg/m^2 for 5 days plus idarubicin 10 mg/m^2 for 3 days (3 patients), clofarabine 22.5 mg/m^2 for 5 days plus idarubicin 10 mg/m^2 for 3 days (3 patients), and clofarabine 30 mg/m^2 for 5 days plus idarubicin 10 mg/m^2 for 3 days (5 patients). DLTs at this level were increased bilirubin, increased ALAT and headaches. The MTD was determined to be clofarabine 22.5 mg/m^2 and idarubicin 10 mg/m^2. Five patients responded in the CI treatment arm (22%) resulting in 3 CRs and 2 CRps. The CIA arm (median age 56 years; range 23-78 years) contained 8 primary refractory and 12 abnormal cytogenetics patients and evaluated 4 dose levels. In the first dosing level, clofarabine 22.5 mg/m^2 for 5 days plus idarubicin 8 mg/m^2 for 3 days plus cytarabine 1 g/m^2 for 5 days (3 patients), all 3 patients experienced grade 3 or higher toxicities (diarrhea, acute renal failure, and increased bilirubin) necessitating dose de-escalation. The subsequent doses included clofarabine 15 mg/m^2 for 5 days plus idarubicin 6 mg/m^2 for 3 days plus cytarabine 0.75 g/m^2 for 5 days (6 patients), clofarabine 22.5 mg/m^2 for 5 days plus idarubicin 6 mg/m^2 for 3 days plus cytarabine 0.75 g/m^2 for 5 days (6 patients), and clofarabine 30 mg/m^2 for 5 days plus idarubicin 6 mg/m^2 for 3 days plus cytarabine 0.75 g/m^2 for 5 days (6 patients). DLTs seen at the highest dose level were increased bilirubin, diarrhea and mucositis. The MTD was determined to be clofarabine 22.5 mg/m^2 plus idarubicin 6 mg/m^2 plus cytarabine 0.75 g/m^2. The CR rate in the CIA arm was 48%.

In another Phase I dose finding study, Karp et al (42) evaluated escalating doses of clofarabine followed by cyclophosphamide (CY) in 18 adults (median age 51 years) with refractory leukemia (12 AML and 6 ALL). One group received 10 mg/m^2/day of clofarabine (n=12; 9 AML, 3 ALL) while the other was treated with 20 mg/m^2/day of clofarabine (n=6; 3 AML, 3 ALL), and both clofarabine doses were followed with 400 mg/m^2/day of CY on days 1-3 and days 8-10. This resulted in total dosages of either 60 mg/m^2 or 120 mg/m^2 of clofarabine and 2400 mg/m^2 of CY. In order to assess the effect of clofarabine on CY-induced cellular toxicity, the first CY dose was divided between days 0 and 1, and

peripheral blood blasts were obtained prior to and after infusion of CY on days 0 and 1. In addition, bone marrows were obtained at day 14. Both apoptosis and DNA damage were assessed on the peripheral blood blast and bone marrow samples. The use of clofarabine prior to CY appeared to augment both CY-induced DNA damage (by phosphoraylated H2AX) and apoptosis (by sub-2N DNA). Two of the AML patients achieved a CR (10 mg/m^2 group) and 1 additional AML patient achieved a partial response (PR) (20 mg/m^2 group). Four patients (22%; 2 from each dosing cohort) expired due to multi-organ failure (n=2), fungal pneumonia (n=1), and prolonged aplasia (n=1). The dose limiting toxicity in the 20 mg/m^2 group was prolonged marrow aplasia (>60 days). Since the increases in CY-induced cellular toxicity did not appear to be closely related to clofarabine dose, the authors intend to restudy this drug combination using lower total dosages of clofarabine and similar or slightly higher total dosages of CY.

5.2.2.4 Combination Therapy with Clofarabine in Previously Untreated Adult AML

Faderl et al (43) conducted a Phase 2 study to evaluate the use of clofarabine plus cytarabine in 60 patients aged 50 years or older with newly diagnosed, untreated AML. The study was conducted in 2 study groups, the first group were 30 patients with diploid cytogenetics and the second group consisted of 30 patients with abnormal cytogenetics, (excluding inv(16) and translocation t(8;21) or t(15;17)). Clofarabine 40 mg/m^2/day was given as a 1-hour IV infusion for 5 days on days 2-6 followed 4 hours later by cytarabine 1 g/m^2/day as a 2 hour IV infusion for 5 days on days 1-5. Cycles were repeated every 4-6 weeks depending on response. Patients were allowed to receive a maximum of 3 induction cycles with the second and third induction course administering both clofarabine and cytarabine on days 1-5. Responding patients could receive up to 6 additional consolidation courses of clofarabine 40 mg/m^2/day and cytarabine 1 g/m^2/day for 3 days (both days 1-3).
Thirty-one patients overall (52%) achieved CR and 5 (8%) achieved CRp, for an overall response rate (ORR) of 60%. Eighteen of the 30 patients (60%) with diploid cytogenetics achieved CR and 2 patients (7%) achieved CRp for an ORR of 67%. In patients with abnormal karyotypes, 13 out of 30 patients (43%) achieved CR, and 3 out of 30 patients (10%) achieved CRp resulting in an ORR of 53%. Median time to CR was 29 days (range 20-98 days) for all patients. Median time to CRp was 34 days (range 24-75 days) for all patients. Median follow up was 18.2 months (range 10.2-26.5 months), and median remission duration for all patients achieving a CR was 8.1 months (range 0.5-25.4 months). Median overall survival of all patients achieving CR was 23.5 months (range 1.2-26.3 months). The most common side effects were diarrhea, nausea, vomiting, headaches, skin rashes (including palmar-plantar erythrodysesthesia), facial flushing, and liver abnormalities including hyperbilirubinemia and elevations of ALT and/or AST. The most common grade 3 or higher adverse events were rash (including one patient with Stevens-Johnson syndrome), hyperbilirubinemia, and ALT/AST elevations. There were a total of 9 (16%) deaths: 4 (7%) patients died during the first

induction course; 4 (7%) died in the second induction course; and 1 patient died on day 12 of the first consolidation course. Most of the deaths were attributed to sepsis-related complications.
A Phase I/II study combining clofarabine (30 mg/m^2/day for 5 days) and cytarabine (100 mg/m^2/day administered as a continuous infusion for 7 days) was conducted by Foran et al (44) in 4 patients (age range 61-77) with newly diagnosed AML . Two patients achieved a CR (50%), and 2 patients died of infectious complications. Due to the 2 deaths, the protocol has been amended to decrease the dose of clofarabine by 25% (22.5 mg/m^2/day for 5 days).
Faderl et al (45) studied the efficacy of clofarabine plus low-dose, subcutaneous cytarabine (LDAC) vs clofarabine in patients ≥60 years (median age 71, range 60-83) with untreated AML and high-risk MDS. Patients were randomized to an induction cycle (maximum of 2 cycles) of either 30 mg/m^2 of clofarabine for 5 days or 30 mg/m^2 of clofarabine for 5 days with 20 mg/m^2 of LDAC for 14 days. Consolidation courses (maximum of 12) could be given every 3 weeks to 6 weeks with the same daily dosages given for fewer days (clofarabine for 3 days and LDAC for 7 days). Most patients (55%) had an antecedent hematologic disorder (MDS, chronic myleomonocytic leukemia, non-Hodgkin's lymphoma) or other malignancy, and 50% of patients had abnormal cytogenetics. Of the 70 evaluable patients, 39 patients achieved a CR and 2 a CRp for an overall response rate of 59%. The difference between the response rates between the 2 treatment arms was statistically significant (P≤0.05) in favor of the clofarabine with LDAC group. Most adverse events were less than grade 2. The following adverse events were grade 3 or higher in one or both of the study groups: diarrhea, hyperbilirubinemia, mucositis, elevated SGPT and SGOT, skin rashes, acute renal failure, elevated creatinine, fatigue, atrial fibrillation, and elevated alkaline phosphatase. Fifteen patients (21%) died during induction. Myelosuppression was universal and myelosuppression-related infectious complications were common.
A Phase I, non-randomized, dose escalation study involving 37 elderly AML patients (median age 67 years) in 5 different combination dosing cohorts was conducted by Burnett et al (46). All patients received daunorubicin 50 mg/m^2 on days 1, 3, and 5 and either 15, 20, 25, or 30 mg/m^2 of clofarabine on days 1-5. Dose limiting toxicities at clofarabine 30 mg/m^2 plus daunorubicin 50 mg/m^2 were oral toxicity, renal toxicity, diarrhea, cardiac toxicity, and increased bilirubin. Once the MTD for the two dose combination was determined, gemtuzumab was added, and the MTD for the three drug combination was determined to be clofarabine 20 mg/m^2 days 1-5 plus daunorubicin 50 mg/m^2 days 1,3, and 5 plus gemtuzumab 3 mg/m^2 day 1. The overall response rate for all dose levels combined was 65% (CR or CRi), with 3 out of 5 CRs at the MTD for the 3 drug combination.

5.2.2.5 Combination Therapy with Clofarabine in Previously Untreated and Salvage Adult AML

Agura et al (47, 48) conducted a single center, Phase II study of combination therapy with clofarabine and intermediate dose cytarabine in 30 treatment-naïve elderly patients with heart disease or

relapsed adult AML patients at high risk of anthracycline toxicity. Eligible patients received 5 consecutive days of clofarabine 40 mg/m^2 over 1 hour followed by 1000 mg/m^2 of cytarabine four hours later. Patients also received IV fluids at 150 mg/m^2/h, bumetanide 2-4 mg per day as needed to maintain initial body weight (≤1 kg of the starting weight), and dexamethasone 10 mg intravenous push daily as part of their chemotherapy regimen. Retreatment with clofarabine was allowed up to a total of 4 treatment cycles.

Thirty patients enrolled with a mean age of 64 years (range 38-82 years). At baseline, 13 patients (43%) had a history of cardiovascular disease (previous myocardial infarction, bypass grafting and/or cardiomyopathy). Twenty-nine of the 30 patients received at least 1 cycle (1 patient died within 24 hours of therapy initiation due to disease progression); 5 patients received 2 clofarabine cycles (48). A histological response was seen in 68% of evaluable patients (17/25; 5 patients were deemed inevaluable due to early death), including CR in 14 patients (56%) and PR in 3 patients (12%). Twenty-seven patients were found to have intermediate or unfavorable cytogenetics, and only 1 patient possessed favorable cytogenetics at baseline. Within the intermediate or unfavorable population, 13 patients achieved a complete cytogenetic remission. Adverse events reported in more than 10% of patients included: grade 3 or 4 neutropenia (100%), grade 3 or less edema (70%), diarrhea (60%), grade 3 or less rash (57%), nausea (40%), elevated transaminases (33%), mucositis (20%), headache (20%), atrial fibrillation/flutter (17%), and elevated bilirubin (10%). There were no case reports of treatment-related cardiac toxicity (48).

5.3 Recently completed studies by HOVON/SAKK in adults (18-60 yr age) with AML

The most recent study of the HOVON and SAKK cooperative groups (ie, HOVON/SAKK AML 42) in previously untreated adult patients with acute myeloid leukemia (AML) has accrued approximately 1000 patients of whom 640 patients took part in the granulocyte-colony stimulating factor (G-CSF) priming study. The evaluation of G-CSF priming in induction in the earlier HOVON/SAKK AML-29 study, being the predecessor of the AML-42 study, had shown a favourable effect of G-CSF priming on the risk of relapse following complete remission as well as an improvement of disease free survival (55). In a subgroup analysis the benefit was particularly apparent as an advantage in intermediate risk AML (ie, in 72% of patients) related to improved overall survival (OS), event free survival (EFS) as well as disease free survival (DFS). However the good-risk and poor-risk subgroups counted relatively small numbers of accrued cases prohibiting a more robust analysis as regards the value of G-CSF priming.

The randomization for induction as regards the G-CSF priming question of the HOVON 29 study has been carried over to the successor HOVON 42 study of which the results are expected end of 2009. Therefore at this time it is not yet possible to definitely settle the value of G-CSF priming in induction but as soon as the results of the latter randomisation will become available it can be decided whether G-CSF priming will become a standard component of remission induction therapy in adults with AML.

Other questions addressed by HOVON/SAKK in the adult age group of age less than 60 years involved the dose of cytarabine in induction and autologous SCT as postinduction therapy. In patients of age 60+ other questions were addressed (see also 5.4.3).

5.4 This new HOVON/SAKK study in adults with AML: rationale and design

5.4.1 Remission induction therapy and rationale

The backbone of 2 cycles of remission induction chemotherapy followed by a third consolidation cycle of chemotherapy (cycle III) or autologous SCT or allogeneic stem cell transplantation (alloSCT) has been stable. Also the choice of the chemotherapeutic regimen of cycles I-III has been maintained. In the successive HOVON/SAKK AML-4, -29 and -42 studies a new agent has been added with the objective of improving the efficacy of the induction treatment. The approach has been to embark first on a feasibility phase when the new agent was introduced in the frontline of treatment and then secondly, following confirmation of feasibility, proceed towards the full prospective phase III study. A similar stepwise approach has been pursued in the parallel studies in AML in patients of older age where for instance in the recent study AML-43 two dose levels of daunomycin were compared in induction cycles I and II.

Clofarabine, a promising new anti-leukemic agent (see above) will be added as an intravenous injection for 5 days to standard induction cycles I and II. The drug is currently used at 40 mg/m^2 as monotherapy in patients of age less than 60 years with AML and in combination at 20-30 mg/m^2. The launch of the new study will begin with a feasibility part A in which the additive effect of Clofarabine will be tested at three carefully selected dose levels. Depending on the outcome of part A of the study and after selection of the feasible dose level (10 mg/m^2, 15 mg/m^2 or 20 mg/m^2), the part B of the study, ie the phase III of the study, will be opened. The study will be undertaken as a controlled trial. The study will directly compare the clinical value of standard remission induction chemotherapy cycles I and II with the same treatment to which Clofarabine is added on days 1-5 of both cycles.

5.4.2 Post induction therapy: allogeneic stem cell transplantation (alloSCT)

Following an analysis of alloSCT in the respective AML-4, AML-29 and AML-42 HOVON/SAKK studies, disease free survival (DFS) appeared significantly better for alloSCT in both intermediate risk and unfavourable risk AML. In addition, a meta-analysis was performed using the combined dataset of the HOVON/SAKK, MRC, EORTC and BGMT studies. The findings from the latter four studies were highly concordant with a strong reduction of relapse of approximately 50% in each study and in each of the cytogenetic subcategories. Furthermore, in the meta-analysis a statistically significant OS benefit of 12% for all patients without favourable cytogenetics was demonstrated. While relapse was also reduced in patients with a favourable risk profile, a counterbalancing non-relapse mortality (NRM)

or transplant related mortality (TRM) of approximately 20% precluded a positive effect of alloSCT on survival. Therefore, alloSCT depending on donor availability will generally remain the consolidation therapy of choice for patients with AML of intermediate risk and for patients with AML of unfavourable prognostic risk (i.e. poor and very poor-risk in the new study), but it deserves to be reassessed for low risk patients in the context of risk adapted strategies (see below). Similarly to the different risk factors that predict for relapse, TRM as such is also subject to a number of risk factors.
Which prognostic factors that siginificantly predict for higher TRM should be taken into account? About 10 years ago, five key factors were identified (Gratwohl et al, Lancet:1998). They include stage of the disease, age of the patient, time interval from diagnosis to transplant, degree of histocompatibility and donor recipient gender combination. They form since the basis for the EBMT risk score. Initially, the risk score was established and validated in several independent series of CML patients. Moreover, the score was recently also validated in patients with AML (Gratwohl et al Cancer 2009).
Recently, the Seatlle group has also developed a risk score, the so-called hematopoietic cell transplantation comorbidity index (HCT-CI) based on the number of co-morbidities (Sorror et al Blood 2005, 2006). The score was also validated in AML and indicated a TRM of approximately 10-15% in patients without co-morbidities, a TRM of 15-25% in patients with scores 1-2, and a TRM of > 35% in patients with a score ≥ 3.
The current study will apply both the EBMT and Seattle HCT-CI scores.

Applicability

AlloSCT has an important role, not only as postinduction therapy for patients in first complete remission. It is the most potent salvage therapy for patients with refractory disease and for patients with relapse. The consequences are clear.
Timing of the transplant becomes as important as the decision to proceed or not to proceed to alloSCT. In order to plan a deliberate risk adapted strategy, alloSCT has to be discussed with the patient at diagnosis. A donor search has to be initiated, if alloSCT is considered as an option. Donor search includes the search within the family and subsequently an unrelated donor search, if no HLA- identical sibling donor is found or if the potential donor is not eligible for medical or personal reasons. Recent data suggest, that well matched unrelated donor transplants (10/10 antigen, A-, B-, C-, DR- and, DQ- matched) provide similar outcome as HLA- identical family donor transplants (Schetelig et al, JCO 2008). Cord blood stem cells with sufficient matching criteria and cell numbers might also be considered in experienced centres for selected poor-risk or very-poor risk patients.
In order to be able to proceed to unrelated donor alloSCT shortly after the second course of chemotherapy, the search for a related and subsequently unrelated donor should be performed shortly after diagnosis and initiation of chemotherapy (first cycle). Therefore, decisions should be

based on an estimation of the risk of the underlying leukaemia and a careful estimation of the risk of TRM, based on the EBMT-score and the Seattle co-morbidity-score (HCT-CI).

AlloSCT in first CR

Based on the above mentioned considerations and data it is proposed in the current study to proceed to alloSCT in general if the risk of TRM can reliably be estimated as follows. This refers to patients with a related HLA- matched family donor or an unrelated donor. A 10/10 HLA-A, B-, C-, DR-, DQ-matched unrelated donor is considered equivalent to an HLA identical sibling donor.
In patients with good-risk AML and those with intermediate risk with an a priori risk of relapse of maximally 40-50% the risk of TRM should be carefully assessed at preferably less than 20%. On the other hand in the poor risk and very poor risk categories with a relapse risk of 70% or more, obviously a greater TRM can be accepted. In addition the quantitative survival advantage following alloSCT should be significant enough to compensate for the reduced quality of life that follows an alloSCT in comparison to autoPBSCT. For further information see 7.2.2.

AlloSCT for relapsed disease

Patients with relapse will, by definition, be off study. No formal guidance is given in this protocol. In general, the decision between transplant and non-transplant strategy should be based on a similar risk assessment: risk of the transplant and risk of the disease. The choice of therapy might be different for a patients with a good risk disease, according to the HOVON score (Breems et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse, J.Clin. Oncol. 2005, 23, 1969-1978) and a high risk donor, than for a patient with a high risk disease and a low risk donor.

Patients older than 60 years, application of reduced intensity conditioning

For patients above age 60 years in first CR with an identified HLA- matched related or unrelated donor, the EBMT protocol will apply. Patients will be randomised to alloSCT in first CR or the conventional non-alloSCT therapy (see protocol). Information and donor search at diagnosis will be identical as for all other patients. Recipients of alloSCT will receive reduced intensity conditioning (RIC) as developed in Seattle. Currently, many centers apply RIC also in patients in between 40 and 60 years of age or, alternatively, in patients for whom a greater TRM is anticipated. The current protocol leaves the type of conditioning at the discretion of the individual centers.

5.4.3 Post induction therapy: autologous peripheral blood stem cell transplantation (autoPBSCT)

For patients with intermediate-risk or unfavourable risk AML who have no suitable donor available or who for medical reasons will not proceed to alloSCT, autologous PBSCT following high-dose cytotoxic therapy will be the treatment of choice. This is based on the arguments that some studies have

reported improved disease free survival following autoPBSCT (51, 52). In this respect we note that the question as regards the clinical value of autoPBSCT in AML first remission is not definitely settled. Results from different studies do not demonstrate a consistent benefit but suggest that autoPBSCT provides better or at least as effective therapy (in terms of DFS/reduced relapse rate) than chemotherapy for consolidation. The final analysis of the large randomized study of autoPBSCT versus 3rd cycle of chemotherapy (within HOVON/SAKK- 29 and 42 studies) is planned for 2009. The interim analysis done in 2007 on 500 patients showed a significantly better disease-free survival in favour of the autoPBSCT arm but no significant advantage as regards overall survival. AutoSCT will be employed as a backbone consolidation therapy in the current protocol (51, 52).

5.4.4 Postinduction therapy: third cycle of chemotherapy

A third cycle of Mitoxantrone/Etoposide chemotherapy will be applied to all patients unless they proceed to allo/autoSCT (eg due to insufficient transplant collection, medical contraindications).

5.5 Cytogenetic prognostic markers and use of the autosomal Monosomal karyotype

Patients with the socalled core binding leukemias (ie AML with translocation t(8;21)(q22;q22) or inv(16)(p13;q26)/t(16;16(p13;q26)) (1, 6) have a relatively favourable prognosis (6, 7, 8, 36). Patients with deletions of chromosomes 5 and 7 or deletions of their long arms (-5, 5q-, -7, 7q-) and patients with complex karyotypes (eg those with 3 unrelated clonal cytogenetic abnormalities) have been shown to have a poor prognosis (6, 7, 8). A recent HOVON/SAKK study has challenged the latter distinction and has proposed an improved prognostic score based upon the socalled monosomal karyotype (MK) (54).

In the latter study cytogenetics and survival were analysed in 1,975 patients with newly diagnosed AML aged 15-60 years entered onto four successive HOVON/SAKK trials. When two or more autosomal chromosomal monosomies were present (n=116), the OS estimate at 4 years was 3%±2 only (‘very poor’). Hyperploidy or loss of sex chromosomes (-X,-Y) did not impact on prognosis. Structural chromosomal abnormalities added prognostic value to the poor outcome only in association with a single autosomal monosomy (OS 4%±2 versus 24%±7; ‘very poor’). These observations resulted in the proposed chromosomal 'monosomal karyotype' for predicting prognosis of AML. MK refers merely to the presence of two or more distinct autosomal chromosome monosomies or one single autosomal monosomy in the presence of one or more structural chromosomal abnormalities. In a direct comparison MK provides better prognostic prediction than a traditionally defined complex karyotype of at least 3 unrelated chromosomal abnormalities. The 'MK' enables (in addition to CN and CBF) the prognostic classification of 2 new cytogenetic aggregates of AML with chromosome abnormalities that includes an unfavourable-risk 'MK' negative (MK-) group of AML (OS 26%±2) and a very-unfavourable risk category that is 'MK' positive (MK+) (OS: 4%±1).

In addition the abn3q26 (ref 15) and t(6;9)(p23;q34) appear to confer a profound negative effect on prognosis (55).

5.6 Molecular diagnostics and genetic markers

Besides cytogenetics, age expresses independent prognostic value. In order to refine the prognostic predictive value of these classifications, additional parameters will need to be introduced into these models. One common prognostic parameter has been the rapidity of attaining a CR. Patients achieving a CR following the first induction cycle of chemotherapy (early CR) have a significantly better outcome than those with a CR attained after induction cycle II (late CR) especially in patients with AML with normal cytogenetics.(7) High white blood cell counts (WBC), when considered in combination with favorable cytogenetics, recognize an unfavorable subset among t(8;21) AML(8), and also among patients without cytogenetic abnormalities. More recently, various new molecular markers have been identified that allow for dissecting these composite risk categories. For instance, FLT3-IND (FMS-like tyrosine kinase 3-internal tandem duplications) have been recognized as a remarkably common genetic abnormality in AML. FLT3-ITD represent activating mutations of the FLT3, a hematopoietic receptor. AML with FLT3-ITD are seen in 15-30% of pediatric and adult patients. FLT3-ITD are associated with significantly greater risk of relapse and reduced survival in some studies, while certain other studies with large numbers of patients did not reproduce the prognostic value of FLT3-ITD for survival which makes the established value of FLT3-ITD as a prognostic marker questionable. Interestingly, FLT3 mutations are mainly seen in the largest AML category of intermediate cytogenetic risk. Hence, detection of FLT3-ITDs offers an important possible addition to recognize a new subset of poor risk AML (9,10). Another recurrent Asp835 point mutation of the FLT3 receptor, seen in approximately 5-10% of de novo AML, has not (or not yet) been correlated with prognosis. NPM 1 (Nucleophosmin 1) mutations have been identified for the first time in 2004 (11,12,13). They are prevalent in a high number of approximately 35% of cases of AML and these define a more favourable subset, especially among the subset of FLT3-ITD AMLs (eg 11, 12,13). EVI-1 (ecotropic virus integration site 1) is an oncogene overexpressed in AML with translocations of 3q26 and characterizes a notoriously poor risk AML (14). Recently it was shown that EVI-1 mRNA overexpression in AML in the absence of 3q26 cytogenetic abnormalities also predicts for notably bad prognosis (15). Some of these AML appear to carry cryptic 3q anomalies. Thus EVI-1 appears to define an intracellular pathway of poor therapy response in approximately 10% of cases.
C/EBPA (CCAAT enhancer-binding protein alpha) is a transcription factor that has a key role in myelopoiesis. C/EBPA mutations have been found in patients with AML in a few percent of cases. The latter mutations define AML with relatively good risk leukemia (20, 21, 22). Point mutations of the hematopoietic receptor c-*KIT* are seen in 30% of patients with abn(16) AML and t(8;21) AML. AMLs with abn(16) and t(8;21) represent leukemias of favourable prognosis. The presence of c-*KIT* mutations among this subgroup defines those with an enhanced risk of recurrence (23). The research

area of molecular prognostic factors in AML is evolving fastly. Some other potential markers of possible interest (eg ERG, BAALC, WT1) were briefly introduced in paragraph 5.1
Many of these genetic aberrations have been investigated in retrospective analysis so that the selection of cases that has entered these analyses has not been clarified. These markers will now need to be evaluated as regards their prognostic impact in multicenter prospective studies.

5.7 Gene expression profiling on DNA microarrays and diagnostics in AML

In approximately 40% of cases of AML no cytogenetic or molecular markers have been discovered yet. Although investigators will continue their search for genetic aberrations in AML, novel techniques and molecular approaches will be developed and made instrumental for disclosing the genetic variations in AML more fully. Particularly gene expression profiling (GEP) can be expected to add an essential and indispensable integrated element in the diagnostic and therapeutic decision making process in the foreseeable future (39-43).
Validation of the high-throughput gene expression approach in prospective studies would be important for head-to-head comparison with other concurrent molecular and cytogenetic diagnostic methods as well as for assessing the prognostic value of unique expression signatures. Gene expression profiling will also serve the objective of defining the minimal sets of predictor genes for certain prognostically defined AML subclasses.

5.8 Risk Group Classification in Current Study

Risk		Definition
Good	GR1 GR2 GR3 GR4	t(8;21) or *AML1-ETO*, WBC≤20 inv16/t(16;16) or *CBFB-MYH11* MK-, CEBPA+ MK-, FLT3ITD-/NMP1+, CRe
Intermediate	IR1 IR2	t(8;21) or *AML1-ETO*, WBC>20 CN –X –Y, WBC≤100, CRe
Poor	PR1 PR2 PR3	CN –X –Y, WBC≤100, not CRe CN –X –Y, WBC>100 CA, non CBF, MK-, no abn3q26, EVI1-
Very Poor	VPR1 VPR2 VPR3	Non CBF, MK+ Non CBF, abn3q26 Non CBF, EVI1+

For further explanation please see appendix D

5.9 Assessment of minimal residual disease (MRD)

The term minimal residual disease (MRD) refers to the ‘occult’ low amount of leukemia that may persist during remission in the absence of clinical or haematological evidence of disease. MRD

detection in acute myeloid leukemia (AML) using PCR based techniques for molecular markers is applicable only in a minority of cases. Recently, the level of minimal residual disease (MRD) was established as a prognostic factor that predicts relapse. Immunophenotypical detection of MRD is based upon the presence of leukemia-associated immunophenotypes (so called LAP's,), which are unusual or aberrant immunophenotypes that distinguish leukemic cells from normal hematopoietic cells. LAP's refer to cross-lineage antigen expression (eg the expression of lymphoid markers on myeloid cells), the asynchronic antigen expression (eg the coexpression of early markers with mature myeloid markers), overexpression of antigens (eg relatively high expression levels of particular myeloid or lymphoid markers), and/or ectopic expression (eg the expression of particular antigens that normally are not expressed on hematopoietic cells). The method of detection MRD is quite easy to perform and is sensitive, with a detection ability of 1 malignant cell among 1,000 to 10,000 normal cells, but it requires detailed immunophenotypical knowledge of normal bone marrow cell differentiation. Bone marrow (BM) after different courses of therapy (61-67), stem cell transplants (68) and sequential follow-up bone marrow sampling (69) have been used for MRD assessment.
For this study, BM and blood samples will be obtained from all patients at diagnosis, at the end of the first and second cycles of induction therapy, in complete remission (CR) and at relapse (see Appendix G).

6 Study objectives

6.1 Primary objectives

For part A of the study:

- To determine the feasibility of Clofarabine when given at three possible dose levels together with standard induction cycles I and II in patients with AML/ RAEB with IPSS≥1.5 in a prospective comparison to standard induction cycles I and II without Clofarabine.

For part B of the study:

- To evaluate the effect of Clofarabine at the selected feasible dose level when combined with remission induction chemotherapy cycles I and II as regards clinical outcome ("event-free survival") in comparison to remission induction cycles I and II with no addition of Clofarabine in a phase III study.

6.2 Secondary objectives

For part A of the study:

- To investigate the clinical efficacy of Clofarabine in combination with remission induction chemotherapy cycles I and II with regard to complete remission rate at different dose levels of Clofarabine.

For part B of the study:

- To investigate the clinical efficacy of Clofarabine with regard to the complete remission rate, disease free survival (DFS), risk of relapse and overall survival (OS) when combined with remission induction chemotherapy cycles I and II in all patients.
- To investigate the clinical efficacy of Clofarabine when combined with remission induction chemotherapy cycles I and II in molecularly and cytogenetically distinguishable subsets with regard to the complete remission rate, disease free survival (DFS), risk of relapse and overall survival (OS).
- To investigate the tolerance and toxicity of Clofarabine in combination with remission induction chemotherapy cycles I and II.
- To assess the effect of Clofarabine on peripheral CD34 cell numbers for autologous peripheral blood transplantation.
- To determine the prognostic value of molecular markers and gene expression profiles of the leukemia assessed at diagnosis.
- To evaluate the treatment effects according minimal residual disease (MRD) measurements following therapy by standardized sampling of marrow/blood.
- To evaluate the outcome of allogeneic sibling or unrelated donor SCT and autologous SCT in cytogenetically and molecularly defined prognostic subgroups of patients.

7 Study design and rationale

Part A: A prospective feasibility study of remission induction chemotherapy combined with Clofarabine at a maximum of 3 dose levels (10, 15, 20 mg/m^2).
Part B: Subsequent to completion of the feasibility study (part A), the value of Clofarabine at the selected dose level when combined with standard induction chemotherapy will be investigated in a phase III randomized study.

The choice of the chemotherapeutic regimens of remission induction cycles I and II has been maintained during the successive HOVON/SAKK AML-4, -29 and -42 studies. In each of these trials a new additional agent has been supplemented to the latter two cycles with the objective of improving the efficacy of the induction treatment (eg less relapse, better DFS). The approach that has been pursued, was to embark on an initial feasibility phase when the new agent was introduced in the frontline of treatment and following the evaluation of feasibility part of the study proceed towards the full prospective phase III study. A similar stepwise approach has been pursued in the parallel studies in AML in patients of older age where in the recent study AML-43 two dose levels of daunomycin were compared in induction cycles I and II.

Clofarabine is a promising new agent that will be added as a 1 hour infusion to standard induction cycles I and II. The drug has been used as monotherapy and in combination chemotherapy in relapsed AML and in elderly patients with AML. The launch of this new study will begin with a feasibility part A in which the additive effect of Clofarabine will be tested at three selected dose levels. After final selection of the feasible dose level (10 mg/m^2, 15 mg/m^2 or 20 mg/m^2 for 5 days), the part B of the study (i.e. the phase III of the study) will be opened. This randomized study will directly compare the clinical effects of standard remission induction chemotherapy cycles I and II with the same treatment to which Clofarabine is added for 5 days to both induction cycles I and II.

7.1 Part A vs Part B

7.1.1 Part A: Dose selection

Although Clofarabine has been administered to patients with acute leukemia and found to be feasible, it is unknown whether this particular combination of Clofarabine and induction chemotherapy will lead to other or earlier toxicities. For this reason the drug will now need to be taken to the relevant setting of upfront treatment of newly diagnosed patients. The study will start with cohort 1 of 10 mg/m^2. Decisions regarding feasibility and dose escalation to the next cohort, continuation or stopping are based on the incidence of DLT (Dose Limiting Toxicity) and the duration of myelosuppression, and will be performed according to the rules defined in chapter 17. The National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events v 4.(CTCAE) will be used to grade toxicities.

Incidences of DLTs in patients treated on both study arms will be compared. DLTs are defined as follows:

- Death
- Any non hematological toxicity CTCAE grade ≥ 4

occurring within 30 days after start of cycles I or II and before the start of the next cycle or a new treatment respectively.
In addition, the duration of myelosuppression defined as the median time to recovery of ANC >0.5x10^9/L will be assessed. DLT and myelosuppression will be used in the decision process for dose escalation, dose reduction and/or dose selection.

If known at randomisation, patients with a good risk profile of AML will be excluded from randomisation in part A of the study in order to avoid unexpected toxicity in those patients.

7.1.2 Part B: Efficacy

Following final dose selection a total number of 800 patients also including all risk subgroups of AML will be randomized to receive the selected dose Clofarabine plus idarubicin/cytarabine or the combination of idarubicin/cytarabine without the study drug Clofarabine (control) in cycle I and amsacrine/cytarabine with or without clofarabine in cycle II. For details see also chapter 17 for statistical considerations.

7.2 Treatment design

7.2.1 Remission induction treatment

The study is designed as a randomized study in which patients will be randomized to receive idarubicin-cytarabine alone or idarubicin-cytarabine combined with intravenously administered Clofarabine (respective study arms A and B - cycle I) and amsacrine-cytarabine alone or amsacrine-cytarabine plus Clofarabine (cycle II). Patients with newly diagnosed AML (except acute promyelocytic leukemia), or RAEB with IPSS ≥1.5 (see appendices A and B), meeting all eligibility criteria will be included.

7.2.2 Post remission treatment according to risk assessment

All patients will receive 2 cycles for induction and then according their prognostic risk assessment (see section 5.4.2 and 5.8) proceed to postremission therapy (chemotherapy cycle III, autoSCT or alloSCT).

Patients in CR, unless they proceed to alloSCT, will undergo stem cell mobilization with G-CSF and stem cell collection after the second induction cycle. Patients with an adequate harvest who fullfill the eligibility criteria will proceed to busulfan-cyclophosphamide ablation + autoPBSCT.

All other patients (considered not eligible for an alloSCT or autoPBSCT) will be offered the chemotherapy cycle III: mitoxantrone and etoposide.

- *Good risk* (GR) patients will receive a third cycle of chemotherapy (cycle III: mitoxantrone plus etoposide). In case the TRM risk following alloSCT is estimated to be very low (≤ 10-15%), alloSCT can be considered. This applies to patients with an optimal donor, with an EBMT risk score of 0 or 1 and a comorbidity score of 0.
- *Intermediate risk* (IR) patients with a HLA matched related or 10/10 molecular matched unrelated donor will proceed to alloSCT if the risk of transplant related mortality of such a transplant is assessed to be less than 20%. This applies to patients with a sibling or a 10/10 matched unrelated donor with an EBMT risk score ≤ 3 and a HCT-CI ≤ 2, If such a transplant is not elected, the autologous transplantation is the second choice. If this is not possible, as the third choice patients will receive a third cycle of chemotherapy (cycle III: mitoxantrone plus etoposide).

- *Poor risk* (PR) patients with a HLA matched related or unrelated donor may proceed to alloSCT as soon as they have entered CR provided the risk for TRM risk < 40 %. This applies to patients with an EBMT risk score ≤ 5. If patients are identified as poor risk following cycle I and logistically there are no impediments, the patient may proceed to alloSCT as soon as possible after cycle I. If an alloSCT is not possible or elected, an autologous transplant is the second option and a third cycle of chemotherapy is the third option
- *Very poor risk* (VPR) patients with a HLA matched related or unrelated donor may proceed to alloSCT as soon as they have entered CR. This applies to patients with an EBMT risk score ≤ 5 and irrespective of HCT-CI. Also, a 9/ 10 unrelated donor, a haploidentical family donor (preferably the mother) or a cord blood with sufficient matching and cell numbers can be considered in experienced centres. If patients are already distinguished as very poor risk following cycle I and logistically it would be possible to immediately proceed to alloSCT at that point, the alloSCT might be planned as soon as possible after cycle I. If an alloSCT is not possible, an autologous transplant is a second and a third cycle of chemotherapy is a third possible option

For further information on risk group definition see appendici E and F.

Comment 1: The choice of the preparative regimen prior to alloSCT and the schedule of immunoprophylaxis of graft-versus-host disease will follow the local institutional protocols.
For centres with no institutional research protocol, standard conditioning with cyclophosphamide/intravenous busulfan and standard GvHD prophylaxis with cyclosporine/short methotrexate is recommended.

Comment 2: It is recommended to start a search for a donor for alloSCT and to get in contact with a transplant centre at diagnosis.

7.2.3 Patients with refractory disease

If patients fail to achieve remission after two cycles of induction chemotherapy, alloSCT from an related or unrelated matched donor might be considered in selected circumstances, but provided at least the transplant can be undertaken following ablative conditioning, a CRi (complete marrow remission without incomplete haematological recovery) can be accomplished pretransplant and the clinical conditions permit to do so. Also, a 9/ 10 unrelated donor, a haploidentical family donor (preferably the mother) or a cord blood with sufficient matching and cell numbers can be considered in selected centres.

8 Study population

8.1 Eligibility criteria

All patients must be randomized before start of treatment and must meet all of the following eligibility criteria.

8.1.1 Inclusion criteria

- Age 18-65 years, inclusive
- Subjects with
  - a cytopathologically confirmed diagnosis of AML according WHO classification (excluding acute promyelocytic leukaemia) **or**
  - a diagnosis of refractory anemia with excess of blasts (RAEB) and IPSS score ≥1.5 **or**
  - patients with therapy-related AML/RAEB **or**
  - patients with biphenotypic leukemia (Appendices A1 and A2).
- 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)$^{-0.203}$ 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
- WHO performance status 0, 1 or 2 (see Appendix I)
- Written informed consent

8.1.2 Exclusion criteria

- Acute promyelocytic leukaemia
- Previous treatment for AML or RAEB, except hydroxyurea
- Concurrent history active malignancy in two past years prior to diagnosis except for:
  - basal and squamous cell carcinoma of the skin
  - in situ carcinoma of the cervix

- Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, infection, hypertension, pulmonary disease etcetera),
- Cardiac dysfunction as defined by:
  - Myocardial infarction within the last 6 months of study entry, or
  - Reduced left ventricular function with an ejection fraction < 50% as measured by MUGA scan or echocardiogram (another method for measuring cardiac function is acceptable), or
  - Unstable angina, or
  - Unstable cardiac arrhythmias
- Pregnant or lactating females
- Unwilling or not capable to use effective means of birth control

9 Treatment

9.1 Dose level of Clofarabine

9.1.1 Part A

Patients will receive the induction treatment cycles as described in section 9.2 and 9.3. In part A of the study Clofarabine is started at a dose level of 10 mg/m^2. If escalation is possible according to the decision rules as described in section 17, a dose level of 20 mg/m^2 is evaluated. If 20 mg/m^2 is not feasible, the intermediate dose level of 15 mg/m^2 is evaluated. If 15 mg/m^2 is not feasible either, we return to 10 mg/m^2. If 10 mg/m^2 is not feasible the trial will be closed.
The dosage is based on the patient's body surface area (BSA), calculated using the actual height and weight before the start of each cycle.

9.1.2 Part B

The Clofarabine dose level for part B is selected in part A.

9.2 Remission induction treatment cycle I

Arm A (standard of care – comparator arm): Idarubicin and conventional-dose Cytarabine (Ara-C)

Time	Agent	Dose/day	Route of administration	Days
13.00	Idarubicin	12 mg/m^2	3 hr infusion	Days 1, 2, 3
13.00 9.00	Cytarabine (Ara-C)	200 mg/m^2	18 hr infusion 24 hr infusion	Days 1 thru 5 Days 6 and 7

Arm B (investigational arm): Idarubicin and conventional-dose Cytarabine (Ara-C) plus Clofarabine.

Time	Agent	Dose/day	Route of administration	Days
9.00	Clofarabine	10,15 or 20 mg/m^2, see section 9.1)	1 hr infusion	Days 1 thru 5
13.00	Idarubicin	12 mg/m^2	3 hr infusion	days 1, 2, 3
13.00 9.00	Cytarabine (Ara-C)	200 mg/m^2	18 hr infusion 24 hr infusion	days 1 thru 5 days 6 and 7

Cytarabine (Ara-C) to be dissolved in 500 mL 0.9% NaCl or 5% glucose (D5W).
Idarubicin, in vials of red orange lyophilized powder, containing 5 mg or 10 mg, to be dissolved in 1 ml sterile water per mg Idarubicin.
Clofarabine will be administered in a 1 hour infusion diluted in 0,9% NaCl or Normal Saline or 5% dextrose injection (D5W) through a freely flowing separate iv line or separate iv port lumen if a central line is in place. The administration will begin 4 hours before the start of the cytarabine infusion to have an optimal effect on the production of the Ara-C triphosphate metabolite and at a 2-hour interval after the end of the 18-hr cytarabine infusion to avoid pharmacokinetic interfence of renal excretion.

Assessment of response after Cycle I is described in 11.3.2.
Patients in CR who have already been identified as belonging to the poor and very poor risk groups (Appendix D) and for whom an allogeneic donor is available and in whom from a practical point of view an early SCT can be undertaken, have the option to proceed to AlloSCT already after cycle I.
Patients assessed as RD, should start as soon as possible with cycle II.
All other patients continue with cycle II according to 9.3.

9.3 Remission induction treatment cycle II

Cycle II will be started as soon as possible when the marrow still shows more than 15% blasts at day 17-21 (with day 1 being start cycle I), and also in case of blasts of less than 15% but with hematopoietic regeneration (platelets above 100x10^9/L; ANC > 1.0x10^9/L). If the hematopoietic regeneration takes more than 56 days, Clofarabine will not be given in cycle II in arm B. A bone marrow evaluation should be done before cycle II is started.

Arm A (standard of care – comparator arm): Amsacrine and intermediate-dose Cytarabine.

Time	Agent	Dose/day	Route of administration	Days
9.00	Amsacrine	120 mg/m^2	1 hr infusion	days 4, 5, 6
10.00 and 22.00	Cytarabine (Ara-C)	1000 mg/m^2 q 12 hrs	3 hr infusion	days 1 thru 6 (12 doses)

Arm B (investigational arm): Amsacrine and intermediate-dose Cytarabine plus Clofarabine.

Time	Agent	Dose/day	Route of administration	Days
9.00	Amsacrine	120 mg/m^2	1 hr infusion	days 4, 5, 6
10.00 and 22.00	Cytarabine (Ara-C)	1000 mg/m^2 q 12 hrs	3 hr infusion	days 1 thru 6 (12 doses)
18.00	Clofarabine	10,15 or 20 mg/m^2, see section 9.1)	1 hr infusion,	Days 1 thru 5

Cytarabine (Ara-C) to be dissolved in 500 ml 0.9% NaCl or 5% glucose.

Amsacrine to be dissolved in 500 ml glucose 5% in glass bottles. Contact with plastic syringes or bottles should be avoided.

Clofarabine will be administered in a 1 hour infusion diluted in 0,9% NaCl or Normal Saline or 5% dextrose injection (D5W) through a freely flowing separate iv line or separate iv port lumen if a central line is in place. The administration will begin 4 hours before the start of the evening cytarabine infusion to have an optimal effect on the production of the Ara-C triphosphate metabolite and at a 5-hour interval after the end of the 3-hr cytarabine infusion in the morning to avoid pharmacokinetic interference of renal excretion.

Assessment of response after Cycle II is described in 11.3.2. Patients in CR will proceed to cycle III, autologous SCT or allogeneic SCT depending on their prognostic risk assessments

9.4 Peripheral blood stem cell mobilization and collection

9.4.1 Peripheral blood stem cell mobilization

Granulocyte-colony-stimulating factor (G-CSF) (filgrastim) 5 µg/kg will be given subcutaneously twice daily to all patients after cycle II, i.e., patients being treated in both arms A and B of the study, except in patients who will certainly proceed to HLA matched allogeneic SCT, and except in patients who are already known to be good risk. G-CSF treatment will be started after cycle II chemotherapy at the onset of recovery of granulocytes of 0.5 x 10^9/L or more, and continued until the last day of apheresis. Patients not in CR after cycle I should first have a marrow evaluation. G-CSF for mobilization should not be started and may be terminated prematurely when marrow smears taken after cycle II show clearly persistent leukemia (more than 15% of blasts) or when significant numbers of leukemic blasts appear in the blood. In that instance bone marrow cytology should be examined and leukopheresis for peripheral blood stem cell collection will be cancelled.

9.4.2 Procedure of peripheral blood progenitor cell collection

Timing of apheresis; as soon as PMN begin to rise to values of 2 x 10^9/L or more and significant numbers of CD34 positive blood cells appear, peripheral blood cells will be collected in one to four leukapheresis sessions (i.e., until the collection of at least 5 x 10^6 CD34+ cells/kg). G-CSF will be discontinued following completion of peripheral blood stem cell harvest. If an insufficient total number of cells has been collected, an autologous marrow may be collected or a second PBPC collection may be attempted. If no adequate PBPC or marrow graft can be obtained cycle III will be delivered.

9.4.3 Procedure for hematopoietic cell cryopreservation

Procedure for hematopoietic cell cryopreservation is according to local procedures

9.5 Post induction therapy with chemotherapy cycle III

Patients in continued CR receiving consolidation treatment with cycle III (see section 7.2.2) will receive this treatment as soon as hematopoietic repopulation (platelets > 100x10^9/L and ANC > 1.0x10^9/L) has taken place.

Agent	Dose/day	Route	Days
Mitoxantrone	10 mg/m^2	30 min infusion	days 1 thru 5
Etoposide	100 mg/m^2	1 hr infusion	days 1 thru 5

Mitoxantrone to be dissolved in 100 mL 0.9% NaCl or 5% glucose or 5% dextrose (D5W).
Mitoxantrone is supplied as blue sterile parenteral solution containing 30 mg in 15 mL vials.
VP-16 (Etoposide) to be dissolved in 500 mL 0.9% NaCl immediately prior to use.

No dose modification should be applied. Cycle III can be postponed in case of intercurrent septic or metabolic complications.

9.6 Post-induction treatment: Busulfan-Cyclophosphamide and autologous PBSCT

Patients in continued CR receiving busulfan-cyclophophamide followed by autologous PBSCT (see section 7.2.2) will receive this treatment as soon as hematopoietic repopulation (platelets > $100x10^9$/L and ANC > $1.0x10^9$/L) has taken place.

Agent	Dose/day	Route	Days
Busulfan *or* Busilvex	1 mg/kg q 6 hrs 0.8 mg/kg q 6 hrs	p.o. 2 hr infusion (i.v)	-7, -6, -5, -4 -7, -6, -5, -4
Cyclophosphamide	60 mg/kg	1 hr infusion (i.v.)	-3, -2
Phenytoin	5 mg/kg q 6 hrs	p.o.	-9, -8, -7 thru –4
SCT infusion			0

Busulfan (oral) - 4 mg/kg/day (total 16 mg/kg) divided into q 6 hours (1 mg/kg/dose oral). A 70 kg man will, for instance, receive 280 mg/day or 70 mg q 6 hrs.

Since administration of high-dose busulfan has been temporarily associated with the development of generalized seizures, prophylactic administration of **Phenytoin** (5 mg/kg/dose p.o. q 6 hrs beginning 2 days before the first dose of busulfan (= day -9), then 5 mg/kg/day p.o. daily through day -4) is recommended. Also Diazepam as an anticonvulsant agent may be used.

Cyclophosphamide - (60 mg/kg) will be infused in 500 mL NS (0.9% NaCl) or 5% glucose over 1 hour. Mesnum 300 mg/m^2 will be administered at -10 min prior to cyclophosphamide infusion, +4 hrs, +8 hrs and +12 hrs following Cyclophosphamide infusion on days -3 and -2. Patients will be hydrated with D5'NS (5% glucose, 0.45% NaCl + 20 mEq KCl/L + 5 mg furosemide/L) i.v. at 200 cc/hr for 72 hrs beginning 2 hrs before the first Cyclophosphamide dose. KCl will be further supplemented in case of hypokalaemia. An average urinary flow of at least 100 cc/hr will be maintained during 48 hrs following the beginning of the cyclophosphamide infusion. Furosemide will be added during this period depending on fluid in- and output status. Before Busulfan and Cyclophosphamide infusions, patients will be premedicated with antiemetics

Infusion of stem cells (SCT)

On day 0 all cryopreserved stem cells will be thawed and infused per intravenous route in approximately 15-30 min. depending on the total volume. Please note that the cells are reinfused through a saline infusion set. The graft will be checked for cell count, bacterial and fungal cultures, prior to administration. If the number of cells collected exceeds a value of $10x10^6$ CD34 positive cells per kg, this number will be considered an upper limit and the additional cells will not be reinfused. The empty bottle/bag will be sent for bacteriology.

9.7 Post-induction treatment: allogeneic SCT

Allogeneic SCT will be carried out according to the standard guidelines and general procedures operational in the local allogeneic bone marrow transplantation centers. Details will be documented on the CRF.

9.8 Special management orders

- Before treatment a *central venous catheter* may be placed. As a rule, patients will receive parenteral alimentation, when they have insufficient oral caloric intake.
- Extremely careful hand washing by all members of health care team is required
- Reverse barrier nursing of patients and decontamination of the GI tract will be applied according to local protocols in the various centres. *Antimicrobial prophylaxis* will be continued at least until granulocyte counts have increased to a minimum of $0.5x10^9$/L.
- *Menstruating premenopausal* females will be started on anovulatory drugs; for instance Orgametril (Lynestrenol) 5 mg, if necessary 10 mg, p.o. q.d. or Deproprovera (Medroxy progesterone acetate) 150 mg, if necessary 300 mg i.m., e.g. q 6 wks.
- All *men and pre-menopausal women* should use adequate contraception during the study. Sperm should be frozen before the start of treatment from men who wish to have children.
- *Hematological supportive care* will involve prophylactic platelet transfusions when counts are below $10x10^9$/L (to prevent hemorrhage) as well as therapeutic transfusions when clinically indicated (34). In case of HLA sensitization, patients will receive HLA compatible platelet transfusions whenever necessary. Filtrated packed red blood cells will be given to keep hematocrit above 30%. Irradiation of blood products (25 Gy) must commence from start of cycle II. Otherwise collections of PBPC may contain viable transfused lymphocytes that may cause graft-versus-host disease. After SCT all blood products will be irradiated with 25 Gy.
- Attempts should be made, prior and during chemotherapy, to control any medical problems, such as *metabolic abnormalities and infections*. Electrolytic abnormalities should be controlled. Patients with fever should receive empirical treatment with broad-spectrum antibiotics. They should be adjusted according to the results from the sensitivity studies, whenever a pathogen has been isolated.

9.9 Study drug information

9.9.1 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 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.

9.9.2 Storage and Handling

Vials containing undiluted clofarabine for injection should be stored at controlled room temperature. The commercial expiry period for Clolar (clofarabine) is 36 months at room temperature. Ongoing stability studies will continue to confirm the appropriate quality of drug product used for clinical trials beyond 36 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, diluted in 0,9% sodium chloride.

9.9.3 Special considerations for administration of clofarabine

Clofarabine is excreted primarily by the kidneys. Additionally, the liver is a known target organ for clofarabine toxicity. 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.
In pediatric studies, during or shortly after clofarabine administration a few patients developed signs and symptoms consistent with capillary leak syndrome. In these heavily pretreated patients, it has been difficult to separate potential drug-related cases of capillary leak syndrome from concurrent medical conditions such as infection/sepsis, progressive disease, or other underlying problems resulting from prior antileukemic therapies.
In case of suspected capillary leak syndrome during clofarabine infusion it is recommended to interrupt or hold clofarabine administration as clinically indicated. If the patient's condition stabilizes or improves, clofarabine administration may resume. Pretreatment with steroids (hydrocortisone 100 mg/day) is recommended for all subsequent doses during the remainder of that treatment cycle and for all subsequent treatment cycles.

Clofarabine is moderately emetogenic. Therefore, standard anti-emetic therapy (such as prochlorperazine or a 5HT3 antagonist, or/and dexamethasone) should be administered prior to therapy.

9.9.4 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.

10 End of protocol treatment

Reasons for going off protocol treatment are:

- Completion of protocol treatment (either cycle III or autologous PBSCT or allogeneic SCT)
- No CR after cycle II
- Excessive extramedullary drug toxicity preventing continuation of treatment
- Bone marrow hypoplasia preventing continuation of treatment
- Death
- Relapse after initial CR (i.e., before completion of treatment)
- Lack of patient compliance (especially refusal to continue treatment)
- Major protocol violation
- Pregnancy

11 Required clinical evaluations

Required investigations at entry should be no older than 14 days prior to randomization unless otherwise noted.

All investigations should be recorded in the patient's medical file.

11.1 Required investigations

	Baseline (and before start cycle I)	**Treatment before cycle 2, 3 and/or auto/allo SCT and 1 month after start cycle 3 or auto/allo SCT**	**FU**
Medical history	X	X	X
Adverse events		X	
Physical examination	X	X	X
Hematology	X	X[1)2)]	X[2)]
Blood chemistry[3)]	X	X[4]	X[5)]
Bone marrow aspirate (see 11.3.2 and Appendix G)			
Morphology	X	X[6)7)]	X[7)]
BM immunophenotyping	X		
Cytogenetics	X	X[8)]	X[8)]
Additional blood/marrow sampling for:			
Molecular diagnostics (Appendix G)	X		
Gene expression profiling (Appendix HG)	X		
MRD assessment (Appendix G)	X	X[8)]	X[8)]
Bone marrow biopsy			
Histopathology	X		
Specific investigations			
HLA typing of patient and family	X[10)]		
Coagulation tests	X		
Chest X-ray	X	o.i.	o.i.
ECG	X	o.i.	o.i.
Cardiac ejection fraction	o.i		
Dental examination	o.i		
Virological tests	X		
Microbiological tests	X[9)]		

o.i. on indication

1) Peripheral blood will be collected to record blast %, ANC value and platelet count at the time of each chemotherapy evaluation. These values will be recorded on a CRF.

2) Peripheral blood will also be collected until peripheral blood recovery (three times -weekly), and thereafter according to 11.4, to record recovery of ANC and platelets on a CRF.
3) Complete blood chemistry and coagulation test results are to be collected to record as an adverse event on an Adverse Event form, if applicable.
4) - creatinine, sodium, potassium, uric acid, calcium, glucose twice weekly until discharge;
- AST, ALT, alkaline phosphatase, "γGT, bilirubin (direct and indirect), LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly
5) only creatinine, AST, ALT, Alk. Phos, γ-GT, bilirubin
Blood chemistry should be followed up until resolution of adverse events (at least once-monthly)
6) Bone marrow aspirate for response assessment from day 17 weekly during treatment (see 11.3.2 and Appendix G –Table 1)
7) o.i. and if patient in first CR: at 4, 8, 12, 18, 24, 36, 48 months as long as the patients is in CR
8) Blood and bone marrow sampling according to Table 1 Appendix G Cytogenetics only in case of abnormalities.
9) according to local bacteriology guidelines
10) Family typing in order to search for an HLA identical family donor; proceed to unrelated donor search, if no donor is identified

11.2 Observations prior to start treatment

Study subjects will be screened for eligibility before randomization. The following assessments will be made within 14 days prior to randomization, unless otherwise noted:

- Medical history, including previous chemotherapy or radiotherapy, antecedent hematological or oncological disease, previous exposure to insecticides, prior and present other diseases, fatigue, bleedings, infections
- Physical examination including body weight, height, splenomegaly, signs of extramedullary leukemia, WHO performance status
- Hematology including hemoglobin, platelets, WBC and WBC differential within 3 days prior to randomization
- Blood chemistry, including serum creatinin, urea, sodium, potassium, uric acid, calcium, glucose, bilirubin, AST, ALT, alkaline phosphatase, gamma GT, LDH within 3 days prior to randomization
- Chest X-ray
- Cardiac ejection fraction, measured by MUGA or echocardiogram (another method for measuring cardiac function is acceptable) if clinically indicated
- ECG
- Dental examination and X-ortopantogram if clinically indicated
- Surveillance cultures of throat, stools and urine according to local bacteriological guidelines
- Virology including tests (PCR or serology) for cytomegalovirus (CMV) infection, HIV (human immunodeficiency virus), hepatitis A, B and C
- Coagulation studies including protrombin time (PT), partial thromboplastin time (PTT), fibrinogen
- Bone marrow aspirate for:
  - cytology and cytochemistry to establish WHO and FAB subtype of AML or MDS
  - immunological phenotyping to verify myeloid leukemia and assessment of minimal residual

disease (leukemia associated phenotype) (see Appendix G)
- cytogenetics (cell culture and banding analysis)
- molecular analysis for *AML1/ETO, CBFB/MYH11, BCR/ABL, Flt3-ITD, CEBPA* and *NPM1* gene mutations and *EVI1* expression and if possible other available or emerging interesting genetic markers (mutations of *KIT*, *WT1*, *FLT3*-point mutations, gene expression markers *ERG, BAALC* and other potentially relevant markers),
- whole genome transcriptional profiling (gene expression profiling) (see Appendix G)

♦ Bone marrow biopsy for histopathology in case of dry tap

11.3 Observations during treatment (before start cycles and/or at evaluation previous cycle)

♦ Daily interim history and physical examination, when hospitalized; thereafter as clinically indicated: weight at start cycle, infections, medication given.,

♦ Blood cell count, quantitative platelets daily, and WBC count and differential at least every other day when hospitalized until discharge, thereafter once weekly

♦ Blood chemistry including creatinin, AST, ALT, bilirubin (direct and indirect), LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly for toxicity assessment

♦ Chest X-ray as clinically indicated .

♦ ECG as clinically indicated

♦ Surveillance cultures according to local bacteriology guidelines

♦ Bone marrow aspirate for response assessment see 11.3.2.
Marrow sampling for minimal residual disease assessments see Appendix G.

11.3.1 Toxicity assessment

During and following each cycle, toxicity has to be carefully examined and evaluated. During the clinical phase a daily assesment of toxicities will be performed. After discharge patients will be followed twice weekly and the same investigations will be performed. The toxicity assesment includes the following:

♦ Complete history of symptoms and complaints

♦ Complete physical examination, with special emphasis on neurological symptoms

♦ Laboratory examination of hemogram, electrolytes, liver enzymes and kidney parameters twice weekly

♦ Chest X-ray as clinically indicated

♦ Electrocardiography when indicated

♦ DLT assessment after induction cycle I and II (see 16.2).

Toxicities will be scored according to the most recent version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4 (see Appendix H)

11.3.2 Bone marrow and blood evaluation and response assessment during treatment

After the first treatment cycle, starting at day 17, the response will be assessed by bone marrow aspiration, blood evaluation and extramedullary disease status evaluation (see Appendix C). If and as long as the marrow is not conclusive a new marrow will be taken as clinically indicated, but at least at weekly intervals. If the marrow shows evidence of resistant disease after cycle I, cycle II may be started as soon as possible without waiting for peripheral blood recovery (PBR). In all other cases blood evaluation will be repeated until PBR.

After cycle II response assessment will be after recovery of blood counts, ie usually at day 28 if CR had been attained after cycle I. Otherwise it will be done similarly as after cycle I, i.e. starting at day 17). If and as long as the marrow is not conclusive a new marrow will be taken as clinically indicated, but at least at weekly intervals.

Cytogenetics may be used in patients when karyotypic markers are available to document remission, or when a relapse is suspected (see also Appendix G)
Marrow sampling for minimal residual disease assessments prior to cycle II, prior to cycle III or allo/autoSCT, in autologous transplant and 4 month after cycle III or allo/autoSCT (=after repopulation) will be performed (see Appendix G)

11.4 Observations during follow up

Outpatient visits to the clinic are planned twice weekly until full hematological recovery or CR.
Thereafter visits are planned as follows:
Outpatient visits to the clinic are planned according to the following schedule:

- Year 1: Subjects will be seen once each month.
- Years 2 and 3: Subjects will be seen at least at 3 months intervals.
- Years 4 and 5: Subjects will be seen once every 4-6 months.
- Beginning with year 6: Subjects will be followed according to the local scheme of the institute but not less than one time per year.

In this schedule time is measured from the date of completion of protocol treatment.
At each clinical visit the following examinations will be done:

- Interim history and physical examination
- Hemoglobin, WBC count and differential, platelet count, erythrocyte count, reticulocyte count
- Creatinin, AST, ALT, alkaline phosphatase, γGT, bilirubin
- Chest X-ray when clinically indicated

- Bone marrow aspirations for morphology will be done as clinically indicated, but at least at 4 months, 8 months, 12 months, 18 months, 24 months, 36 months and 48 months after last cycle or auto/allo SCT, as long as the patient is in CR.
- Marrow sampling for minimal residual disease assessments see appendix G.

12 Toxicities

Clofarabine

Clofarabine is an investigational drug*. For detailed information on clofarabine toxicology and pharmacology please refer to the clofarabine Investigator's Brochure* (Genzyme).
Toxicities will be scored according to the NCI Common Terminology Criteria of Adverse Events, version 4.0.
Like the toxicities of many chemotherapeutic agents the use of clofarabine is commonly associated with myelosuppression, infectious complications (eg febrile neutropenia, septicaemia, pneumonia, and others), alopecia, anorexia, vomiting, nausea, diarrhea, headache, oral mucositis. Also liver abnormalities (hyperbilirubinemia, elevations of AST/ALT), renal abnormalities (creatinine elevations, renal insufficiency), skin rash, dermatitis, hand-foot syndrome, atrial fibrillation have been reported as side effects of clofarabine.

Idarubicin

Congestive heart failure is a major complication of anthracyclines, frequently observed after high cumulative doses. The total planned dose of Idarubicin is 36 mg/m^2. These doses are considerably lower than those associated with congestive heart failure. Cardiotoxicity has also been observed with Amsacrine, enhanced by hypokalemia and previous anthracycline drugs, and after high dose Cyclophosphamide (usually more than 7.6 g/m^2) administered for conditioning regimen of SCT.
Other non-hematological drug toxicities of idarubicin are: hair loss, mucositis, cardiomyopathy, nausea, vomiting, colitis, infertility.

Cytarabine (Ara-C)

Conventional-dose: 200 mg/m^2: anorexia, nausea, vomiting, hepatic dysfunction, skin rash, pneumonitis, fever.
*Intermediate-dose: 1 g/m^2 and high-dose: 2 g/m^2 in additio*n: stomatitis, rash, fever, conjunctivitis (prevented by the use of methylcellulose or steroid eye drops), somnolence, and in few cases, cerebellar toxicity. Intermediate-dose Ara-C and high-dose Ara-C must be stopped immediately in case of nystagmus or dysarthria.

Amsacrine (AMSA)

Nausea, vomiting, mucositis, skin rash, phlebitis or infusion pain (when drug infused without dilution), hepatic dysfunction, arrythmia, seizures, infertility.

Mitoxantrone

Alopecia, mucositis, nausea, vomiting, diarrhoea, elevations of hepatic enzymes, lethargia, peripheral neuropathy.

VP-16 (Etoposide)

Nausea, vomiting, mucositis, hepatic dysfunction, neurotoxicity, skin rash.

Busulfan

Interstitial pneumonitis, hepatic dysfunction, erythematous skin rash, myastenia symptoms, cataract, infertility, alopecia, epileptic seizures (to be prevented by phenytoin prophylaxis), atrophic bronchitis, adrenal hypofunction.

Cyclophosphamide

Bone marrow depression, fluid retention, cardiomyopathy (at doses greater than 7.6 g/m^2 fatal heart failure), diarrhoea, hemorrhagic cystitis (prevented by forced diuresis or Mesna), alopecia, diffuse macropapular rash.

Following conditioning and autologous PBSCT

Autologous PBSCT is rarely associated with chills, fever and nausea, which can be prevented with oral antihistaminics and/or alizapride. Following the infusion, patients will experience a period of severe pan-cytopenia of 2-6 weeks duration and therefore risks of fever, infections or hemorrhages, which will require transfusion and microbiological support. In addition they will enter a 1-3 week period of gastro-intestinal symptoms (nausea, diarrhoea) due to the chemotherapy. This may also include a period of oral mucositis (stomatitis). Veno-occlusive disease may occur, but occurs in less than 10% of patients when the exclusion criteria regarding cardiac liver function abnormalities are considered. Infertility frequently ensues following high-dose therapy and stem cell transplantation. Hair loss is a side effect, which most patients will already show due to preceding conventional antileukemia chemotherapy.

G-CSF (granulocyte-colony stimulating factor)

Fever, diarrhoea, abdominal pain, vomiting, skin rash, headaches, bone pain and injection site reactions have been reported following the use of G-CSF.

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 and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse reaction (AR)

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

Serious adverse event (SAE)

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

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

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

Unexpected SAE

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

Suspected unexpected serious adverse reaction (SUSAR)

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

13.2 Reporting of (serious) adverse events

Adverse event

All AEs of CTCAE grade 2 or higher, with the exception of alopecia, nausea/vomiting and progression of the disease under study, have to be reported on the Adverse Events CRF.
Adverse events 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.
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.
Adverse events occurring after 30 days should also be reported if considered to be at least suspected to be related to the study drug.

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

- a standard procedure for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- the administration of blood or platelet transfusion. Hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable serious adverse event.
- a procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- a procedure that is planned (i.e., planned prior to starting of treatment on study; must be documented in the 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 SAE reports within 24 hours of receipt to the prinicipal investigator, the study central datamanager, to the safety desk and the product manufacturer (for clofarabine: the Pharmacovigilance group of Genzyme). The safety desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR).
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.

Any suspected unexpected serious adverse reactions (SUSARs) arising from this trial will be reported expedited by HOVON to the investigators, to Genzyme, and to all applicable Ethics Committees and Health Authorities within the timelines required by the EU Clinical Trial Directive.
The manner of SUSAR reporting will be in compliance with the procedures of the Ethics Committees and Health Authorities involved.

Genzyme 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 will be available in a separate document at the HOVON datacenter.

14 Endpoints

14.1 Part A: of the study (Dose level selection)

14.1.1 Primary endpoint

Occurrence of DLT and duration of myelosuppression of the combination of Clofarabine at three selected dose levels.

DLT is defined as

- Death
- Any non hematological toxicity CTCAE grade ≥ 4,

occurring within 30 days after start of cycles I or II and before the start of the next cycle or a new treatment respectively.

The duration of myelosuppression is defined as the median time to recovery of ANC > $0.5x10^9$/L.

DLT and myelosuppression will be used in the decision process for dose escalation, dose reduction and/or dose selection (see 17.1).

14.1.2 Secondary endpoint

- Response and especially CR to chemotherapy cycles I and II

14.2 Part B: of the study (Efficacy)

14.2.1 Primary endpoint

Event-free survival (EFS) (i.e., time from registration to induction failure, death or relapse whichever occurs first).

14.2.2 Secondary endpoints

- Response and especially CR (including CRi) to chemotherapy cycles I and II
- Overall survival (OS) measured from the time of registration
- Disease-free interval (duration of the first CR) measured from the time of achievement of CR to day of relapse or death from any cause (whichever occurs first).
- Occurence of toxicities and treatment related mortality (according to Appendix H)
- Time to hematopoietic recovery (ANC 0.5 and $1.0x10^9$/L; platelets 50 and $100x10^9$/L) after each treatment cycle.
- Number of platelet transfusions and last day of platelet transfusion after each cycle.

15 Registration and Randomization

15.1 Regulatory Documentation

The following documents must be provided to the HOVON Data Center before shipment of 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 induction treatment. Patients can be randomized via the Internet via TOP (Trial Online Process; https://www.hdc.hovon.nl/top) or at the HOVON Data Center of the Erasmus MC Rotterdam – location Daniel 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 randomization:
Protocol number

Institution name
Name of caller/responsible investigator
Local patient code (optional)
Sex
Date of birth
Date of diagnosis of AML or RAEB
Date written informed consent
Eligibility criteria

All eligibility criteria will be checked with a checklist.
Patients will be randomized, stratified by center and disease (AML vs RAEB) 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 CRFs

Data will be collected on Case Report Forms (CRFs) 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) and myelosuppression duration a separate CRF (DLT-form) will be used. This DLT-form must be filled out for every patient, 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 weekly after start of cycle I and II if no DLT occurred. DLTs should be reported until day 30 or until start next treatment. Duration of myelosuppression must be reported on the DLT form until ANC recovery or until start next treatment (if not yet recovered). Investigators will weekly receive a reminder for sending in a new DLT form.

16.3 End of trial

End of trial is defined according the last patient being out ('off protocol treatment'). This will be at 7 months following enrollment and randomization of the last patient into the study. Subsequently the patients will be followed for progress reports for 10 years

17 Statistical considerations

All analyses will be done according to the intention to treat principle.
The purpose of the first part of the study is to determine which dose level of Clofarabine is feasible. In the second part of the study the effectiveness of treatment with Clofarabine at the selected dose level is investigated.

17.1 Part A: Dose level selection

In this study, a maximum of three dose levels of Clofarabine will be considered. The study starts at a dose level of 10 mg/m^2, and if possible escalating to 20 mg/m^2. If 20 mg/m^2 is not feasible we return to the intermediate dose level of 15 mg/m^2, and we return to 10 mg/m^2 if 15 mg/m^2 is not feasible as well. At each dose level the decision to stop or escalate will be made on the basis of (a) the incidence of Dose Limiting Toxicities (DLTs) in the arm treated with Clofarabine versus the incidence of DLTs in

the control arm and (b) the duration of myelosuppression in the Clofarabine arm compared to the control arm.

DLT is defined as “Death or Any non hematological toxicity CTCAE grade ≥ 4” occurring within 30 days after start of cycles I or II and before the start of the next cycle or a new treatment respectively. The duration of myelosuppression is defined as the median time to recovery of ANC > $0.5x10^9$/L. Applying the criteria to the patients treated in the HOVON/SAKK-29 and 42 studies with standard dose chemotherapy, who are comparable to the patients in the control group of this trial we find:

20% of the patients experience DLT in cycle I

5% of the patients stop after cycle I with no DLT

14% of the patients experience DLT in cycle II

So, overall 31% of the patients experience DLT

The median time to recovery of ANC >$0.5x10^9$/L is 29 days following cycle I and cycle II

In the decision rules, the number of DLTs in both arms and the number of days at risk for DLT are taken into account. Each patient is at risk for a maximum of 2*30= 60 days. If no early decision to stop or escalate can be made, on the moment the maximum number of 15 patients in the Clofarabine arm have completed induction therapy, the active dose level is chosen as the dose level to continue Part B of the trial.

DLTs have to be reported within 24 hours and investigators weekly receive a questionnaire for patients who are still at risk. The decision rules will be checked once a week, and the trial will be closely monitored in the mean time.

The precise decision rules are presented in the table below. A patient has completed induction therapy if the patient (a) is known not to have experienced DLT 30 days after start of cycle II, (b) is known not to have experienced DLT 30 days after start of cycle I and known not receive cycle II, or (c) has experienced DLT.

Number of patients who have completed induction treatment in the Clofarabine arm	Excess DLTs on Clofarabine arm		DLT Incidence Rate Ratio		ANC Recovery Hazard Ratio	Dose Escalation / Reduction
n < 10	>3	*and*	>3		(Any value)	Stop/reduce
	≤3	*or*	≤3			Continue
10 ≤ n < 15	(Any value)		>2***	*or*	>1.9	Stop/reduce
			1.3 to 2	*and*	≤1.9	Continue
			≤2	*and*	1.2 to 1.9	
			<1.3	*and*	<1.2	Escalate*
n ≥ 15	(Any value)		>1.6	*or*	>1.7	Stop/reduce
			1.4 to 1.6	*and*	≤1.7	Continue **
			≤1.6	*and*	1.5 to 1.7	
			<1.4	*and*	<1.5	Escalate*

* At the highest dose level the decision to escalate means continuation at that dose level for evaluation of efficacy in Part B.

** Continue means here that no decision to stop or escalate can be made after 15 patients in arm B completed induction therapy, and we continue with part B of the trial at the present dose level.

*** If no DLTs in the standard arm are present, the calculated DLT incidence ratio will be infinite. Although unlikely, in such a case the decision to stop the trial or reduce the dosage will be made only when a minimum of DLTs in the Clofarabine arm are present: >3 DLTs if 10 or 11 evaluable patients in the experimental arm are present, > 4 with 12-14 evaluable patients, and >5 with at least 15 evaluable patients.

Excess DLTs on the Clofarabine arm are defined as the number of DLTs on the Clofarabine arm minus the number on the control arm.

DLT Incidence Rate Ratio is the DLT incidence rate on the Clofarabine arm divided by the DLT incidence rate on the control arm. An incidence rate is defined as the total number of DLTs observed at a particular dose (max of one per patient) divided by the total number of days “at risk” for a DLT summed over all patients in that particular cohort.

The ANC Recovery Hazard Ratio is determined from a Cox proportional hazards model with

- all observations including cycles I and II

- the event indicator variable equals 1 when a patient recovers, i.e. ANC >0.5x10^9/L, after a cycle before start of the next treatment; with the time to event the number of days between the start of the cycle and the date of recovery, i.e. the first date that ANC >0.5x10^9/L
- censoring (ie, the event indicator variable equals 0) if the patient dies, or starts a new treatment without previous recovery, or at the date of last known ANC in case of missing ANC values after that date.
- The hazard ratio is calculated as the hazard ratio of recovery in the conventional arm compared to the Clofarabine arm. A hazard ratio >1 implies a poorer recovery in the Clofarabine arm.

For each row, the condition as characterized in the first four columns should be present to take a decision to stop (and return to the previous dose level if possible), escalate, or continue.
These decision rules lead to the following characteristics where we assume that the true probability of DLT in the control arm is 31% and the median recovery time for ANC > 0.5x10^9/L is 29 days as expected; an increase of 20% in the Clofarabine arm corresponds to a median recovery time of 35 days. An expected accrual of 150 patients a year (12.5/month) is used in the calculations.

Absolute increase of DLT in Clofarabine arm	Increase of duration of recovery time	Decision	Percentage	Mean (range) of number of patients entered in both arms
0%	0%	Stop/reduce	32%	38 (14-64)
		Continue	3%	54 (42-62)
		Escalate	65%	46 (32-63)
	20%	Stop/reduce	55%	45(14-69)
		Continue	7%	55(43-67)
		Escalate	38%	49(31-70)
15%	0%	Stop/reduce	55%	38(12-64)
		Continue	6%	51(41-63)
		Escalate	39%	46(31-66)
	20%	Stop/reduce	71%	40(11-64)
		Continue	8%	53(40-68)
		Escalate	21%	47(36-64)

So, for example the probability of escalating in a situation in which the true incidence of DLT and true recovery time of ANC in both arms are as expected, is 65%. And, the decision to stop in the situation that the incidence of DLT is increased with 15% and the duration of recovery time for ANC > 0.5x10^9/L is increased with 20% in the Clofarabine arm compared to the control arm is 71%.

If a decision to stop is implied by the decision rules we return to a lower dose level until a decision is made by the DSMB, if not possible the trial is put on hold. Until the DSMB has confirmed a decision to escalate, the trial remains open at the active dose level.
The decision rules serve as guidelines for the DSMB (see section 17.2.2). As soon as a decision can be made according to the above defined rules, a report will be generated and sent to the DSMB for final recommendations. The report contains a tabulation of the number of patients recruited, the number of evaluable patients, the number of DLTs, a specification of the DLTs and their outcome, the ANC>0.5x10^9/L recovery results, and the incidence and intensity of the other reported AEs, split by arm, treatment cycle and dose level.

17.2 Part B: Efficacy

17.2.1 Patient numbers and power considerations

Based on the results of the HOVON/SAKK AML 42 trial we expect the following:

Accrual rate:	200 patients per year
EFS at three years in the control arm:	31 %

The target number of patients for this part of the study is 800 to be accrued in approximately 4 years. After entry of the last patient an additional follow up of 1 year is planned before the first final analysis. The target number of 800 patients will give a power of 87% with a two-sided test at 5% significance level to detect an improvement in EFS with hazard ratio HR=0.76, which corresponds to an increase of EFS at 3 years from 31% to 41%.

17.2.2 Interim analyses and safety monitoring

Three interim analyses are planned, primarily to guard against unfavourable results in the induction treatment arm with Clofarabine. Results of the interim analyses will be presented confidentially only to the DSMB. Only if the DSMB recommends that the study should be stopped or modified, the results will be made public to the principal investigators for further discussion. Interim analyses are planned after inclusion of the 100th, 200th and 400th patient at the final dose level.
The main endpoint for the interim analyses is the overall failure rate on induction treatment. A patient counts as a failure in induction therapy if one of the following conditions apply:

- the patient does not complete cycle II
- the patient does not achieve CR on induction
- the patient dies due to side effects of cycle I or II.

At each interim analysis, a detailed report will be generated and presented to the DSMB. The report includes by treatment arm the number of entered patient and at that time evaluable patients, treatment given, the number of events and event types (no CR, relapse, death), and split by cycle the duration of hematological recovery, and the incidence of side effects and infections (CTCAE grade). The DSMB is free in her public recommendations to the principal investigators and her confidential recommendation to the trial statistician, but the following guidelines apply:

- A higher failure rate on induction treatment in the Clofarabine arm with a P-value <0.10, or if the 95% confidence interval of the hazard ratio associated with EFS excludes a reduction of the hazard rate with 25%, are good reasons to recommend the stopping of the trial or recommendations for modifications.
- A benefit in terms of event free survival (EFS) or overall survival (OS) in the Clofarabine arm is in general no reason to recommend early stopping of the trial, unless the associated P-value is very extreme (P<0.001) and the number of evaluable patients in each arm is at least 150.

17.2.3 Efficacy analysis

Induction randomization

Main endpoint for the comparison of standard chemotherapy with or without Clofarabine is EFS from time of registration to induction failure (failure to reach CR on induction therapy), death or relapse whichever occurs first. Secondary endpoints are CR rate and overall survival. Actuarial estimates of competing risks (no CR, relapse after CR, death in first CR) will be made for each treatment arm. Formal tests for the difference in EFS between the two induction treatment arms will be done with Cox regression analysis, stratified by risk group and adjusting for type of postremission treatment using time dependent covariates.

Evaluation of AlloSCT

The outcome of patients treated with AlloSCT will be determined by calculation of the probabilities of relapse and death in first CR after AlloSCT as competing risk and the survival probability. Estimates will be made separately by type of transplant (HLA identical sibling or MUD or umbilical cord blood or haplo-identical SCT), by age group, by diagnosis (AML versus MDS) and by risk group and molecularly defined subtypes depending on available numbers of subgroups. The results will be pooled with the data of similar patients in the previous AML studies.

The outcome of the poor risk patients treated with AlloSCT in PR will be determined by calculation of the probabilities of reaching CR, relapse and death after AlloSCT.

Since there is no randomization between AlloSCT and other consolidation treatment, the effect of AlloSCT cannot be estimated in a proper unbiased way. As an approximation an analysis will be done based on donor availability. Data will be collected for each patient (below the age of 55) in CR after

cycle II with intermediate and poor risk concerning the availability of an HLA identical sibling donor or a matched unrelated donor.
A comparison will be made of the outcomes of the patients by donor availability, irrespective of the actual treatment in first CR. These data will also be submitted to the data of the AML Collaborative Group for a meta-analysis.

17.2.4 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of side effects and infections with CTCAE grade 2 or more (see Appendix H) by treatment arm and cycle or type of SCT. Time to hematological recovery after each treatment cycle or SCT will be analyzed by actuarial methods. Actuarial competing risk estimates of probability of death will be split by cause of death where a difference will be made between death due to or after relapse or induction failure and death due to side effects of the treatment, overall and separately by treatment arm and cycle.

17.2.5 Additional analysis

Additional analyses involve the analysis of prognostic factors, especially age, cytogenetic abnormalities and risk group with respect to CR rate, EFS, OS and DFS. Logistic and Cox regression analysis will be used for this purpose.
Furthermore, the efficacy of Clofarabine on CR rates and EFS will be evaluated for different subgroups separately. These subgroups will be defined on the basis of prognostic relevant characteristics, especially age and riskgroup. The results will be interpreted with caution, as multiple testing and possibly low power per separate subgroup limit the value of subgroup analyses. The appearance of spurious significant differences due to multiple testing will be taken into account.

17.3 Data Safety Monitoring Board

An independent Data and Safety Monitoring Board (DSMB) will be appointed, consisting of two international clinical hematologists with a broad background in AML therapeutics as well as an independent statistician. The DSMB will give recommendations about dose escalations, 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 studycoordinators. 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 at the current dose level. The study coordinators make the final decision.

18 Ethics

18.1 Accredited ethics committee or Institutional review board

The study protocol and any susbstantial amendment will be approved by an accredited Ethics Committee or Institutional Review Board.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the current version of the ICH-GCP Guidelines, the EU directive for Good Clinical Practice (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for ensuring that the study will be conducted in accordance with the protocol, the ethical principles of the Declaration of Helsinki, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory requirements.

18.3 Patient information and consent

Written Informed Consent of patients is required before registration. The procedure, the risks and the therapy options 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 Data Center and 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
AGT	O6 Alkylguanine alkyl transferase
AHD	antecedent hematologic disorder
ALL	Acute Lymphoblastic Leukemia
AlloSCT	Allogeneic stem cell transplantation
ALT	Alanine Amino Transferase
AML	Acute Myelogenous Leukemia
ANC	Absolute Neutrophil Count
Ara-C	Cytarabine, cytosine arabinoside
ASCO	American Society of Clinical Oncology
ASH	The American Society of Hematology
AST	Aspartate Amino Transferase
AutoPBSCT	Autologous peripheral blood stem cell transplantation
AutoSCT	Autologous stem cell transplantation
BAALC	brain and leukaemia Cytoplasmic
BM	Bone Marrow
BMT	Bone Marrow Transplant
CBF	core-binding-factor
CCAAT	cytidine-cytidine-adenosine-adenosine-thymidine
CEBPA	CCAAT binding factor alpha
CFC	Colony Forming Cells
CI	Continuous Infusion
CI	Confidence interval
Cl	Chloride
CLL	chronic lymphocytic leukemia
CMV	Cytomegalovirus
CN	normal cytogenetics
CNS	Central nervous system
CO2	Carbondioxide
CR	Complete Remission
Cre	Complete Remission Early (after induction cycle 1)
Cri	Complete Remission with incomplete blood count recovery
CRp	Complete Remission without platelet recovery

CRF	Case Report Form
CT	Computerized Tomography
CTC	Common Terminology Criteria
CTCAE	Common Terminology Criteria for Adverse Events
CY	cyclophosphamide
dCK	deoxycytidine kinase
DFS	Disease free Survival
DLT	Dose Limiting Toxicity
DNR	Daunorubicin
DSMB	Data and Safety Monitoring Board
EBMT	European Group for Blood and Marrow Transplantation
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	Event Free Survival
EMEA	European Medicines Agency
EP	European Pharmacopeia
ERG	Ets Related Gene
EVI1	ecotropic virus integration 1
FAB	French American British (cytological classification)
FISH	Fluorescent in situ hybridization
FLT3(-IND)	FMS-like tyrosine kinase III(-internal tandem duplications)
GCP	Good Clinical Practice
GEP	Gene Expression Profiling
GFR	glomerular filtration rate
G-CSF	Granulocyte-Colony Stimulating Factor
GI	Gastro-intestinal
GR	Good Risk
GvHD	Graft versus Host Disease
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
HR	Hazard ratio
Ht	Hematocrit
HOVON	Dutch/Belgian Hematology-Oncology Cooperative Group
HPLC	high performance liquid chromatography
HRC	Hematocytology Review Committee
ILLN	Institutional Lower Limit of Normal

IPSS	International Prognostic Score System (for myelodysplastic syndromes)
IR	Intermediate Risk
IRB	Institutional Review Board
ITT	Intention to Treat
(I)ULN	(Institutional) Upper Limit of Normal
IV	Intravenous
K	Potassium
LAP	Leukemia Associated Phenotype
LDAC	low-dose, subcutaneous cytarabine
LD50	Lethal Dose 50%
LDH	Lactate Dehydrogenase
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MDR	Multi Drug Resistance
MDR-1	Multi Drug Resistance-1 gene
MDS	Myelodysplastic Syndrome
MK	Monosomal karyotype
MRD	Minimal residual disease
MTD	Maximum tolerated dose
MUD	Matched unrelated donor
MUGA	Multiple Gated Acquisition
Na	Sodium
NPM1	nucleophosmin-1
ORR	Overall response rate
OS	Overall Survival
PMN	Polymorphonuclear
PB	Peripheral Blood
PBPC	Peripheral Blood Progenitor Cells
PBR	Peripheral Blood Recovery
PCR	polymerase chain reaction
PK	Pharmacokinetics
PR	Partial Response
PR	Poor Risk
PS	Performance status
RAEB	Refractory Anemia with Excess of Blasts

SAE	Serious Adverse Event
SUSAR	Suspected unexpected serious adverse reaction
SCT	Stem cell transplantation
SGOT	Serum glutamic aminotransferase
SGPT	Serum glutamic pyruvic transaminase
TRM	Treatment related mortality
ULN	Upper limit of normal
VPR	Very Poor Risk
WBC	White Blood Cell
WT1	Wilms tumor gene
WHO	World Health Organization

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A1 WHO classification for acute myeloid leukemias

- Definition AML: ≥ 20% myeloblasts in blood or in bone marrow
- Abnormal promyelocytes in acute promyelocytic leukaemia, promonocytes in AML with monocytic differentiation and megakaryoblasts in acute megakaryocytic leukaemia are considered blast equivalents
- First, AML should be classified as AML with recurrent cytogenetic abnormalities. If this is not applicable the leukaemia is classified as AML with multilineage dysplasia or therapy related and if this subtype is also not applicable as AML not otherwise categorised.

WHO code	**Category**	Subcategory and short description
9896	**Acute myeloid leukaemia with recurrent genetic abnormalities**	AML with t(8;21)(q22;q22);(*AML/ETO*)*
9871		AML with inv(16)(p13q22) or t(16;16)(p13;q22); (*CBFß*/*MYH11*)*
9866		Acute promyelocytic leukaemia; AML with t(15;17)(q22;q12)(*PML*/*RARα*) and cytogenetic variants
9897		AML with 11q23 (*MLL*) abnormalities
9895	**Acute myeloid leukaemia with multilineage dysplasia**	Dysplasia should be present in ≥ 50% of 2 or more cell lineages
9920	**Acute myeloid leukaemia and myelodysplastic syndromes, therapy-related**	Alkylating agent-related Topoisomerase type II inhibitor-related Other types These types of AML and MDS may be classified if appropiate in a specific morphologic or genetic category with the qualifying term “therapy related”
Acute myeloid leukaemia not otherwise categorized:		
9872	**AML minimally differentiated**	≤ 3% of blasts positive for Sudan Black B or myeloperoxidase at least one of the following myeloid markers present: CD13, CD33, CD117 in absence of lymphoid markers CD3, CD22 and CD79a
9873	**AML without maturation**	Blasts ≥ 90% of bone marrow nonerythroid cells (i.e. excluding also lymphocytes, plasmacells, macrophages and mast cells) >3% of blasts positive for Sudan Black B or myeloperoxidase At least two of the following myelomonocytic markers present: CD13, CD33, CD117 and/or MPO
9874	**AML with maturation**	≥ 10% maturing granulocytic cells in the bone marrow (i.e. promyelocytes, myelocytes and mature neutrophils) < 20% bone marrow monocytes
9867	**Acute myelomonocytic leukaemia**	≥ 20% neutrophils and precursors of marrow cells ≥ 20% monocytes and precursors of marrow cells
9891	**Acute monoblastic and monocytic leukaemia**	≥ 80% of the leukemic cells are monoblasts, promonocytes and monocytes
9840	**Acute erytroid leukaemia**	**Erythroleukaemia (erythroid/myeloid)** Erythroblasts: ≥ 50% of bone marrow cells Blasts: ≥ 20% of the bone marrow nonerythroid cells
		Pure erythroid leukaemia Erythroblasts: > 80% of bone marrow cells No evidence of a significant myeloblastic component

9910	**Acute megakaryoblastic leukaemia**	≥ 50% of the blasts are of megakaryocytic lineage Blasts express CD41 and/or CD61
9870	**Acute basophilic leukaemia**	Primary differentiation to basophils; mature basophils are usually sparse
9931	**Acute panmyelosis with myelofibrosis**	acute panmyeloid proliferation with accompanying fibrosis Involves all the major myeloid cell lines, i.e. the granulocytes, erythroid cells and megakaryocytes % of blasts and micromegakaryoblasts is variably increased -No or minimal splenomegaly
9930	**Myeloid sarcoma**	Tumour mass of myeloblasts or immature myeloid cells occuring in an extramedullary site or in bone
9801	**Acute leukaemias of ambiguous lineage**	**Undifferentiated acute leukaemia** Blasts lack markers considered specific for a given lineage including CD79a, CD22, CD3 and MPO
9805		**Bilineal acute leukaemia** Leukaemia with a dual population of blasts with each population expressing markers of a distinct lineage, i.e. myeloid and lymphoid or B and T
		Biphenotypic acute leukaemia Blasts coexpress myeloid and T or B lineage specific antigens or concurrent B and T lineage antigens

*Rare cases show < 20% myeloblasts; these should be classified as AML

A2 WHO classification for myelodysplastic syndromes

WHO code	Disease	Blood findings	Bone marrow findings
9980	Refractory anaemia (RA)	Anaemia No or rare blasts	Erythroid dysplasia only < 5% blasts < 15% ringed sideroblasts
9982	Refractory anaemia with ringed sideroblasts (RARS)	Anaemia No blasts	Erythroid dysplasia only < 5% blasts ≥ 15% ringed sideroblasts
9985	Refractory cytopenia (RC)	with multilineage dysplasia (RCMD)	
		Cytopenias (bicytopenia or pancytopenia No or rare blasts No Auer rods < 1 x 10^9/L monocytes	Dysplasia in ≥ 10% of the cells of two or more myeloid cell lines < 5% blasts No Auer rods < 15% ringed sideroblasts
		with multilineage dysplasia and ringed sideroblasts (RCMD-RS)	
		Cytopenias (bicytopenia or pancytopenia No or rare blasts No Auer rods < 1 x 10^9/l monocytes	Dysplasia in ≥ 10% of the cells of two or more myeloid cell lines < 5% blasts No Auer rods ≥ 15% ringed sideroblasts
9983	Refractory anaemia with excess of blasts (RAEB)	with excess blasts-1 (RAEB-1)	
		Cytopenias < 5% blasts No Auer rods < 1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 5-9 % blasts No Auer rods
		with excess blasts-2 (RAEB-2)*	
		Cytopenias 5-19% blasts Auer rods ± < 1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 10-19 % blasts Auer rods ±
9989	MDS unclassified	Cytopenias No or rare blasts No Auer rods	Unilineage dysplasia: one myeloid cell line < 5% blasts No Auer rods
9986	MDS associated with isolated del(5q)	Anaemia Usually normal or increased platelet count < 5% blasts	Normal or increased megakaryocytes with hypolobulated nuclei < 5% blasts No Auer rods Isolated del(5q) cytogenetic abnormality

**Patients with 5-19% blasts in the blood and <10 % blasts in the bone marrow are also placed in the RAEB-2 group*

A3 FAB classification of AML

Cytological criteria for the diagnosis of acute myeloid leukemia: French-American-British-(FAB) classification

FAB subtype	
	For all AML subtypes the following criteria apply: ♦ Blasts ≥ 30% of bone marrow nucleated cells, except for M3 ♦ ≥ 3% of blasts positive for Sudan BlackB or Myeloperoxidase, except for M0 and M7
M0	♦ < 3% of blasts positive for Sudan Black B or Myeloperoxidase ♦ at least one of the following myeloid markers present: CD13,CD33, CD15, CDw65 ♦ in absence of lymphoid markers CD3 and CD22
M1	♦ Blasts ≥ 90% of bone marrow nonerythroid cells (i.e. excluding also lymphocytes, plasma cells, macrophages and mast cells) ♦ Maturing granulocytic cells (i.e. promyelocytes towards polymorphonuclear cells ≤ 10% of nonerythroid cells ♦ (pro)monocytes ≤ 10% of nonerythroid marrow cells
M2	♦ Blasts 30-89% of bone marrow nonerythroid cells ♦ Maturing granulocytic cells (i.e. promyelocytes to polymorphonuclear cells) > 10% of nonerythroid cells ♦ Monocytic cells (i.e. monoblasts to monocytes) < 20% of nonerythroid cells
M2E	♦ Analogous to M4E, but lacking clear monocytic differentiation
M3	♦ Promyelocytes (most hypergranular) > 30% of bone marrow nucleated cells
M3V	♦ Promyelocytes (hypogranular or microgranular) > 30% of bone marrow nucleated cells
M4	♦ Granulocytic cells (myeloblasts to polymorphonuclear cells) ≥ 20% of nonerythroid cells plus one of the following criteria • Monocytic cells (monoblasts to monocytes) ≥ 20% of nonerythroid cells Or • Peripheral blood monocytes ≥ 5 x 10^9/l Or • Elevated urinary lysozymes ≥ 3 x normal value
M4E	♦ Same as M4, but with ≥ 5% abnormal eosinophils (basophilic granulae)
M5A	♦ Blasts ≥ 30% of bone marrow nonerythroid cells ♦ Bone marrow monocytic component ≥ 80% of nonerythroid cells ♦ Monoblasts ≥ 80% of bone marrow monocytic component
M5B	♦ Blasts ≥ 30% of bone marrow nonerythroid cells ♦ Bone marrow monocytic component ≥ 80% of nonerythroid cells ♦ Monoblasts < 80% of bone marrow monocytic component
M6	♦ Erythroblasts ≥ 50% of bone marrow nucleated cells ♦ Blasts ≥ 30% of bone marrow nonerythroid cells
M7	♦ > 30% of bone marrow nucleated cells are megakaryoblasts CD41 or CD61 positive or ♦ Platelet specific peroxidase reaction (electron microspcopy) ♦ < 3% of blasts positive for Sudan Black B or Myeloperoxidase

B International Prognostic Score System (IPSS) for MDS
(ref. 4)

The **IPSS score** is calculated by summation of the score values (see table below) for categories of the prognostic variables for a patient. IPSS groups are defined as follows:

Low : 0

Int-1 : 0.5-1.0

Int-2 : 1.5-2.0

High : ≥ 2.5

	Score value				
Prognostic Variable	0	0.5	1.0	1.5	2.0
BM blasts (%)	<5	5-10	--	11-20	21-30
Karyotype*	A	B	C		
Cytopenias**	0/1	2/3			

*Karyotype

A : normal, -Y, del(5q), del(20q)

C : complex (≥ 3 abnormalities in the same clone)
or chromosome 7 abnormalities

B : all other (or not done)

**Cytopenias

Hb < 6.2 mmol/L

ANC < 1.5×10^9/L

Platelets < 100×10^9/L

Note: this score only (!) applies to the determination the IPSS, not for the risk classification (Appendix D)

C Response criteria for AML and RAEB

HOVON-AML/MDS Response criteria (modified from the International Working Group Criteria[53, 54])

1. Disease status and response criteria

Note that the kind of cells considered equivalent to blasts and included in the calculation of last percentages depends on the WHO classification of diagnosis.

1.1. Morphologic leukemia-free state ('marrow remission'):

Bone marrow with spicules and a count of at least 200 nucleated cells, <5% blasts, and no Auer rods. Also no extramedullary disease.

In case of biopsy, when spicules are absent in the aspirate, no clusters of blasts should be present.

1.1.a Complete hematological remission (CR):

Morphological leukaemia-free state **and** absolute neutrophil count (ANC) $\geq 1.0 \times 10^9$/L , platelet count $\geq 100 \times 10^9$/L (i.e. 72h after last transfusion)

(The presence of blasts in the peripheral blood does not argue against and is compatible with a complete remission).

1.1.b Morphological complete remission with incomplete blood count recovery (CRi)

CRi implies the presence of a morphological leukaemia-free state **but** incomplete recovery of the absolute neutrophil count (ANC) $<1.0 \times 10^9$/L and/or platelet count $<100 \times 10^9$/L (i.e. 72 h after last transfusion)

1.1.c Cytogenetic remission

This criterion will be assessed only in case of pre-existent cytogenetic abnormalities (at diagnosis): disappearance of all cytogenetic abnormalities in a marrow karyotypic analysis of at least 16 metaphases

1.2. Partial remission (PR)

ANC $\geq 1.0 \times 10^9$/L and/or, platelet count $\geq 100 \times 10^9$/L (i.e. 72 h after last transfusion).
Blasts in the bone marrow should decrease 50% **and** reach a value between 5 and 25%. If blasts ≤ 5% but Auer Rods are present this should be considered PR

2. Treatment failure

Subjects who do not enter CR (phase III) or PR (phase I-II) following induction will be classified according to the type of failure (document on CRF) as described below:

2.1. Resistant disease

Subject has persistent leukaemia in the blood or bone marrow and/or persistent extramedullary disease. Persistant disease can only be assessed in patients surviving ≥ 7 days after completion of the final dose of cycle I

2.2. Aplastic death

Death with cytopenia and marrow aplasia in patients with no evidence of active leukemia surviving ≥ 7 days after completion of the final dose of cycle I

.

2.3. Indeterminate cause:

- patient dies <7 days after the last day of induction chemotherapy
- patient dies ≥7 days after the last day of induction chemotherapy. No signs of leukaemia in the most recent bloodsmear. No bone marrow evaluation available
- patient dies without completion of the first course of therapy

3. Relapse Criteria

Relapse after complete remission is defined as:
recurrence of blasts in the marrow of ≥ 5% (excluding increased blasts in the context of regenerating marrow)
recurrence of leukemic blasts in the peripheral blood
recurrence of leukemia at an extramedullary site
recurrence of pre-treatment characteristic signs of morphological dysplasia
recurrence of Auer rods

Note:
After recent treatment and no circulating blasts: if the bone marrow contains 5-15% blasts bone marrows should be repeated after an interval of at least one week to exclude the possibility of an increase of blasts due to early myeloid regeneration. The repeat evaluation should provide information to distinguish persistent leukemia or relapse versus myeloid regeneration. This applies to situations after cycle I or cycle II when a complete remission has not previously been established and has to be determined for the first time.

D Prognostic relapse risk group definition

Patients are classified in 4 risk groups according to the table below.

Risk		Definition	% pts at baseline	% pts with CR & consolidation
Good	GR1	t(8;21) or *AML1-ETO*, WBC≤20	5 %	7 %
	GR2	inv(16)/t(16;16) or *CBFB-MYH11* gene	6 %	7 %
	GR3	MK-, *CEBPA*+	7 %	8 %
	GR4	MK-, *FLT3ITD-/NPM1+,* CRe	11 %	13 %
Intermediate	IR1	t(8;21) or *AML1-ETO*, WBC>20	2 %	2 %
	IR2	CN –X –Y, WBC≤100, CRe	17 %	21 %
Poor	PR1	CN –X –Y, WBC≤100, not CRe	10 %	8 %
	PR2	CN –X –Y, WBC>100	5 %	4 %
	PR3	CA, non CBF, MK-, no abn3q26, EVI1-	16 %	15 %
Very Poor	VPR1	Non CBF, MK+	9 %	5 %
	VPR2	Non CBF, abn3q26	2 %	1 %
	VPR3	Non CBF, EVI1+	9 %	9 %

The table gives the % distribution of each risk subgroup of all patients at diagnosis and of all patients that have reached CR and have received consolidation treatment.

- The core-binding factor (CBF) leukemias involve AML's with cytogenetic abnormality t(8;21)(q22;q22) or the *AML1-ETO* fusion gene and the cytogenetic abnormalities inv(16)(p13q22) or t(16;16)(p13;q22) or the related fusion gene *CBFB-MYH11*.
- If cytogenetics unknown, consider as CN
- Monosomal karyotype (MK) refers to AML with two or more autosomal monosomies or a single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities
- MK-: monosomal karyotype negative
- MK+: monosomal karyotype positive
- CN –X-Y: cytogenetically normal or only loss of X or Y chromosome
- CA: cytogenetically abnormal
- CRe: attainment of early CR, ie after cycle I
- EVI1+ refers to high EVI1 mRNA expression
- FLT3-ITD-/NMP1+ : FLT3-ITD mutant negative (FLT3ITD-) but NPM1-mutant positive (NPM1+): Fms-like tyrosine kinase receptor-3 internal tandem duplications (FLT3-ITD) and nucleophosmin-1 (NPM!) mutations often go together as dual genetic anomalies in the same AML.
- *To exclude ambiguities in the classification patients should be classified in the following hierarchical order: first patients with CBF abnormalities in GR1, GR2 or IR1, of the remaining patients the MK+ patients in VPR1, followed by the abn3q26 patients in VPR2 subsequently the CEBPA+ patients in GR3 and the FLT3ITD-/NPM1+ patients in GR4, subsequently the EVI1+ patients in VPR3. The remaining patients are classified in PR1, IR2, PR2 and PR3.*

The above risk classification is based on

(a) an analysis of the data of 1975 patients from the previous HOVON/SAKK AML studies for patients up to 60 years of age (4, 4A, 29 and 42), registered before January 1, 2004 and with successful cytogenetic analysis

(b) an analysis of the data of a subset of 424 patients for which also marker information and microarray expression data were available.

(a) Risk based *only* on cytogenetics, WBC at diagnosis and early/late CR.

The data of 1975 patients from the previous HOVON/SAKK AML studies for patients up till 60 years of age (4, 4A, 29 and 42), registered before January 1, 2004 and with successful cytogenetic analysis have been analysed. This analysis led to the identification of a subgroup of patients with a very poor prognosis: patients with two or more autosomal monosomies or a single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities (monosomal karyotype positive MK+ patients) (37). This group overlaps to a large extent with the group of patients that are classically considered as poor risk by most authors (i.e. complex karyotype, -5,-7, 5q-, 7q-, t(6;9), abn3q, inv(3) or t(9;22)). However classically poor risk patients that are not MK+ do not have such a poor prognosis. Their prognosis is similar to that of patients with other cytogenetic abnormalities, while MK+ patients that do not fall in the category of classically defined poor risk exhibit the same very poor prognosis as the other MK+ patients. Only the MK- patients with an abn3q26 showed a poor prognosis, though somewhat better than the MK- patients. The analysis confirmed that patients with CBF abnormalities have a favorable prognosis, except for the t(8;21) patients with a WBC>20 at diagnosis. Patients without cytogenetic abnormalities (CN) or only loss of X or loss of Y showed a better prognosis than the MK- patients with non CBF cytogenetic abnormalities (CA), except when they had a very high WBC>100 at diagnosis. Patients with an early CR (CRe), i.e. after induction cycle I showed a better prognosis than patients with a late CR. Given all these factors age did not show an impact on prognosis. All these factors have been integrated in a risk classification. This risk classification is in essence for patients who reach a CR on protocol. The risk classification can be used for risk adapted consolidation treatment decisions. Patients without cytogenetic data should be considered as cytogenetically normal (CN) for the risk classification as the OS and DFS of these patients without cytogenetic data in the previous studies was most similar to the OS and DFS of the CN patients.

Risk		Definition	% pts at baseline (n=1975)	% pts with CR & consolidation (n=1251)
Good	GR1	t(8;21), WBC≤20	5 %	7 %
	GR2	inv(16)/t(16;16)	6 %	7 %
Intermediate	IR1	t(8;21), WBC>20	2 %	2 %
	IR2	CN –X –Y, WBC≤100, CRe	28 %	34 %
Poor	PR1	CN –X –Y, WBC≤100, not	15 %	13 %
	PR2	CRe CN –X –Y, WBC>100	8 %	7 %
	PR3	CA, non CBF MK- , no abn3q	25 %	24 %
Very Poor	VPR1	Non CBF, MK+	9 %	5 %
	VPR2	Non CBF, abn3q26	2 %	1 %

Table gives the % distribution of each risk subgroup at diagnosis and at consolidation after attainment of CR

(b) Risk based in addition on marker and microarray expression data

For a smaller set of 424 patients also gene marker information and microarray expression data were available. Analysis of these data were consistent with results by others: AML with *CEBP*A mutations and AML with *FLT3ITD-/NPM1+* (ie *NPM1* mutation without FLT3-ITD mutation) have a favorable prognosis, while leukemias with high *EVI1+* mRNA expression show a very poor

prognosis. Combination of the cytogenetic, the WBC , the early or late CR and the molecular information led to the extended risk classification shown at the beginning of this appendix.

A summary of the OS and EFS of the patients in the previous HOVON/SAKK AML studies is shown in the table below for each of the risk (sub)groups. The most relevant estimates are OS2 and EFS2 which are the 5 year overall survival and event free survival measured from the start of consolidation treatment and which are restricted to patients who have reached a CR on protocol after cycle I or II and who received consolidation treatment. These are the patients for which a choice must be made between consolidation with chemotherapy cycle III, an autologous transplant or an allogeneic transplant. Estimates from diagnosis have been added for completeness, although at diagnosis knowledge about the achievement of (early) complete remission is still unavailable.

Risk			From diagnosis			From start consolidation	
			CR1	EFS1	OS1	EFS2	OS2
Good			**94***	**51**	**65**	**58**	**76**
	GR1	t(8;21), WBC≤20	94	59	68	66	75
	GR2	inv(16)/t(16;16)	93	44	68	50	77
	GR3	MK-, *CEBPA*+	84	48	61	59	67
	GR4	MK-, *FLT3ITD-/NPM1*+, CRe	100*	51	57	59	61
Inter-mediate			**99***	**42**	**51**	**48**	**55**
	IR1	t(8;21), WBC>20	87	32	46	35	50
	IR2	CN –X –Y, WBC≤100, CRe	100*	43	51	48	55
Poor			**75***	**19**	**25**	**27**	**33**
	PR1	CN –X –Y, WBC≤100, not CRe	69*	17	23	24	31
	PR2	CN –X –Y, WBC>100	74*	23	27	32	37
	PR3	CA, non CBF,MK-,no abn3q26, *EVI1*-	79	20	25	27	33
Very Poor			**60**	**3**	**7**	**7**	**12**
	VPR1	Non CBF, MK+	48	2	4	6	9
	VPR2	Non CBF, abn3q26	65	8	19	8	12
	VPR3	Non CBF, *EVI1*+	79	10	17	10	16

Table gives the outcome of therapy for each of the prognostic risk subgroups as regards CR, EFS and OS (from diagnosis) or from consolidation (EFS2, OS2)

CR1 % patients reaching CR after cycle I or cycle II

EFS1 actuarial probability of event free survival 5 year from diagnosis

OS1 actuarial probability of overall survival 5 year from diagnosis

EFS2 actuarial probability of event free survival 5 year from start consolidation

OS2 actuarial probability of overall survival 5 year from start consolidation

* Note that the risk classification includes early CR (after cycle 1) or late CR as a criterion for some classes. This has an impact on the estimates from diagnosis.

(C) ALLOSCT RISK

The acceptable risk of the AlloSCT is different for each relapse risk group and depends of the EBMT risk score and the HCT-CI score. This is described in paragraph 7.2.2. The EBMT risk score and HCT-CI risk score are given in Appendix E and Appendix F.

E. Risk of TRM after AlloSCT – EBMT score

EBMT risk score

If a donor is available, the EBMT risk score is calculated by counting up the scores given for each item.

Disease stage	
First complete remission	0
Second complete remission	1
All other	2
Age of patient	
< 20y	0
20-40y	1
> 40y	2
Time interval from diagnosis to transplant	
< 12 months	0
> 12 months	1(does not apply for 1st CR; always 0 for 1st CR patients)
Histocompatibility	
HLA- id sibling	0
Other	1
Donor recipient gender combination	
Other	0
Female donor for male recipient	1
Other factor:	
HLA 10/10 high resolution matched unrelated	- 1

TRM by Allo-SCT-EBMT score

In case disease stage is first CR and time interval from diagnosis to transplant is less than 12 months

	EBMT score 0 points	EBMT score 1-2 points	EBMT score 3-4 points
TRM	10-15%	15-25%	30-40%

(Gratwohl et al Cancer 2009)

F. Risk of TRM after AlloSCT – Comorbidity index

AlloSCT-Comorbidity Index (HCT-CI) as developed in Seattle

Comorbidity	Definitions of comorbidities included in the new HCT-CI	HCT-CI scores
Arrhythmia	Atrial fibrillation/flutter, sick sinus, ventricular arrhythmia	1
Cardiac	Coronary artery disease[a], congestive heart failure, MI or EF ≤ 50%	1
IBD	Crohn disease or ulcerative colitis	1
Diabetes	Requiring treatment with insulin or oral hypoglycemics but not diet alone	1
CVD	Transient ischemic attack or cerebrovascular accident	1
Psychiatric disturbance	Depression or anxiety requiring psychiatric consult or treatment	1
Hepatic, mild	Chronic hepatitis, bili > ULN and ≤ 1.5 x ULN , or AST/ALT > ULN and ≤ 2.5 x ULN	1
Obesity	Patients with a body mass index > 35 kg/m^2	1
Infection	Requiring continuation of antimicrobial treatment after day 0	1
Rheumatologic	SLE, RA, polymyositis, mixed CTD, or polymyalgia rheumatica	2
Peptic ulcer	Requiring treatment	2
Moderate/severe renal	Serum creatinine > 177 µmol/L, on dialysis, or prior renal transplantation	2
Moderate pulmonary	DLco and/or FEV1 66%-80% or dyspnea on slight activity	2
Prior solid tumor	Excluding nonmelanoma skin cancer	3
Heart valve disease	Except mitral valve prolapse	3
Severe pulmonary	DLco and/or FEV_1 ≤ 65% or dyspnea at rest or requiring oxygen	3
Moderate/severe hepatic	Liver cirrhosis, bilirubin > 1.5 x ULN, or AST/ALT > 2.5 x ULN	3
Total score		……..

MI indicates myocardial infraction; IBD, inflammatory bowel disease; EF.ejection fraction; CVD, cerebrovascular disease; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT alanine aminotransferase.

[a] One or more vessel-coronary artery stenosis requiring medical treatment, stent, or bypass graft.

Non-relapse mortality at 2 yrs according to HCT-Comorbidity Index

	Non-relapse mortality (%) by HCT-CI		
	0	1-2	≥ 3
Sorror et al. Blood 2006			
training set (n=708)			41-43
validation set (n=346)	14	19-22	40-41
Sorror et al. Blood 2007	7	19-21	27-37

G INSTRUCTIONS FOR LABORATORY INVESTIGATIONS (DIAGNOSTICS AND SIDE STUDIES)

Content

I. Table 1 (a+b) with summary of sampling time points for diagnostics and side studies

The tables below give an overview of sampling time points and can be used in the patient file.

Patient study number…….

Patient name code…….

Table 1a:

BM aspirate	Diagnostic/Side study	Baseline	treatment			
			cycle 1	**cycle 2**	**cycle 3 or auto/allo SCT**	**month 1 (after start cycle 3 or auto/allo SCT)=after repopulation**
Morphology	**Diagnostics (local)**	☐	from day 17 weekly till cycle 2	☐ 30days after diagnose (before start cycle 2)	☐ before start auto/allo SCT or cycle 3	☐
Smears for HRC review	**Diagnostics (local)**	☐				
Immunophenotyping	**Diagnostics (local)**	☐				
Cytogenetics	**Diagnostics (local)**	☐		**only in case of abnormalities:** ☐ 30days after diagnose (before start cycle 2) (optional)	**only in case of abnormalities:** ☐ before start auto/allo SCT or cycle 3 (optional)	
Molecular analysis	**Diagnostics (local or Rotterdam)**	☐				
Gene expression profiling	**Side study (Amsterdam)**	☐				
MRD assessment	**Side study (Amsterdam)**	☐		☐ 30days after diagnose (before start cycle 2)	☐ before start auto/allo SCT or cycle 3	

Patient study number…….

Patient name code…….

Table 1b:

BM aspirate	Diagnostic/Side study	follow up fase (= months after start cycle 3 or auto/allo-SCT)							
		month 4	month 8	month 12	month 18	month 24	month 36	month 48	In case of relapse
Morphology	**Diagnostics (local)**	☐	☐	☐	☐	☐	☐	☐	☐
Smears for HRC review	**Diagnostics (local)**								
Immunophenotyping	**Diagnostics (local)**								☐
Cytogenetics	**Diagnostics (local)**	**only in case of abnormalities** (optional) ☐							☐
Molecular analysis	**Diagnostics (local or Rotterdam)**								☐
Gene expression profiling	**Side study (Amsterdam)**								
MRD assessment	**Side study (Amsterdam)**	☐							☐

II. Summary of amounts of bone marrow (BM) and peripheral blood (PB) needed for local and/or external diagnostics and side studies

II.1. Hematocytologic analysis (local)

This is performed at the local institute according to local procedures using slides of BM and PB. Time points for hematocytologic analysis at diagnosis and response evaluation during treatment and follow-up are summarized in Table 1 above.
For review by Haematology Review Committee (HRC) to confirm WHO classification extra slides of BM and PB must be send to Erasmus MC Daniel den Hoed in Rotterdam (see AD II.1.1).

Guideline:

Sampling moment	**BM Sampling**	**PB Sampling**
At diagnosis	6 slides	4 slides

II.2. Immunophenotyping (local)

This is performed at diagnosis at the local institute according to local procedures using flowcytometry performed on BM en PB. Time points for immunophenotyping are indicated in Table 1.

II.3. Cytogenetic analysis (local)

This is performed at the local institute according to local procedures using amounts of BM and/or PB chosen at the local institute.

Guideline:

Sampling moment	BM Sampling	PB Sampling
At diagnosis	7 ml heparin BM	7 ml heparin PB
Only in case of abnormality: 30 days after diagnosis (before start cycle 2)	7 ml heparin BM	7 ml heparin PB
Only in case of abnormality: before start auto/allo SCT or cycle 3	7 ml heparin BM	7 ml heparin PB
Only in case of abnormality: 4 month after start cycle 3 or auto/allo	7 ml heparin BM	7 ml heparin PB
In case of relapse	7 ml heparin BM	7 ml heparin PB

II.4. Molecular diagnostics (local or Rotterdam)

This is performed at the local institute (local molecular diagnostics laboratory), except when novel assays necessary for HOVON-SAKK 102 have not yet been implemented locally. In that case samples should be sent directly to the EMC in Rotterdam (See Ad III.1.2).

Guideline:

Sampling moment	BM Sampling	PB Sampling
At diagnosis	7 ml EDTA BM	3 x 7 ml EDTA PB

II.5. Side Studies (Amsterdam)

Similar to HOVON-SAKK42A, HOVON-SAKK81 and HOVON-SAKK92, BM and PB should be send directly to the VUMC in Amsterdam (where the patient material is further processed for use in MRD as well as gene expression profiling studies, see Ad IV.4)

Guideline:

Sampling moment	BM Sampling	PB Sampling
At diagnosis*	20 ml heparin BM or (in case of dry tap) 20 ml heparin PB	10 ml EDTA PB
30 days after diagnosis (before start cycle 2)	10 ml heparin BM	10 ml EDTA PB
Before start auto/allo SCT or cycle 3	10 ml heparin BM	10 ml EDTA PB
Auto SCT	5 ml transplant	
4 month after start cycle 3 or auto/allo	10 ml heparin BM	10 ml EDTA PB
In case of relapse	10 ml heparin BM or (in case of dry tap) 10 ml heparin PB	10 ml EDTA PB

* Of the 20ml BM, 10 ml heparin BM will be used for MRD study (immunophenotypic and molecular MRD) and 10 ml heparin BM for gene expression profiling

III. Contact and shipping details

III.1. Shipment to Erasmus MC Rotterdam

III.1.1. Hematocytologic analysis (review)

Send 6 unstained and not fixated bone marrow slides and 4 blood slides, well packed up, together with a copy of the results of immunophenotyping, cytogenetics and molecular diagnostics to:

HOVON Haematology Review Committee (HRC)
T. de Jong
AKC Erasmus MC Daniel den Hoed
Room D1-89
P.O. Box 5201
3008 AE Rotterdam

Samples should be accompanied by a completely filled out registration form that can be downloaded from: www.hovon.nl

For questions please contact:
Trudi de Jong: (g.dejong@erasmumc.nl) +31.10.7041209 or
Dr. Mojca Jongen-Lavrencic (Co-ordinator HRC): m.lavrencic@erasmusmc.nl +31.10.7041367

III.1.2. Molecular Diagnostics

Address: Molecular Diagnostics, Department of Hematology, Erasmus Medical Center Rotterdam Room no. Ee1363, Dr. Molewaterplein 50, Rotterdam Z-H, 3015 GE The Netherlands (Phone: +31.10.704.3975/ Fax: +31.10.704.4745/ PJM Valk/ E-mail: p.valk@erasmusmc.nl)

Arrival of samples should be reported to the Molecular Diagnostics Laboratory (010-7043962).

Samples should be accompanied by a request form that can be downloaded from: www.hovon.nl <log in> > Studies > AML > HO102 (ho102_requestform_moldiag.pdf).

Samples should be shipped with the local courier.

At a later stage the Molecular Diagnostics Laboratory at Sanquin, Department of Immunocytology, Plesmanlaan 125, 1066 CX Amsterdam, The Netherlands (Phone: +31.20.5123390/

Fax:+31.20.5123474/ Rob Dee, Christa Homburg/ r.dee@sanquin.nl;c.homburg@sanquin.nl) will serve as second reference laboratory.

III.2. Shipment to VUmc Amsterdam (side studies)

Sampling conditions

All BM samples should be obtained preferably from the first tap, gathered in heparin-coated tubes and kept at room temperature. In any case it should be indicated whether the sample is from the first or second tap.

Announcement of a sample to VUmc:

Announcements are as in HOVON42A: the announcement of a forthcoming sample should be at least 1 day, preferably before 11:00 pm, prior to bone marrow aspiration. The expected time for a sample to be ready for transport and the place where it can be collected by the courier should be indicated on this announcement. The announcement should be done by phone or by email to:

MRD.info@vumc.nl

This email will arrive in the mailbox of all the members of the MRD-team: Angèle Kelder, Sander Snel, Willemijn Scholten, Arjo Rutten, Monique Terwijn and Gerrit Jan Schuurhuis

If for some reason (e.g. when a new patient presents with leukaemia), a forthcoming sample is announced on the same day (day 0) as the actual bone marrow aspiration, this should be done as early as possible in the morning, since hereafter VUmc has to inform the courier before 11.00 pm on day 0, in order to get the sample delivered at VUmc on day 1. When announced after 11.00 pm on day 0, the courier will collect it on day 1 and deliver it to VUmc on day 2, which is one day late. From this it follows that, whenever possible, we prefer to have bone marrow sampling on Thursday at last, since it can be delivered and processed at VUmc on Friday.

Please follow above mentioned procedure for announcing the sample since the actual MRD measurements should be done at last one day after the bone marrow collection.

Action of VUmc after the announcement of the center:

VUmc will contact the courier and provide these with the required information (location of the center and time of providing the sample). The courier will sent the necessary shipment papers by email on the morning of the transport. There are no financial actions for the participating center since all bills will be send to VUmc. The courier involved will be:

"Special Delivery eXchange" (SDX) in Utrecht, The Netherlands.
Tel: (+31) (0) 30-2410106
Fax: (+31) (0) 30- 2413311
E-mail: info@sdx.nl
Internet: www.sdx.nl

Sending of samples:

The samples have to be packed up (otherwise the courier is not allowed to transport it) together with the shipment papers and labeled with the address:

VU medical Center
Department of Hematology
To: A. Kelder
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands
(Tel: +31) (0) 20-4443836)

You will have to bring the sample to the arranged location (e.g. post office or porter) in the hospital, where it can easily be picked up by the courier for transport to VUmc.

For questions please contact:
Angèle Kelder (a.kelder@vumc.nl) (+31) (0) 20-4443836
Sander Snel (an.snel@vumc.nl) (+31) (0) 20-4443836
Gerrit Jan Schuurhuis (gj.schuurhuis@vumc.nl) (+31) (0) 20-4443838

IV. More detailed description of the diagnostic and side studies

IV.1. Hematocytologic analysis

Hematocytology analysis of bone marrow and peripheral blood should be performed at diagnosis and classified according to WHO and FAB classification. The HOVON Haematology Review Committee (HRC) will review diagnostic slides to confirm diagnosis and classification.

IV.2. Cytogenetic analysis

Conventional cytogenetic analysis should be performed in all patients at diagnosis and during treatment in case of an abnormality. For selected genetic abnormalities the use of molecular techniques will be required. In general, the results of the cytogenetic analysis should be known at approximately 2 weeks after diagnosis. This will permit the risk assessment.

Additional FISH analysis is recommended for the detection of abnormalities, which involve 11q23 (*MLL*) or 3q26 (*EVI1*). For patients with MDS (RAEB, RAEB-t) additional FISH analysis is required for the detection of –5/-7. The HOVON Cytogenetic Working Party will standardize conditions for FISH.

IV.3. Molecular diagnostics

Molecular diagnostics should be performed in all patients at diagnosis In general, the results of the molecular/cytogenetic analysis should be known at approximately 2-3 weeks after diagnosis. This will permit the risk assessment.

(RT-)PCR is obligatory for the detection of *AML1/ETO* (t(8;21)) and *CBFB/MYH11* inv(16), t(16;16)) fusion transcripts, the *FLT3* internal tandem duplication (ITD), mutations in *NPM1*, mutations in *CEBPA* and increased expression of *EVI1*. These analyses are carried out by the molecular diagnostic laboratories of the participating centers or the reference laboratories. The assays are carried out at diagnosis and the required sensitivity (positive cells diluted in negative cells) of all these assays is at least $1/10^2$.

Quality control rounds for these abnormalities will be conducted by the Dutch Network for Molecular Diagnostics of Hematological Malignancies (MODHEM).

Selected References Molecular Diagnostics

1. Levis M, Small D. FLT3: ITDoes matter in leukemia. Leukemia. 2003;17:1738-1752.
2. Nakao M, Yokota S, Iwai T, et al. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia. 1996;10:1911-1918.

3. van Waalwijk van Doorn-Khosrovani SB, Erpelinck C, Meijer J, et al. Biallelic mutations in the CEBPA gene and low CEBPA expression levels as prognostic markers in intermediate-risk AML. Hematol J. 2003;4:31-40
4. Preudhomme C, Sagot C, Boissel N, et al. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood. 2002;100:2717-2723.
5. Barjesteh van Waalwijk van Doorn-Khosrovani S, Erpelinck C, van Putten WL, et al. High EVI1 expression predicts poor survival in acute myeloid leukemia: a study of 319 de novo AML patients. Blood. 2003;101:837-845.
6. van der Reijden BA, Massop M, Tonnissen E, et al. Rapid identification of CBFB-MYH11-positive acute myeloid leukemia (AML) cases by one single MYH11 real-time RT-PCR. Blood. 2003;101:5085-5086.

IV.4. Side Studies

The side studies in the HOVON-SAKK AML 102 protocol include both the assessment of minimal residual disease (MRD) and Gene expression profiling (see below for details). Material should be sent to the VU University Medical Center (VUMC), where further processing of the material for MRD analysis and gene expression profiling will occur.

IV.4.1. Assessment of minimal residual disease (MRD)

You can find background information regarding immunophenotypic MRD and detailed procedures on the website: http://www.vumc.nl/afdelingen/hematologie/onderzoek/mrd/

Patient samples collected at diagnosis and follow-up for MRD analysis are processed as follows: first, a sufficient amount of cells is measured directly for immunophenotypic MRD. Second, material is frozen for molecular MRD analysis: for DNA analysis, 200 ul whole bone marrow is frozen at -20ºC, for RNA analysis, 10x 10^6 WBCs (in 150 ul) are frozen (-80ºC) in 1 ml RNAbee.

For additional questions please contact:

Angèle Kelder (a.kelder@vumc.nl) (+31) (0) 20-4443836
Sander Snel (an.snel@vumc.nl) (+31) (0) 20-4443836
Gerrit Jan Schuurhuis (gj.schuurhuis@vumc.nl) (+31) (0) 20-4443838

Selected References MRD

1. Venditti, F. Buccisano F, G. Del Poeta, L. Maurillo, A. Tamburini, C. Cox, A. Battaglia, G. Catalano, B. Del Moro, L. Cudillo, M. Postorino, M. Masi, S. Amadori. Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood 96: 3948-3952 (2000).

2. J.F. San Miguel, M.B. Vidriales, C. Lopez-Berges, J. Diaz-Mediavilla, N. Gutierrez, C. Canizo, F. Ramos, M.J. Calmuntia, J.J. Perez, M. Gonzalez, A. Orfao. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood 98: 1746-1751 (2001).
3. E.L. Sievers, B.J. Lange, T.A. Alonzo, R.B. Gerbing, I.D. Bernstein, F.O. Smith, R.J. Arceci, W.G. Woods, M.R. Loken. Immunophenotypic evidence of leukemia after induction therapy predicts relapse: results from a prospective Children's Cancer Group study of 252 patients with acute myeloid leukemia. Blood 101: 3398-3406 (2003).
4. W. Kern, D. C. Voskova, W. Schoch, S. Hiddemann, T. Schnittger, Haferlach T. Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. Blood 104: 3078-3085 (2004). Epub 2004 Jul 29.
5. N. Feller, M.A. van der Pol, A. van Stijn, G.W.D. Weijers, A.H. Westra, B.W. Evertse, G.J. Ossenkoppele, G.J. Schuurhuis. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukemia. Leukemia 18: 1380-1390 (2004).
6. N. Feller, G.J. Schuurhuis, M.A. van der Pol, G. Westra, G.W.D. Weijers, A. Van Stijn, P.C. Huijgens, G.J. Ossenkoppele. High percentage of CD34-positive cells in autologous AML peripheral blood stem cell products reflects inadequate in vivo purging and low chemotherapeutic toxicity in a subgroup of patients with poor clinical outcome. Leukemia 17: 68-75 (2003). See also Commentary by T de Witte. Leukemia 17: 39-40 (2003).
7. Venditti, L. Maurillo, F. Buccisano, G. Del Poeta, C. Mazzone, A. Tamburini, M.I. Del Principe, M. Consalvol, P. De Fabritiis, L. Cudillo, A. Picardi, A. Franchi, F. Lo-Coco, S. Amadori, F.L. Coco. Pretransplant minimal residual disease level predicts clinical outcome in patients with acute myeloid leukemia receiving high-dose chemotherapy and autologous stem cell transplantation. Leukemia 17: 2178-2182 (2003).
8. N. Feller, M.A. van der Pol, A. van Stijn, G.W.D. Weijers, A.H. Westra, B.W. Evertse, G.J. Ossenkoppele, G.J. Schuurhuis. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukemia. Leukemia 18: 1380-1390 (2004).

IV.4.2. Gene expression profiling

AML blasts at diagnosis and at relapse will be analyzed for gene expression profiles on the Affymetrix platform.

Whole genome transcriptional profiling with Affymetrix HGU Plus2.0 GeneChips (will be carried out to establish the level of over 47,000 transcripts and variants, representing 19.000 unique genes). The aim of this exploratory analysis is to further develop a molecular classification of AML, validate prognostic signatures identified in previous studies and identification of novel candidate markers that predict patient response to treatment.

For questions about the gene expression profiling analyses please contact:

Dr. Peter Valk (p.valk@erasmusmc.nl) (+31) (0) 10-7043975

Selected References Gene Expression Profiling

1. Haferlach T, Kohlmann A, Kern W, et al. Gene expression profiling as a tool for the diagnosis of acute leukemias. Semin Hematol. 2003;40:281-295.
2. Kohlmann A, Schoch C, Schnittger S, et al. Molecular characterization of acute leukemias by use of microarray technology. Genes Chromosomes Cancer. 2003;37:396-405.
3. Schoch C, Kohlmann A, Schnittger S, et al. Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. Proc Natl Acad Sci U S A. 2002;99:10008-10013.
4. Valk PJ, Verhaak RG, Beijen MA, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med. 2004;350:1617-1628.
5. Bullinger L, Dohner K, Bair E, et al. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. N Engl J Med. 2004;350:1605-1616.
6. Debernardi S, Lillington DM, Chaplin T, et al. Genome-wide analysis of acute myeloid leukemia with normal karyotype reveals a unique pattern of homeobox gene expression distinct from those with translocation-mediated fusion events. Genes Chromosomes Cancer. 2003;37:149-158.
7. Ross ME, Mahfouz R, Onciu M, et al. Gene Expression Profiling of Pediatric Acute Myelogenous Leukemia. Blood. 2004; 104: 3679-3687
8. Lacayo NJ, Meshinchi S, Kinnunen P, et al. Gene Expression Profiles at Diagnosis in de novo Childhood AML Patients Identify FLT3 Mutations with Good Clinical Outcomes. Blood. 2004.
9. Yagi T, Morimoto A, Eguchi M, et al. Identification of a gene expression signature associated with pediatric AML prognosis. Blood. 2003;102:1849-1856.
10. R.G.W.Verhaak, C.S.Goudswaard, W.van Putten, M.A.Bijl, M.A.Sanders, W.Hugens, A.G.Uitterlinden, C.A.J.Erpelinck, R.Delwel, B.Löwenberg, P.J.M.Valk – Mutations in nucleophosmin NPM1 in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood 2005, 106, 3747-3754.
11. Wouters BJ, et al Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1, BLOOD 2007; 110: 3706 - 3714
12. Wilson CS, George S. Davidson, Shawn B. Martin, Erik Andries, Jeffrey Potter, Richard Harvey, Kerem Ar, Yuexian Xu, Kenneth J. Kopecky, Donna P. Ankerst, Holly Gundacker, Marilyn L. Slovak, Monica Mosquera-Caro, I-Ming Chen, Derek L. Stirewalt, Maurice Murphy, Frederick A. Schultz, Huining Kang, Xuefei Wang, Jerald P. Radich, Frederick R. Appelbaum, Susan R. Atlas, John Godwin, and Cheryl L. Willman.Gene expression profiling of adult acute myeloid leukemia identifies novel biologic clusters for risk classification and outcome prediction.Blood, Jul 2006; 108: 685 - 696

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H Common Terminology Criteria for Adverse Events

The grading of toxicity and adverse events will be done using the most recent version of the NCI Common Terminology Criteria for Adverse Events, CTCAE version 4. A complete document may be downloaded from the following sites:

http://ctep.cancer.gov/reporting/ctc.html
http://www.hovon.nl under 'Studies' > 'Algemene studie-informatie'
'Trials' > 'General information about studies'

I ZUBROD-ECOG-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