Randomized induction concerning the value of G-CSF priming in adult patients (≤ 60 yrs of age) with acute myelocytic leukemia (AML) or refractory anemia with excess of blasts (RAEB, RAEB-t) with IPSS score ≥ 1.5

A phase III study.

PROTOCOL

Study Coordinators : B. Löwenberg
M.A. Boogaerts
E. Vellenga
J. Gmür
T. Pabst

Statistician : W.L.J. van Putten

Datamanagers : S. Ramnarain
L. el Jarari

Registration : HOVON Data Center
Erasmus MC - Daniel den Hoed
P.O. Box 5201
3008 AE ROTTERDAM
The Netherlands
tel. +31.10.4391568
fax +31.10.4391028
https://www.hdc.hovon.nl/top

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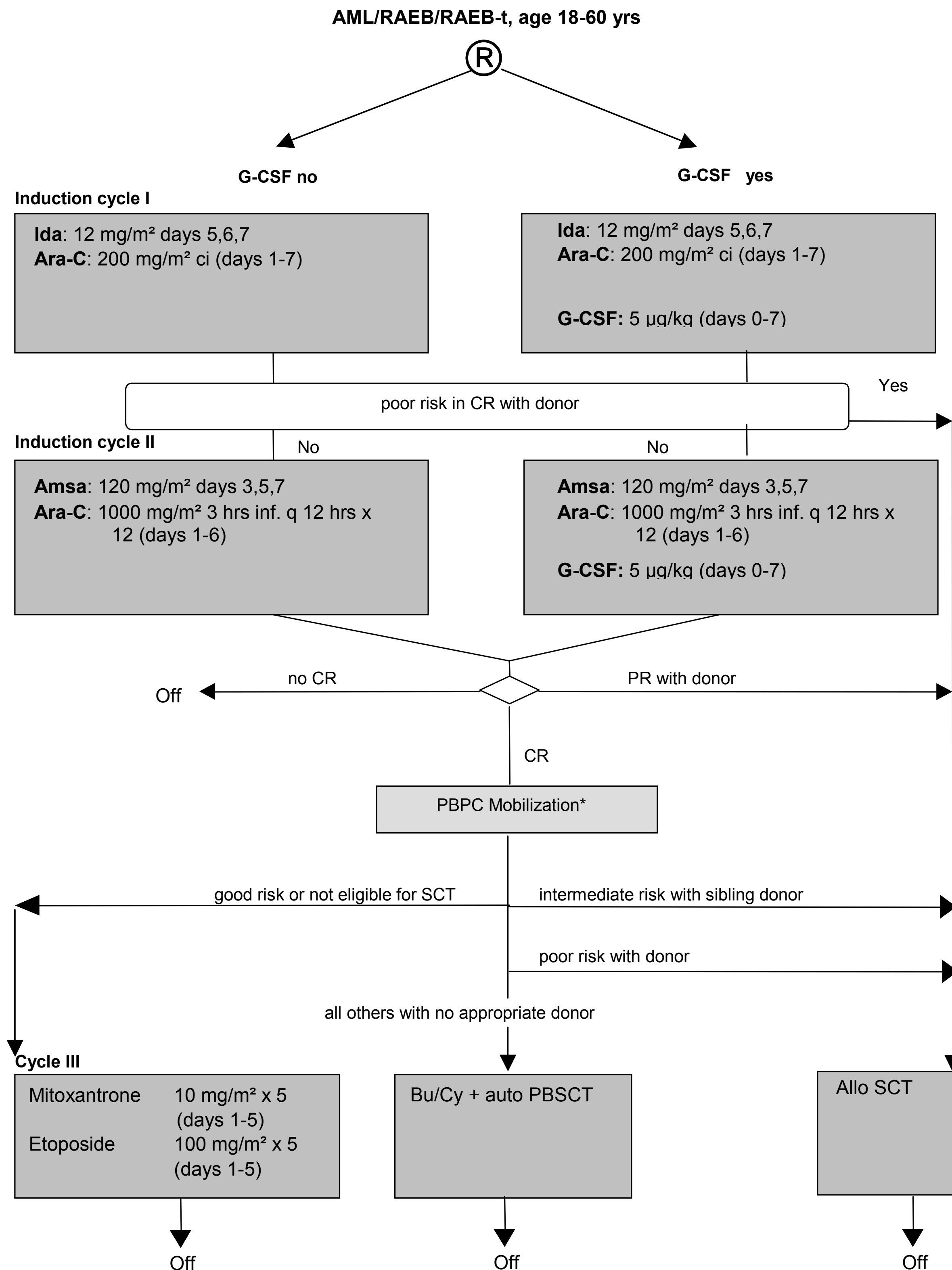
1 Scheme of study



*unless known as good risk or to proceed to Allo SCT

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3 Synopsis

Study phase	Phase III
Study objectives	• Evaluation of G-CSF priming in induction cycles I and II. • Evaluation of the effect of allogeneic sibling or unrelated donor SCT in subgroups of patients with AML of intermediate or poor risk. • Assessment of molecular markers and gene expression profiles at diagnosis • Assessment of minimal residual disease with immunophenotyping and molecular analysis during patient follow up
Patient population	Patients with AML (except FAB M3 or t(15;17)), RAEB or RAEB-t with IPSS ≥ 1.5, previously untreated, age 18-60 years (incl.)
Study design	Prospective, multicenter, randomized with randomization between yes or no G-CSF priming.
Duration of treatment	Expected duration of induction cycles I and II inclusive evaluation is about 3 months. Consolidation treatment will take an additional 1-3 months.
Number of patients	Total number registered: 1440 167 patients entered before first amendment; 820 randomized for standard vs escalated dose Ara-C since first amendment, of which 368 also randomized between yes/no G-CSF priming and 190 patients randomized between between cycle III and PBSCT; 453 patients registered and randomized after amendment 5 between yes/no G-CSF priming
Adverse events	Adverse events will be documented if observed, mentioned during open questioning, or when spontaneously reported.
Planned start and end of recruitment	Start of recruitment: I 2001 End of recruitment: II 2008

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Study Coordinators	B. Löwenberg	Erasmus MC
	J. Maertens	University Hospital Leuven
	E. Vellenga	University Medical Center Groningen
	T. Pabst	University Hospital Bern
Writing Committee	S.M.G.J. Daenen, E. Vellenga	University Medical Center Groningen
	A.W. Dekker, L.F. Verdonck	University Hospital Utrecht
	P.C. Huijgens, G.J. Ossenkoppele	VU University Medical Center
	J. van der Lelie	Amsterdam Medical Center
	H.C. Schouten	University Hospital Maastricht
	G.E. de Greef, B. Löwenberg, P. Sonneveld, J.M.J.M. Zijlmans	Erasmus MC
	P.W. Wijermans	Leyenburg Hospital, The Hague
	S. Wittebol	Hospital Eemland, Amersfoort
	M.R. Schaafsma	Medisch Spectrum Twente, Enschede
	J. Maertens, G.E.G. Verhoef	University Hospital Leuven
	A. Ferrant	Cliniques Universitaires St. Luc, Brussels
	T. Pabst, M.F. Fey	University Hospital Bern
	J. Gmür	Zentrum für ambulante Chirurgie und Medizin, Zürich
	E. Jacky	University Hospital Zürich
	A. Gratwohl	University Hospital Basel
	M. Theobald	Johannes Gutenberg Universität, Mainz
Cytogenetics review	Drs A Nieuwint	VU University Medical Center
	Dr C. Mellink, AMC	Amsterdam Medical Center
	Dr HB Beverloo	Erasmus MC
Cytological and immunophenotype review	M.B. van 't Veer	Hematocytology Review Committee, University Hospital Rotterdam
Statistician	W.L.J. van Putten	HOVON Data Center, Rotterdam
Datamanagement	S. Ramnarain, L. el Jarari	HOVON Data Center, Rotterdam
Serious Adverse Events (SAEs) notification	HOVON Data Center	fax: +31 10 4391028

4.1 Cytological and immunophenotype review

4 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. M.B. van ‘t Veer, Hematocytology Review Committee, Erasmus MC – Daniel den Hoed, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands, at the time of registration. Confirmation of diagnosis is not necessary for randomization and start of treatment.

4.2 Cytogenetic review

Each cytogeneticist, responsible for the cytogenetic analysis of the AML 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 3 months to the HOVON Data Center for central review.

5 Introduction

In this section of the amended protocol, parts that are no longer relevant for the remainder of the study will be preceded with the note “Only till amendment 5”. Other sections have been modified when needed.

5.1 Previous study in adults with newly diagnosed AML

The previous study of the HOVON and SAKK cooperative groups (HOVON/SAKK AML 29) in previously untreated adult patient with acute myeloid leukemia (AML) has accrued 1000 patients of whom 640 patients took part in the G-CSF priming study. The evaluation of the study as regards the use of G-CSF in induction has shown a favourable effect of G-CSF priming on the risk of relapse following complete remission as well as an improved disease free survival (see B. Löwenberg et al, NEJM 2003). In a subgroup analysis the benefit was particularly apparent as an advantage as regards improved overall survival, event free survival as well as disease free survival in intermediate risk AML (= 72% of patients). The randomization for induction has been closed per December 1, 1998, and the final evaluation came only recently available with 50 months median follow up. Patients in the study were also randomized for the post induction question. This question involves a randomization of a post-induction chemotherapy cycle versus autologous peripheral blood stem cell transplantation. Patients with an HLA identical sibling donor have the option of an allogeneic stem cell graft unless they are classified as “good-risk AML”.

Only till amendment 5:

In the AML-42 study of the HOVON and SAKK cooperative groups the post-remission randomization of peripheral blood stem cell transplantation (PBSCT) versus chemotherapy will be extended. This will be done with the purpose of increasing numbers of patients and enhancing the power of the final analysis and permitting also an analysis of the value of PBSCT among distinct prognostic subgroups. From the recent AML collaborative intergroup analysis (AMLCG, AML Collaborative Group, Oxford, September 1998) involving several major cooperative study groups in acute myeloid leukemia, it appears that relapse is reduced following autologous bone marrow transplantation. However, because of an increase of the treatment related mortality there is no or a minor evidence of an improvement of the overall survival. Certain major questions remain to be sorted out: does an overall survival benefit of autologous stem cell transplantation exist for prognostic subsets of patients with AML, and would the use of peripheral stem cell grafts (instead of bone marrow) reduce the treatment related mortality, while maintaining the advantage of a reduction in the risk of relapse. The last question is addressed by the HOVON/SAKK 29 with currently over 200 patients randomized, which as yet, is insufficient for definitive conclusions. Therefore it would be useful to continue collecting data on the issue of autologous peripheral stem cell transplantation in AML, in order to have greater numbers both overall and in specific subsets patients.

5.2 Refractory anemia with excess of blasts (RAEB) and RAEB-in-transformation

The myelodysplastic syndromes are a heterogeneous group of hematopoietic disorders. In 1982 the French-American-British (FAB) Group has presented a classification which distinguishes RAEB and RAEB-t as prognostically most unfavourable forms[(9)]. RAEB and RAEB-t frequently evolves to AML and survival prospects resemble those of AML[(9,10,18)]. The similarity between RAEB, RAEB-t and AML has also been emphasized by a recent World Health Organization Steering Group that has proposed to include RAEB-t in the diagnosis AML[(8)]. The International Prognostic Score Index (IPSS) for MDS appreciates the heterogeneity of prognosis of all forms of MDS and distinguishes low-risk, intermediate-low, intermediate-high, high-risk classes of patients[(10)]. RAEB and RAEB-t with IPSS of $\geq$1.5 fall in the high-risk category and these patients are generally treated according to AML-type therapy approaches[(18,19)].

5.3 Remission induction

only till amendment 5:

In the next study a new induction question will be addressed. An important question concerns the intensity of the chemotherapy induction schedule . Two studies have applied high-dose cytarabine (Ara-C) in induction[1,2] . Both studies have shown similar overall complete response rates in both treatment arms. However, remissions were more rapidly attained. Thus, the results of these studies provided evidence for a greater probability of complete remission following one (the first) induction cycle. They also provided support for a reduced probability of relapse among complete responders following the high-dose cytarabine induction arm. However this benefit was offset by the disadvantage of more hematological and CNS (central nervous system) toxicity. As a result there was no advantage as regards overall survival. Another phase III study has applied high-dose Ara-C post induction[3]. The results of the latter study indicated an advantage for high-dose Ara-C post remission, i.e., a reduced probability of relapse (in adults less than 60 years). The EORTC has recently piloted high-dose Ara-C in induction (3 g/m^2 x 8) in combination with VP16 and DNR and demonstrated acceptable toxicity and satisfactory stem cell mobilization for autologous transplantation (personal communication). A preliminary comparison of trials with more intensive induction programs with those of less intensive induction schedules suggests that the more intensive induction programs result in better survival (AMLCG, Oxford 1998). However, these latter analyses are not based on complete individual patient data and comprise a mixed bag of induction schedules and therefore only provide circumstantial evidence in favour of more intensive induction therapy. Even though extensive experience exists in various trial groups with higher doses of cytarabine in induction, the HOVON/SAKK groups have recently completed a pilot study among 33 patients with untreated AML in order to assess the feasibility of the new schedule. The dose level of cytarabine in cycle I was 1000 mg/m^2 and in cycle II it was set at 3000 mg/m^2. The results of the latter study show that the dose-intensified cycle I does not lead to more side effects, prolonged cytopenia or more deaths. After cycle II, platelet recovery, not neutrophil recovery, was delayed. Platelets recovered to 50 x 10^9/l at a median of 40 days as compared to a historical value of 28 days. There was also more non-hematological toxicity after cycle II, especially more grade III-IV infections, and the successful mobilization of progenitor cells after cycle II was less than after standard Ara-C. However, it should be noted that patients entering the pilot study appeared older than those of the previous HOVON-SAKK AML-29 study and there were no good risk (as assessed by cytogenetics) cases among them, suggesting that a relatively high-risk population had been entered into the pilot study. Nevertheless, after two cycles the cumulative CR rate was entirely satisfactory (70%). Based on these data, we elected to set the doses of cytarabine (only till amendment 5) in the experimental arms of the new study respectively at 1000 mg/m^2 (cycle I) and

2000 mg/m^2 (cycle II). The incidence of adverse events will be monitored in the AML-42 study (see paragraph 13).

Since the availability of the final analysis of the G-CSF priming part of the AML-29 study and the evidence for a positive effect of priming on relapse and disease free survival, it has been decided to add the priming question again to the current study as well. This will be done for a number of reasons. First of all, the HOVON-SAKK AML-29 study was indicative of a disease-free survival advantage in favour of the priming approach but the overall survival, although showing a trend in favour of priming, was not significantly different. Therefore a confirmatory study is considered desirable for establishing G-CSF priming as an accepted treatment modality in AML.
Second, the benefit of G-CSF priming was restricted to the intermediate prognostic risk category of AML. It would be of interest to see whether this effect in relation to risk class can be reproduced in another study.
Only till amendment 5:
Thirdly, in the AML-29 study 200 mg/m2 cytarabine (cycle I) and 2000 mg/m2 cytarabine (cycle II) have been employed but the dependence of G-CSF chemotherapy priming on cytarabine dose level, has remained an important question for future study. Is the priming effect of G-CSF restricted to the lower dose levels of cytarabine, or is the effect also sorted out in the context of 2000 mg/m2 or higher levels? The HOVON-SAKK AML-42 study offers an opportunity to address the latter question as well (only till amendment 5; see 5.6).

5.4 Risk Groups

5.4.1 Good risk AML

From the previous HOVON-SAKK AML studies as well as several other trials it has become clear that a certain subset of patients with AML who enter remission, have a comparatively low probability of relapse[4,13-17]. For instance, patients with translocation t(15;17) or FAB M3 and no leucocytosis have a favourable prognosis. However, these patients are treated with ATRA and are not included in this study. Other patients with a low probability of relapse are patients with favourable cytogenetic abnormality t(8;21) together with a WBC of 20 x 10^9 or less at diagnosis and patients with inv(16) or del(16). These patients have a probability of remaining in CR of approximately 70% (Appendix G). These “good-risk” patients representing approximately 10% of complete remitters, apparently benefit from modern chemotherapy, so that there appears no compelling need for immediate transplantation. As a matter of fact the hazards of intensive transplantation programs probably do not outweigh the risk of relapse in these individuals. For

instance HLA matched allogeneic marrow transplantation, even though it reduces the probability of relapse, is associated with a 20-25% mortality due to non-leukemic factors and autologous marrow transplantation in multicenter studies with an approximately 10% procedure related mortality. Therefore, in good risk patients the option of transplantation will be reserved for those relapsing.

5.4.2 Poor risk AML/poor risk RAEB/RAEB-t

At the other end of the spectrum is the unfavourable prognostic subgroup, which includes approximately 33% of the cases among patients between 15-60 yrs of age (see Appendix G). These unfavourable cases are defined in the first place by the presence of unfavourable cytogenetic abnormalities involving three or more different clonal abnormalities (complex abnormalities), monosomies of chromosomes 5 or 7, deletions of the long arms of chromosomes 5 (del 5q) or 7 (del 7q), abnormalities of the long arm of chromosome 3, abnormalities of the long arm of chromosome 11 (abn 11q23), translocation t(9;22) or translocation t(6;9). Their relapse rate is approximately 80% and survival at 5 yrs is less than 25%. This group is about 8% of the patients reaching CR. A second subset with a high risk of relapse are the patients not classified as good risk who reach CR only after the second cycle (see Appendix G for a more precise definition). This last group is about 25% of the patients reaching CR. Together these two groups form about 33% of complete remitters. Other potentially relevant factors, e.g., FAB-M0 AML, FAB-M6 AML, FAB-M7 AML will not be considered high risk since prognostic estimations as yet are based upon small series of patients and their independent prognostic value (e.g., independent from cytogenetics) needs additional investigation. For patients in first CR with unfavourable prognosis further improvements may be sought for by the introduction of allogeneic stem cell grafts as early as possible.

As patients with secondary AML, i.e., AML evolving from an antecedent hematological disorder (e.g. MDS of at least 6 months duration) or following a history of chemotherapy for a non-leukemic condition, have often been treated on separate protocols, their prognostic value has not been independently assessed in comparison with other hematological or cytogenetic parameters in multivariate analysis. These patients will therefore be classified according to the same criteria as patients with primary AML. The same approach will be followed as regards patients presenting with MDS (RAEB or RAEB-t).
The results of a variety of studies (MRC, CALG-B and HOVON-SAKK cooperative groups) consistently indicate that the subset of AML with t(9;22) or with the fusion gene BCR-ABL have an

extraordinary poor prognosis (high relapse, minimal survival). Thus AML t(9;22) will be included in the poor risk category.

5.4.3 Intermediate risk AML

Any patient not meeting the criteria of AML good-risk nor poor-risk (see above paragraph 5.4.1 and 5.4.2, and Appendix G) will be considered as intermediate risk.

5.5 Post remission stem cell transplantation

There is evidence e.g. from the AMLCG (AML Collaborative Group) Intergroup Analysis, that in patients receiving stem cell allografts relapse is reduced and survival improved in patients with intermediate risk and poor risk AML. Thus in these individuals as first options the possibilities of HLA identical sibling and HLA identical unrelated donor transplants will be offered respectively.
Only till amendment 5 (see 5.6):
If these options are not available (because of age, no matched donor), patients will be randomized between additional chemotherapy and autologous stem cell transplantation . In recent years autologous bone marrow transplantation schedules have increasingly been applied to patients with AML in first CR and current evidence would suggest a limited antileukemic superiority of auto BMT over chemotherapy. However, the advantage of auto BMT appears offset by three negative factors: 1) auto BMT is associated with enhanced (approximately 10%) mortality, 2) hematopoietic repopulation following auto BMT is generally slow and 3) auto BMT in clinical practice (due to refusal, collection of inadequate graft, early relapse), can be offered to only a minority (30-40%) of CR patients. This as a consequence restricts the applicability of auto BMT[4]. Thus the superiority of auto BMT over chemotherapy has not been definitely established. Auto BMT would make more sense if more patients with AML in CR would have access to it.

Peripheral blood stem cell transplants (PBSCT) when collected at an earlier time during remission may potentially circumvent several of the drawbacks of auto BMT. G-CSF when applied following chemotherapy has proved an effective hematopoietic growth factor for mobilization of hematopoietic stem cells for subsequent collection, storage and reinfusion (peripheral blood stem cell transplantation, PBSCT). PBSCT generally gives better hematopoietic recovery and the withdrawals appear less frequent[5]. Thus PBSCT appears available to more patients and clinically more easy and less toxic to apply. In this study (AML-42) the randomization of intermediate risk and poor risk patients with AML in first CR will continue (Only till amendment 5).

In intermediate risk and poor risk patients the results of treatment of marrow ablative chemotherapy and autologous PBSCT will be compared with the outcome after conventional chemotherapy in a controlled study. In addition, in an uncontrolled comparison the results of allogeneic marrow transplantation will also be evaluated in those who have an HLA matched related or unrelated donor and satisfy the proper age criteria.

5.6 Modifications with amendment 5.

The target number of patients (800 + 20 for compensation for roughly 2% ineligible patients) randomized between standard and escalated dose Ara-C after will be reached beginning of December 2005. At that moment randomization between standard and escalated dose Ara-C will be stopped. Because the G-CSF induction question for treatment of adult AML patients will be carried over to the next protocol, there is no reason to start a new study. Instead this study will be continued with only the priming question by randomisation until the target number of patients for that question will be reached. A preliminary comparison of the outcomes in the arms with standard and escalated dose Ara-c based on the available data indicated no difference in CR rate or EFS or OS between both arms but a moderately increased incidence of side effects in the escalated arm. Therefore - for the time being - the standard dose Ara-C will be the backbone of induction treatment for the remaining part of the study.
A preliminary analysis of the combined data of the HOVON/SAKK AML 29 and the AML 42 concerning the value of autologous PBSCT indicated an improved DFS but no difference in OS. This preliminary analysis is based on the available data of 513 patients (of which 171 randomized in the AML 42). These preliminary results are in line with the results from other studies which indicated an improved DFS following autologous BMT, but no benefit in terms of OS. After 10 years of randomization between consolidation with cycle III or autologous PBSCT in the AML 29 and 42 together, this randomization will now be closed for patients entered after the activation of the amendment 5. Given the improved DFS in the autologous PBSCT arm, this treatment will be the consolidation treatment of choice for intermediate and high risk patients without a suitable allogeneic donor for the remainder of the AML 42.

Molecular diagnostic information was already collected in the first part of the AML 42. However, new prognostically important markers (*FLT3-ITD, EVI-1, NPM1,* C/EBP-alpha) have appeared in the meantime, which are not yet routinely determined by all participants. [21-23] For the remainder of this study the assessment of molecular markers and shipment of samples to laboratories will be closely monitored in collaboration with the MODHEM. Details are in Appendix F.

Gene expression profiling (GEF) on DNA microarrays may provide additional prognostic and diagnostic information in AML, especially in the approximately 40% of cases of AML without cytogenetic or molecular markers.
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 (GEF) can be expected to add an essential and indispensable integrated element in the diagnostic and therapeutic decision making process in the foreseeable future.[24,25] 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. All participants are requested to send in samples for GEF assessment . Details are in Appendix J.

Minimal residual disease (MRD) detection in acute myeloid leukemia (AML) using PCR based techniques is applicable only in a minority of cases. MRD detection using multiparameter flowcytometry, using aberrant phenotypes defined at diagnosis, is applicable in roughly 80% of the cases and has been shown to offer a strong prognostic factor independent of other prognostic factors in both adult and childhood AML. [26-30] This prognostic factor is not derived at diagnosis but along the course of the treatment. Assessments of MRD in bone marrow (BM) samples taken sequentially after different courses of therapy [26-30, 32-33], and in harvests of stem cell transplants[31] have been investigated. This technique will now be introduced in the remainder of the AML 42 protocol. In all cases bone marrow sampling includes diagnosis, after each cycle of therapy and, whenever possible, at relapse. In addition autologous stem cell transplants will be analyzed. Details are in Appendix K.

6 Study objectives

- To study in a randomized comparison the use of granulocyte-colony stimulating factor (G-CSF) for priming during induction cycles I and II as regards the complete remission rate, disease free survival, risk of relapse and overall survival.
- To evaluate the outcome of allogeneic sibling SCT in intermediate risk patients and compare the results with those after chemotherapy and ablation + PBSCT

- To assess the value of early allogeneic family donor and unrelated donor SCT in patients with poor risk AML in comparison to the results in those treated with chemotherapy and ablation + PBSCT
- To determine the prognostic value of molecular markers and gene expression profiles of the leukemia assessed at diagnosis
- To assess minimal residual disease following therapy by standardized sampling of marrow/blood

7 Study design

Details of all treatments (dose and schedule) are given in 9.1-9.5.

7.1 Remission induction

- Patients with AML (Appendix A.1), except those with FAB M3 or t(15;17) or with a PML/RAR alpha or a variant RAR alpha fusion gene and patients with RAEB or RAEB-t with an IPSS of ≥1.5 (Appendix B), meeting all eligibility criteria (see 8.1) will be randomized on entry between:

Arm 1 induction treatment without G-CSF priming
Arm 2 induction treatment with G-CSF (filgrastim) priming

The induction treatment consists of
Cycle I: idarubicin and conventional dose cytarabine
Cycle II amsacrine and intermediate dose cytarabine

Arm 1 is the same induction schedule as used in the previous HOVON/SAKK AML 29 study and AML 42 prior to amendment..
Patients will be evaluated for response after cycle I and cycle II. All patients who have not attained a complete remission (CR) after cycle II, will go off study.

7.2 Risk assessment and post remission treatment

All CR patients will be distinguished according to good risk, intermediate risk, and poor risk features (see appendix G).

- Good risk patients will receive a third cycle of chemotherapy (cycle III: mitoxantrone plus etoposide).
- Intermediate or poor risk patients with a HLA matched family donor will proceed to allogeneic stem cell transplantation.
- Poor risk patients without a HLA matched sibling donor, but with a phenotypically matched unrelated donor may proceed to marrow ablative treatment and allogeneic stem cell transplantation as soon as they have entered CR. If patients are already distinguished as poor risk following cycle I and logistically there are no impediments the patient may proceed to Allo SCT as soon as possible after cycle I.
- All other patients in CR, except patients who refuse stem cell transplantation, will undergo stem cell mobilization with G-CSF and stem cell collection. Patients with an adequate harvest will receive consolidation with autologous PBSCT.
- Patients who are not eligible for autologous or allogeneic SCT will receive cycle III as consolidation treatment.

Poor risk patients in PR after cycle II with a HLA matched family donor or with a phenotypically matched unrelated donor may proceed to allogeneic stem cell transplantation.

8 Study population

8.1 Eligibility for registration

All eligible patients have to be registered and randomized before start of treatment (see 15)

8.1.1 Inclusion criteria

- ♦ Age 18-60 years (incl.)
- ♦ Subjects with a cytopathologically confirmed diagnosis of
  - (a) AML (M0-M2 and M4-M7, FAB classification, appendix A), or
  - (b) with refractory anemia with excess of blasts (RAEB) or refractory anemia with excess of blasts in transformation (RAEB-t) with an IPSS score of $\geq$1.5 (appendix B)
- ♦ Patients with therapy-related AML/RAEB/RAEB-t are eligible provided they have not received chemotherapy during the past 6 months. Also patients with biphenotypic leukemia may be included.

- Subjects with a secondary AML progressing from antecedent myelodysplasia are eligible. Antecedent MDS refers to a condition of at least 4 month duration
- WHO performance status ≤ 2 (see appendix E)
- Written informed consent

8.1.2 Exclusion criteria

- Prior chemotherapy within 6 months of study entry
- Relapse of AML or MDS after induction chemotherapy
- Prior stem cell transplant
- Previous polycythemia rubra vera
- Primary myelofibrosis
- Blast crisis of chronic myeloid leukemia
- AML-FAB type M3 or AML with cytogenetic abnormality t(15;17) or AML with a PML/RAR alpha or a variant RAR alpha fusion gene
- Impaired hepatic or renal function as defined by:
  - ALT and/or AST > 3 x normal value
  - Bilirubin > 3 x normal value
  - Serum creatinin > 3 x normal value (after adequate hydration), (unless these are most likely caused by AML organ infiltration)
- Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, infection, hypertension, etc.)
- 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)
  - Unstable angina
  - Unstable cardiac arrhythmias
- Pregnancy

9 Treatments

9.1 Remission induction treatment cycle I

Arm 1 Idarubicin and conventional-dose Cytarabine

Arm 2 Idarubicin and conventional-dose Cytarabine and G-CSF (filgrastim)

Agent	Dose/day	Route	Days
Idarubicin	12 mg/m^2	3 hr infusion	5, 6, 7
Cytarabine	200 mg/m^2	24 hr infusion	1 thru 7
Filgrastim (Arm 2 only)	5 µg/kg	SC, once daily	0 thru 7

Cytarabine to be dissolved in 500 ml NaCl 0.9% or glucose 5%.
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.
Granulocyte-colony stimulating factor (filgrastim): 5 µg/kg/d for those randomized to G-CSF. See Appendix I for more details about G-CSF dosages.
G-CSF will be postponed or interrupted when WBC >30 x 10^9/l and be resumed when WBC counts have dropped to 20 x 10^9/l.
Assessment of response after Cycle I starts with bone marrow evaluation (aspirate) on day 18-21 and at subsequent times as is described in 11.2.1. Patients in CR who have already been identified as belonging to the poor risk group (Appendix G) and for whom an allogeneic donor is available and in whom from a practical point of view an early SCT can be undertaken, may proceed to Allo SCT at this point. All other patients continue with cycle II.

9.2 Remission induction treatment cycle II

Cycle II will be started when the marrow still shows more than 15% blasts as soon as possible, or in case of blasts of less than 15% as soon as possible following hematopoietic regeneration (platelets above 100 x 10^9/l; ANC > 1.0 x 10^9/l). A bone marrow evaluation should be done before cycle II is started.

Arm 1 Amsacrine and intermediate-dose Cytarabine

Arm 2 Amsacrine and intermediate-dose Cytarabine and G-CSF (filgrastim)

Agent	Dose	Route	Days
Amsacrine	120 mg/m^2	1 hr infusion	3, 5, 7
Cytarabine	1000 mg/m^2 q 12 hrs (x12)	3 hr infusion	1 thru 6
Filgrastim (Arm 2 only)	5 µg/kg	SC, once daily	0 thru 7

Cytarabine to be dissolved in 500 ml NaCl 0.9% or glucose 5%.

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

Granulocyte-colony stimulating factor (filgrastim): 5 µg/kg/d for those randomized to G-CSF. See appendix I for more details about G-CSF dosages.

G-CSF will be postponed or interrupted when WBC >30 x 10^9/l and be resumed when WBC counts have dropped to 20 x 10^9/l.

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

9.2.1 AML with t(9;22)(q34;q11) or BCR-ABL fusion gene

Patients with this cytogenetic or molecular abnormality will have the option to receive in addition to the treatment program of the protocol the kinase inhibitor imatinib mesylate (Glivec, Gleevec) (see appendix H).

9.3 Peripheral blood stem cell mobilization and collection

9.3.1 Peripheral blood stem cell mobilization

- G-CSF will be given to all patients in CR after cycle II, i.e., patients being treated in both arms 1 and 2 of the study, except in patients who will certainly proceed to HLA matched allogeneic BMT, 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 0.1 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 after an interval of 5-7 days. In this instance leukapheresis for peripheral blood stem cell collection will be cancelled.

- G-CSF 10 µg/kg/day (filgrastim) will be given per subcutaneous injection. See Appendix I for more details about G-CSF dosages.

9.3.2 Procedure of peripheral blood progenitor cell collection

- Timing of apheresis; as soon as PMN begin to rise to values of 0.1×10^9/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 2×10^6 CD34+ cells/kg or 10×10^4 CFU-GM per 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, following cyclophosphamide priming (4000 mg/m^2). If no adequate PBPC or marrow graft can be obtained, the patient is not eligible for autologous PBSCT.
- Mononuclear and progenitor cell content in the PBPCT will be assessed by conventional cell counting and flowcytometric analysis of CD34 positive cells; optionally CFU-GM, BFU-E and CFU-Mix content may be evaluated using standard short term clonogenic assays in methylcellulose.

9.3.3 Procedure for cryopreservation

The leukapheresis product will be counted and weighed, centrifuged and resuspended in RPMI-medium to which calparine or heparin is added. The cells are mixed with DMSO to attain a final concentration of 10% and 10% autologous serum at a final concentration of 0.5-1.0 x 10^8 cells/ml and a maximum volume of 250 ml (e.g. in GAMBRO bags). Controlled rate freezing (1 °C/min) will then be performed till -40 °C, then at 5 °C/min to -140 °C. After this, the bags are transferred to liquid N_2-containers and stored until use.

9.4 Post induction treatment with chemotherapy cycle III

Patients in continued CR elected for consolidation treatment with cycle III (see 7.2) will receive this treatment as soon as hematopoietic repopulation (platelets > 100×10^9/l and ANC > 1.0×10^9/l) has taken.

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

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

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

9.5 Post-induction treatment with Busulfan-Cyclophosphamide and autologous PBSCT

Patients in continued CR elected for consolidation treatment with busulfan-cyclophophamide followed by autologous PBSCT 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	1 mg/kg q 6 hrs	p.o.	-7, -6, -5, -4
Cyclophosphamide	60 mg/kg	1 hr infusion i.v.	-3, -2
Phenytoin	5 mg/kg q 6 hrs 5 mg/kg	p.o. p.o.	-9, -8 -7 thru -4
Infusion of SCT			0

Busulfan - 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. The local pharmacist may be asked to prepare the dosage of 1 mg/kg in one pill or prepare a syrup in order to facilitate oral intake.
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. Intravenous administration of Phenytoin may be required if the patient is unable to tolerate oral medications or if a therapeutic level needs to be attained. Anticonvulsant levels should be monitored and the doses adjusted to maintain levels in the therapeutic range.
Cyclophosphamide - (60 mg/kg) will be infused in 500 ml NS (0.9% NaCl) or glucose 5% 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 (glucose 5% NaCl 0.45% + 20 mEq KCl/l + 5 mg Furosemide/l) iv 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, e.g. Metoclopramidechloride (Primperan) 1 mg/kg q 4 hrs i.v. or a 5 HT_3 antagonist.

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, in vitro colony formation, 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 will be sent for bacteriology.

9.6 Post-induction treatment with 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.

9.7 Special management orders

a) Before treatment a central venous catheter may be placed. As a rule, patients will receive parenteral alimentation, when they have insufficient oral caloric intake (see below)

b) Extremely careful hand washing by all members of health care team is required

c) 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 risen to a minimum of 0.5×10^9/l.

d) 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

e) Hematological supportive care will involve prophylactic platelet transfusions when counts are below $10x10^9$/l (to avoid hemorrhages) as well as therapeutic transfusions when clinically indicated (30). In case of HLA sensitization, patients will receive HLA compatible platelet transfusions whenever necessary to avoid hemorrhages. Filtrated packed red blood cells will be given to keep hematocrit above 30%.

f) Irradiation of blood products (25 Gy) must commence from 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

g) Attempts should be made, prior and during chemotherapy, to control any medical problems, such as bleeding, infection and metabolic abnormalities. 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

h) Adequate alimentation appears an essential prerequisite for successful SCT. Therefore parenteral alimentation (through the central catheter) is started on every patient as soon as the enteral alimentation is inadequate. Parenteral alimentation is maintained until normal oral intake can be resumed

10 End of protocol treatment

Reasons for going off protocol treatment are:

1. No CR after cycle II, unless PR with donor
2. Excessive extramedullary drug toxicity preventing continuation of treatment
3. Hypoplastic bone marrow abnormalities preventing continuation of treatment
4. Death
5. Relapse after initial CR (i.e., before completion of treatment)
6. No compliance of the patient (especially refusal to continue treatment)
7. Major protocol violation
8. Patients entered into the protocol who at a later time appear to have AML-FAB type M3, AML t(15;17) or AML with fusion gene PML-RAR alpha or a variant RAR fusion gene, and therefore on, retrospect appear ineligible, will be taken off protocol. They will considered unevaluable
9. Completion of protocol treatment (either cycle III or autologous PBSCT or Allo SCT)

11 Required clinical evaluation

11.1 Observations prior to start of treatment

- History, including exposure to insecticides, previous chemotherapy or radiotherapy, antecedent hematological or oncological diseases
- Physical examination including body weight, height, splenomegaly, signs of extramedullary leukemia
- WHO classification of AML (including WHO classification of MDS if applicable)

- Performance status
- Hemoglobin, hematocrit, reticulocytes, platelets, WBC and WBC differential
- Blood chemistry, including serum creatinin, urea, potassium, uric acid, calcium, glucose, bilirubin, AST, ALT, alkaline phosphatase, gamma GT, LDH
- Surveillance cultures of throat, stools and urine
- Chest X-ray
- Cardiac ejection fraction, measured by MUGA or echocardiogram
- ECG
- Dental examination and X-ortopantogram
- Serology for cytomegalovirus (CMV) infection, HIV (human immunodeficiency virus), hepatitis A, B and C
- Coagulation studies including fibrinogen, fibrin degradation products
- Bone marrow aspiration/biopsy for:
  - cytology and cytochemistry to establish FAB subtype of AML or RAEB or RAEB-t
  - cytogenetics (cell culture and banding analysis)
  - immunological phenotyping to verify myeloid leukemia or RAEB/RAEB-t
  - molecular analysis to assess the molecular leukaemia genotype (see appendix F)
  - minimal residual disease detection (in cases of dry tap with blasts present in PB also PB sample possible; see appendix K)
- Bone marrow biopsy for histopathology
- Bone marrow and peripheral blood for whole genome transcriptional profiling (see Appendix J)

11.2 Observations during treatment

- Daily interim history and physical examination, when hospitalized; thereafter as clinically indicated
- Blood cell count, quantitative platelets daily, and WBC count and differential at least every other day when hospitalized until PBR, thereafter as clinically indicated.
- X-chest as clinically indicated
- Creatinin, Na, K, Cl, CO_2, uric acid, Ca, glucose twice until discharge
- AST, ALT, alkaline phosphatase, gamma GT, bilirubin (direct and indirect), LDH as clinically indicated and at least weekly until discharge
- Surveillance cultures according to bacteriology guidelines
- Bone marrow and peripheral blood for:

- molecular analysis and cytogenetic analysis when cytogenetic abnormalities were evident at diagnosis (see appendix F)
- minimal residual disease detection (PB sample only at relapse in case of dry tap with blasts present in PB; see appendix K)

♦ Bone marrow of autologous stem cell transplant for minimal residual disease detection in case of autologous stem cell transplantation (see appendix K)

11.2.1 Response assessment after Cycle I and Cycle II

Following each cycle, at day 18-21, 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 PBR. In all other cases blood evaluation will be repeated until PBR.

Immunological examination may be done if markers allow discrimination of malignant cells. Cytogenetic or molecular analysis may be used in patients when karyotypic or molecular markers are available to document remission, or when a relapse is suspected (see also Appendix F)

11.2.2 Remission status assessment after consolidation treatment

Following consolidation treatment the remission status will be assessed by blood marrow and blood evaluation and evaluation of extra medullary disease status. Blood evaluation will be repeated until PBR.

11.3 Observations during follow up.

Outpatient visits to the clinic during first CR are planned according to the following schedule, the 1st year at least every month; 2nd and 3rd year at least at 3 months intervals; 4th and 5th year at 4-6 months intervals and thereafter according to the local scheme of the institute. 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, quantitative platelet count, erythrocyte count, reticulocyte count

♦ Creatinin, AST, ALT, alkaline phosphatase, gamma-GT, bilirubin

- Assessment of ventricular function by measuring the cardiac ejection fraction by MUGA scan or electrocardiogram and ECG at 12 months and 24 months
- 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 as long as the patient is in first CR.
- Immunological examination if markers allow discrimination of malignant cells (including MRD, see appendix F).
- molecular analysis and cytogenetic analysis when cytogenetic abnormalities were evident at diagnosis (see Appendix F).

Table for sampling for required cytogenetic and molecular analysis, GEF and MRD.

Analysis and kind of sample / Sample time point	Cytogenetic[0] and Molecular analysis		Gene expression profiling (GEF)		Minimal residual disease (MRD)	
	PB	BM	PB	BM	PB	BM
Diagnosis	X	X				X[1]
Registration and randomisation			X	X		
30 days after diagnosis (ie before cycle II)	X	X				X
Before cycle III	X	X				X
Before Allo/ Auto SCT	X	X				X[2]
Follow up[3]	X[3]	X[3]				X[4]

[0] only when cytogenetic abnormalities were evident at diagnosis
[1] or PB in case of dry tap
[2] also of autologous stem cell transplant
[3] in case of CR 3 months after end of treatment or at relapse
[4] or PB at relapse in case of dry tap

12 Toxicities

All the chemotherapeutic agents used in the protocol cause prolonged pancytopenia during 3-6 weeks and can induce septic or hemorrhagic complications.

Congestive heart failure is a major complication of anthracyclines, frequently observed after high cumulative doses. The 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 BMT.

Non-hematological drug toxicities are:

Idarubicin (Ida)

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 in addition: 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 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). Some degree of veno-occlusive disease may occur, but occurs in less than 10% of patients when the exclusion criteria regarding cardiac liver function abnormalities are considered (paragraph 8.1.2). 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.

Toxicities will be scored according to the NCI Common Toxicity Criteria, version 2.0 (Appendix D).

13 Safety evaluations and adverse events reporting

13.1 Definitions

Adverse event (AE)

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

Adverse reaction (AR)

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

Serious adverse event (SAE)

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

- death

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

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

Unexpected SAE

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

Suspected unexpected serious adverse reaction (SUSAR)

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

Protocol treatment period

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

13.2 Reporting of (serious) adverse events

Adverse event

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

SAE and Unexpected serious adverse event

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

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

All details should be documented on the **Serious Adverse Event and Death Report**. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 2 working days and sent to the HOVON Data Center. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.

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

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

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

13.3 Processing of serious adverse event reports

The HOVON Data Center will forward all reports within 24 hours of receipt to the study coordinator and the study central datamanager. The report of an SAE will be the signal for the central datamanager to ask the investigator or the responsible local datamanager to complete and send as soon as possible all relevant CRF's for the involved patient with details of treatment and outcome.

14 Endpoints

14.1 Endpoints for the comparison of the effect of G-CSF priming in induction treatment

Primary endpoint

1. Event-free survival (i.e., time from registration to induction failure, death or relapse whichever occurs first); the time to failure of patients with induction failure is set at one day.

Secondary endpoints

2. Response and especially CR to chemotherapy cycles I and II
3. Overall survival measured from the time of registration
4. 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).
5. Toxicities and treatment related mortality (according to Appendix D)
6. Time to hematopoietic recovery (ANC 0.5 and 1.0 x 10^9/l; platelets 50 and 100 x 10^9/l) after each treatment cycle.
7. Number of platelet transfusions and last day of platelet transfusion after each cycle.

14.2 Endpoints for the evaluation of Allo SCT

Primary endpoint

1. Disease-free survival measured from the date of Allo SCT to relapse or death from any cause.

Secondary endpoints

2. Overall survival measured from the date of Allo SCT.
3. Probability of relapse and death in first CR from date of Allo SCT calculated as competing risks.
4. Duration of hospitalization as well as transfusion requirements (red cell and platelet transfusions).
5. Time to hematopoietic recovery.
6. Incidence and severity of acute and chronic GvHD

See also 17.2.

15 Registration and randomization

15.1 Registration and randomization for induction treatment

Eligible patients should be registered immediately after diagnosis (on the basis of cytological examination of marrow and blood smears in the participating center), and before the start of chemotherapy. Patients can be registered at the HOVON Data Center of the Erasmus MC – Daniel den Hoed by phone call: +31.10.4391568 or fax +31.10.4391028 Monday through Friday, from 09:00 to 17:00, or via the Internet through TOP (Trial Online Process; https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:

1. Protocol number
2. Institution name
3. Name of caller/responsible investigator
4. Patient's initials or code
5. Patient's hospital record number
6. Sex
7. Date of birth
8. Date of diagnosis of AML or RAEB or RAEB-t
9. WHO performance status
10. White blood cell count (WBC)
11. FAB type of AML or RAEB or RAEB-t
12. Eligibility criteria (see 8.1.1 and08.1.2)
13. Prior hematological or oncological disease
14. Previous chemotherapy or radiotherapy

All eligibility criteria will be checked with a checklist.
Each patient will be given a unique patient study number. Patients will be randomized, stratified by center, diagnosis (AML, MDS) and previous chemo- or radiotherapy (yes, no) with a minimization procedure, ensuring balance within each stratum and overall balance. Patient study number and result of randomization will be given immediately by TOP or phone and confirmed by fax or email.

16 Forms and procedures for collecting data

16.1 CRF's and schedule for completion

Form nr	Title
1	Registration & Randomization Form
2	On Study Form
3	Cytogenetic Form
4	Chemotherapy Form (Cycle I, II, III)
5A	Chemotherapy Evaluation Form
6	*Second Randomization Form (only till amendment 5)*
7	Transplant Form
5B	Transplant Evaluation Form
8	Bone Marrow Evaluation Form
9	Hematological Evaluation Form
10	Off Treatment Form

11 Follow Up Form
12 Side Effects Form
13 Infection Report Form
14 General Comments Form
15 Prolonged Hypoplasia Report Form
16 Imatinib Treatment Form
17 Fish Form

Table for filling out forms

	Forms																	
	1	2	3	4	5A	6	7	5B	8	9	10	11	12	13	14	15	16	17
Registration & randomization	X					X[1]												
On study		X	X[2]						X	X					(X)			X[2]
Cycle I, II, III				X	X				X	X			(X)	(X)	(X)	(X)	(X)	(X[2])
Transplant							X	X		X			(X)	(X)	(X)	(X)	(X)	(X[2])
End of treatment											X	X			(X)			
Follow up												X			(X)	(X)	(X)	(X[2])

(x) fill out if necessary, see instructions

[1] only till amendment 5

[2] by local cytogeneticist

Instructions for completion and sending in of the forms are specified in a separate document together with the forms.

17 Statistical considerations

17.1 Patient numbers and power considerations

From the previous HOVON/SAKK AML 4 and 29 studies we derive the following data:

Accrual rate in HOVON/SAKK AML 29: 200 patients per year.
CR rate 80%
EFS at 3 years 31%
Randomized between PBSCT and III 32% of all registered patients
OS 3 years after 2nd randomization 44%

When the HOVON/SAKK AML 29 will be closed it is expected that about 300 patients will have been randomized between PBSCT and cycle III.

The target number of patients for the randomization between yes or no G-CSF is 800 + 20 patients to compensate for loss of roughly 2% ineligible patients (based on experience). 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 92% with a two-sided test at 5% significance level to detect an improvement in EFS with hazard ratio HR=0.76, which corresponds with an increase of the CR rate with 4% and the EFS at 5 years with 10% from 25% to 35% in the G-CSF priming arm.

17.2 Statistical analysis

All main analyses will be according to the intention to treat principle.

17.2.1 Efficacy analysis

Induction randomization:

Main endpoint for the comparison between yes and no G-CSF priming is the EFS from registration with failure defined as failure to reach CR on induction treatment, relapse after CR or death in first CR. Secondary endpoints are rate of CR and overall survival. Actuarial estimates of competing risks of failure (no CR, relapse after CR or death in CR1) 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 Allo SCT

The outcome of patients treated with Allo SCT in CR1 will be determined by calculation of the probabilities of relapse and death in CR1 after Allo SCT as competing risk and the survival probability. Estimates will be made separately by type of transplant (HLA identical sibling or MUD), by age group, by diagnosis (AML versus MDS) and by risk group. The results will be pooled with the data of similar patients in the previous AML studies.

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

Since there is no randomization between Allo SCT and other consolidation treatment the effect of Allo SCT 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 or 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 CR1. These data will also be submitted to the data of the AML Collaborative Group for a meta-analysis.

17.2.2 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of side effects and infections with CTC grade 2 or more (Appendix D) by treatment arm and cycle or type of SCT. Time to hematological recovery after each treatment cycle or SCT will be analysed by actuarial methods. Actuarial competing risks 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 treatment, overall and separately by treatment arm and cycle.

17.2.3 Additional analyses

Additional analyses involve the analysis of prognostic factors, especially age, cytogenetic abnormalities and risk group with respect to CR rate, DFS, EFS and OS from registration and DFS and OS from second randomization. Logistic and Cox regression analysis will be used for this purpose.
Since the analysis of the effect of G-CSF in the HOVON/SAKK AML 29 suggested (a) an increased death rate on induction treatment in the G-CSF arm, with an increasing trend with age, and (b) the largest favourable effect in the subgroup of patients with intermediate risk, we will look specifically at treatment by covariate interaction for the covariates age and risk group and perform all analyses restricted to the intermediate risk subgroup.

17.3 Interim analyses

Given the ample experience with G-CSF priming treatment, there are no interim analyses planned with regard to the effect of G-CSF priming.

18 Ethics

18.1 Independent ethics committee or Institutional review board

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

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki (South Africa Amendment 1996) and the ICH-GCP Guidelines of 17 January 1997.

18.3 Patient information and consent

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

19 Trial insurance

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

Individual participating centers from outside the Netherlands have to inform the HOVON about the national laws regarding the risk insurance of patients participating in a study. If necessary HOVON will extend the insurance to cover these patients.

19.1 Intergroup studies

The HOVON insurance program does not cover the risk insurance of patients from centers participating within another cooperative group taking part in an intergroup study. The other participating groups will cover the insurance of patients registered/randomized through their offices.

20 Publication policy

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

Authors of the manuscript will include the study coordinator(s), the lead investigators of the major groups (in case of intergroup studies), investigators who have included more than 5% of the evaluable patients in the trial (by order of number of patients included), the statistician(s) and the HOVON 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 study coordinator(s). This is applicable to any individual patient registered/randomized in the trial, or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study end-points unless the final results of the trial have already been published.

21 Glossary of abbreviations

(in alphabetical order)

AE	Adverse Event
ALT	Alanine Amino Transferase
AML	Acute Myelogenous Leukaemia
AMSA	Amsacrin
ANC	Absolute Neutrophil Count
AR	Adverse Reaction
Ara-C	Cytarabine, cytosine arabinoside
AST	Aspartate Amino Transferase
BM	Bone Marrow
BMT	Bone Marrow Transplant
Ca	Calcium
CALGB	Cancer and Leukaemia Group B
CFC	Colony Forming Cells
CI	Continuous Infusion
CMV	Cytomegalovirus
CNS	Central nervous system
CR	Complete Remission
CRF	Case Report Form
CT	Computerized Tomography
CTC	Common Toxicity Criteria
DFS	Disease free Survival
DNR	Daunorubicin
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	Event Free Survival
EORTC	European Organization for Research and Treatment of Cancer
FAB	French American British (cytological classification)
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GEF	Gene Expression Profiling
GI	Gastro-intestinal
GvHD	Graft-versus-Host Disease
Hb	Hemoglobin
Ht	Hematocrit
HLA	Human leukocyte histocompatibility antigen
HOVON	Dutch/Belgian Hematology-Oncology Cooperative Group
HIV	Human Immunodeficiency Virus
IDA	Idarubicin
ILLN	Institutional Lower Limit of Normal

IPSS	International Prognostic Score System (for myelodysplastic syndromes)
IRB	Institutional Review Board
ITT	Intention to Treat
(I)ULN	(Institutional) Upper Limit of Normal
IV	Intravenous
K	Potassium
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
MRD	Minimal Residual Disease
MUD	Matched unrelated donor
MUGA	Multiple Gated Acquisition
Na	Sodium
OS	Overall Survival
PB	Peripheral Blood
PBPC	Peripheral Blood Progenitor Cells
PBR	Peripheral Blood Recovery
PR	Partial Response
RAEB	Refractory Anemia with Excess of Blasts
RAEB-t	RAEB in transformation
SAE	Serious Adverse Event
SAKK	Swiss Group for Clinical Cancer Research
SCT	Stem cell transplantation
WBC	White Blood Count
WHO	World Health Organization

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A. Classification of acute myeloid leukaemias

A.1 FAB classification of acute myeloid leukaemias

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 microscopy) ♦ < 3% of blasts positive for Sudan Black B or Myeloperoxidase

A.2 WHO classification of acute myeloid leukaemias

♦ 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.

A1	**Acute myeloid leukaemia with recurrent genetic abnormalities**	AML with t(8;21)(q22;q22);(*AML/ETO*)*
A2		AML with inv(16)(p13q22) or t(16;16)(p13;q22); (*CBFß/MYH11*)*
A3		Acute promyelocytic leukaemia; AML with t(15;17)(q22;q12)(*PML/RARα*) and cytogenetic variants
A4		AML with 11q23 (*MLL*) abnormalities
B	**Acute myeloid leukaemia with multilineage dysplasia**	Dysplasia should be present in ≥ 50% of 2 or more cell lineages
C	**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:		
D1	**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
D2	**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
D3	**AML with maturation**	-≥ 10% maturing granulocytic cells in the bone marrow (i.e. promyelocytes, myelocytes and mature neutrophils) -< 20% bone marrow monocytes
D4	**Acute myelomonocytic leukaemia**	-≥ 20% neutrophils and precursors of marrow cells -≥ 20% monocytes and precursors of marrow cells
D5a	**Acute monoblastic and monocytic leukaemia**	**Acute monoblastic leukaemia** -≥ 80% of the leukemic cells are monoblasts, promonocytes and monocytes -≥ 80% of the monocytic lineage are monoblasts
D5b		**Acute monocytic leukaemia** -≥ 80% of the leukemic cells are monoblasts, promonocytes and monocytes -the majority of the monocytic lineage are promonocytes
D6a	**Acute erytroid leukaemia**	*Erythroleukaemia (erythroid/myeloid)* -Erythroblasts: ≥ 50% of bone marrow cells -Blasts: ≥ 20% of the bone marrow nonerythroid cells

D6b		**Pure erythroid leukaemia** -Erythroblasts: >80% of bone marrow cells -No evidence of a significant myeloblastic component
D7	**Acute megakaryoblastic leukaemia**	-≥ 50% of the blasts are of megakaryocytic lineage -Blasts express CD41 and/or CD61
D8	**Acute basophilic leukaemia**	-Primary differentiation to basophils; mature basophils are usually sparse
D9	**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
D10	**Myeloid sarcoma**	-Tumour mass of myeloblasts or immature myeloid cells occuring in an extramedullary site or in bone
E1	**Acute leukaemias of ambiguous lineage**	**Undifferentiated acute leukaemia** Blasts lack markers considered specific for a given lineage including CD79a, CD22, CD3 and MPO
E2		**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
E3		**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 and not as RAEB

**see appendix A.3

A.3 WHO classification for myelodysplastic syndromes

	Disease	Blood findings	Bone marrow findings
C1	**Refractory anaemia (RA)**	-Anaemia -No or rare blasts	-Erythroid dysplasia only -< 5% blasts -< 15% ringed sideroblasts
C2	**Refractory anaemia with ringed sideroblasts (RARS)**	-Anaemia -No blasts	-Erythroid dysplasia only -< 5% blasts -≥ 15% ringed sideroblasts
C3a	**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
C3b		**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
C4a	**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
C4b		**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 ±
C5	**MDS unclassified**	-Cytopenias -No or rare blasts -No Auer rods	-Unilineage dysplasia: one myeloid cell line -< 5% blasts -No Auer rods
C6	**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*

B. Criteria for the diagnosis of RAEB and RAEB-t, and IPSS

Cytological Criteria for the diagnosis of RAEB and RAEB-t

Type		Blood	Bone marrow
Refractory anemia with excess of blasts (RAEB)		< 5% blasts Morphological dysplasia ++	5-20% blasts
Refractory anemia with excess of blasts in transformation (RAEB-t)		≥ 5% blasts	< 30% blasts
	or	blasts with Auer rods	< 30% blasts
	or		20-30% blasts
	or		blasts with Auer rods with < 30% blasts

International Prognostic Score System (IPSS) for MDS[18]

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

The IPSS score is calculated by summation of the score values for categories of the prognostic variables for a patient. Risk groups are defined on the basis of this sumscore as:

Low : 0

Int-1 : 0.5-1.0

Int-2 : 1.5-2.0

High : ≥ 2.5

* Karyotype

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

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

Intermediate : all other (or not done)

**Cytopenias

Hb < 6.2 mmol/l

ANC < $1.5x10^9$/l

Platelets < $100x10^9$/l

C. Response criteria for AML and MDS

HOVON-AML/MDS Response criteria for AML and according to the International Working Group Criteria for MDS.(34,35)

1. DISEASE STATUS CRITERIA

Note that the kind of cells considered equivalent to blasts and included in the calculation of last percentages depends on the FAB classification (Appendix A and B).

1.1 Bone Marrow

A1 marrow with spicules and a count of at least 200 nucleated cells, normal maturation of all cell lines and no evidence of dysplasia (*); **and** <5% blasts, **and** no Auer rods. When erythroid cells constitute less than 50% of bone marrow nucleated cells, then the percentage of blasts is based on all nucleated cells; when there are ≥ 50% erythroid cells, the percentage of blasts should be based on the non-erythroid cells.

A2 in case of AML: cellular marrow with maturation of all cell lines; **and** blasts ≥ 5% but ≤ 15%

A2 in case of RAEB/RAEB-t: blasts decreased by ≥ 50% over pre-treatment value, or change to a less advanced MDS FAB classification than pre-treatment. The order from advanced to less advanced is: RAEB-t, RAEB, CMMOL, RA, RARS. Cellularity and morphology are not relevant

A3 Failure to meet criteria for A1 or A2

1.2 Peripheral Blood

B1 Peripheral Blood Recovery (PBR): ANC ≥1.0 x 10^9/l , and transfusion independent platelet count ≥ 100 x 10^9/l (i.e. 48 h after last transfusion);
and no leukemic blasts in the peripheral blood (**)

B2 Failure to meet the criteria for B1

(*) The presence of mild megaloblastoid changes may be permitted if considered to be consistent with chemotherapy effect. However, persisting pre-treatment abnormalities (e.g. pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR or PR.
(**) The presence of <5% in the peripheral blood in combination with A1 bone marrow does not argue against and is compatible with a complete haematological response.

1.3 Extramedullary Disease

C1 None

C2 Any

2.0 RESPONSE CRITERIA

2.1 Morphologic complete remission (CR)

Attainment of A1 marrow status and B1 peripheral blood recovery and C1 extra-medullary disease status .

2.1a Morphologic complete remission with incomplete recovery (CRi)

Attainment of A1 bone marrow status and B2 peripheral blood recovery and C1 extra-medullary disease.

2.2 Treatment failure

Subjects who do not enter CR following induction will be classified according to the type of failure (document on CRF):

- Partial response (PR): Subject only achieves A2 marrow status with B1 peripheral blood status and C1 extramedullary involvement as a best response in any induction cycle. The response of subjects who achieve A1B1C1 status and within 28 days relapse will be considered as PR.
- Induction resistance (RD): Subject has persistent leukaemia in the bone marrow with ≥15% blasts and/or > 5% persistent blasts in the peripheral blood and/or persistent extramedullary disease
- Other induction failure (Ind.F.): Patients who do not meet any of the criteria for CR, PR or RD are classified as other induction failures. This includes patients who die before response could be ascertained or before PBR was achieved.

3.0 Relapse Criteria

Relapse after complete remission for patients with AML, RAEB / RAEB-t 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

D. Common toxicity Criteria

The grading of toxicity and adverse events will be done using the NCI Common Toxicity Criteria, CTC version 2.0, revised march 23, 1998. A complete document (19 pages) may be downloaded from the following sites:

http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdfng/CTC-3test.html
http://www.eortc.be/Services/Doc/ctc/default.htm
http://www.hovon.nl

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

E. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity
1 Symptoms, but nearly ambulatory
2 Some bed time, but to be in bed less than 50% of normal daytime
3 Needs to be in bed more than 50% of normal daytime
4 Unable to get out of bed

F. Cytogenetic and molecular analysis

Conventional cytogenetic analysis should be performed in all patients at diagnosis. For selected genetic abnormalities the use of molecular techniques will be required (see below).
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.

1. Additional FISH analysis is recommended for the detection of abnormalities which involve 11q23 (MLL). For patients with MDS (RAEB, RAEB-t) FISH analysis is required for the detection of –5/-7. Conditions for FISH will be standardized by the HOVON Cytogenetic Working Party.
2. RT-PCR is recommended at diagnosis for the detection of t(9;22) BCR/ABL, t(8;21) AML1/ETO; inv/del(16) CBFβ/MYH11 fusion transcripts with a preferred sensitivity (positive cells diluted in negative cells) of at least $1/10^2$. RT-PCR for detection of t(6;9) DEK/CAN is recommended when available.
3. For diagnosis of additional abnormalities of chromosome 11q23 (such as MLL self fusions) a Southern Blot is required. As this will be retrospectively analyzed in this study, preparations will be made for the possibility for sending samples for central analysis.
4. A variety of molecular markers will be analysed including internal tandem repeat mutations as well as D835 mutations in the receptor of FLT3 (FLT3-ITD and FLT3 D835), kit mutations, NPM1 muations, CEBP-alpha mutations, high EVI-1 expression, RAS mutations
5. AML blasts at diagnosis and remission marrow will be analyzed for gene expression profiles on the Affymetrix platform

Subsequent (follow-up) samples

In all patients peripheral blood and bone marrow samples will be collected for molecular analysis during follow-up in case a complete remission is obtained.

Bone marrow + blood sampling:

1. At diagnosis
2. Day 30 after diagnosis according to the clinical protocol (ie before the beginning of cycle II)
3. Prior to the start of cycle III or the allo/autoSCT
4. In cases of CR, 3 months after end of treatment.
5. At relapse(to recognize eventually occurring phenotypic shifts)

For the HOVON centers diagnostic and follow-up samples should be sent to the local laboratory. If necessary this local lab will forward the samples to a central laboratory as agreed within the Network for Molecular diagnostics.

For questions, please contact Dr P. Valk, Molecular Diagnostic lab, Dept of Hematology, Erasmus MC, Rotterdam (p.valk@erasmusmc.nl, phone: (+31)-10-408 7975/408 7962)))

G. Risk Group Definition

Risk Classification	
Good	AML/RAEB/RAEB-t with ▪ t(8;21)(q22;q22) or AML1/ETO fusion gene and WBC ≤ $20x10^9$/l at diagnosis and no additional unfavourable cytogenetic abnormalities or ▪ inv/del(16)(p13;q22) or MYH11/CBFβ fusion gene and no additional unfavourable cytogenetic abnormalities
Intermediate	AML/RAEB/RAEB-t: ▪ all patients not assigned to Good Risk or Poor Risk
Poor	▪ AML/RAEB/RAEB-t with unfavourable** cytogenetic abnormalities, except those with simultaneous favourable* cytogenetic abnormalities or ▪ AML/RAEB/RAEB-t with a late CR (i.e. CR attained after induction cycle II), except those with favourable cytogenetic abnormalities

* Favourable cytogenetic abnormalities:
- t(8;21)(q22;q22) or AML1/ETO
- t/inv/del (16)(p13;q22) or MYH11/CBFß

** Unfavourable cytogenetic abnormalities:
- complex cytogenetic abnormalities (three or more distinct clonal abnormalities)
- -7, -5 (monosomies of chromosomes 5 or 7)
- del 5q or del 7q
- abnormalities of the long arm of chromosome 3 (abn 3q)
- t(6;9)(q23;q34) or DEK/CAN fusion gene
- t(9;22)(q34;q11) or BCR-ABL fusion gene
- abnormalities of the long arm of chromosome 11 (abn 11q23)

Note
This risk classification differs from the one used in the HOVON/SAKK AML 29. It is based on a landmark analysis of the risk of relapse and the overall survival from start of consolidation treatment of all patients treated in the previous HOVON/SAKK studies AML 4 and 29, who reached CR after cycle I and II and who received consolidation treatment. The outcomes of the analysis were compared with an analysis of pooled data of the AMLCG (AML Collaborative Group) of 4817 patients with age between 15 and 60 years in CR after I or II. The following observations were made.

1. *The previously found unfavourable characteristic of a high WBC for patients with favourable cytogenetics turned out to be restricted in the HOVON / SAKK data after exclusion of the M3 patients to patients with t(8;21). This was confirmed by the AMLCG data, where an optimal cutpoint for WBC of 20 x 10^9/l was found. The prognostic impact of WBC for patients with inv(16) or del(16) was not apparent in the HOVON/SAKK data and less pronounced in the AMLCG data. Patients with unfavourable cytogenetic abnormalities in addition to t(8;21), inv(16) or del(16) (only 6 patients in the HOVON/SAKK group and 35 in the AMLCG series) showed a higher risk of relapse comparable with the patients in the intermediate risk group and are therefore classified as intermediate risk.*
2. *Analysis of the MRC data (ref 13) indicated that (among patients not qualifying for good risk) a late CR (attained after cycle I) by itself was an unfavourable prognostic factor. This was especially true for patients without favourable or unfavourable cytogenetics. This was confirmed by analysis of the HOVON/SAKK data and the AMLCG data. Therefore these patients are included in the poor risk group.*
3. *The selection of unfavourable cytogenetic abnormalities is based on the AMLCG data. Certain abnormalities were too infrequent in the HOVON/SAKK data to allow a separate analysis.*
4. *In the HOVON/SAKK series FAB type M6 also came out as an unfavourable characteristic with a high risk of relapse and death, but this was not confirmed by the analysis of the AMLCG series. Therefore, AML FAB-M6 was not defined as poor risk.*

Summary of landmark analysis HOVON/SAKK series

	N	5-year probabilities		
		Relapse	Death in CR1	OS
Good risk	86	28%	11%	74%
Intermediate risk				
A	17	49%	18%	50%
B	457	50%	10%	43%
Poor risk				
Late CR	207	70%	12%	22%
Unfavourable cytogenetics	70	80%	9%	24%

A: Patients with favourable cytogenetics, but not in the Good Risk group due to either a high WBC (>20) combined with t(8;21) or the presence of additional unfavourable cytogenetic abnormalities.

B: Patients without favourable or unfavourable cytogenetics in CR after cycle I

H. The use of IMATINIB-MESYLATE in Philadelphia-positive AML
and, in particular, within the context of HOVON-42 amended protocol

Background

Philadelphia chromosome (Ph)-positive acute myeloblastic leukemia (AML) constitutes a rare cytogenetic abnormality in adults with AML. Ph+-AML is associated with a bad prognosis. Although remission rates are only slightly inferior to standard-risk AML, relapse is almost universal and long-term survival remains rare. Given the poor outcome with current chemotherapy consolidation programs, allogeneic stem cell transplantation is recommended for these patients in first remission or as soon as feasible. Even with transplantation the impact on outcome is limited and new therapeutic concepts are urgently needed. One of the most promising developments has been the recent introduction of the tyrosine kinase inhibitor Imatinib mesylate.

Imatinib (Glivec, also Imatinib mesylate [Gleevec]; formerly STI571) is a rationally designed, potent selective competitive inhibitor of the BCR-ABL protein-tyrosine kinase. In an ascending-dose phase 1 study, Imatinib induced substantial and durable responses with minimal toxicity, at daily doses of 300 mg and higher, in nearly all patients with chronic phase CML, results that were recently confirmed in a phase 2 study **(1, 2)**. Imatinib was also shown to have significant antileukemic activity in patients with accelerated-phase CML **(3)** or in myeloid blast crisis **(4)**, although results were inferior to results in chronic phase. In patients with lymphoid blast crisis (LyBC) or Ph^+ ALL entered in the initial phase 1 study, Imatinib at daily doses of 300 to 1000 mg induced hematologic responses in 14 of 20 patients (70%), including 4 complete hematologic responses (20%) and 7 marrow responses (35%), **(5)**. A recent phase II study in 56 patients with relapsed or refractory Ph^+ acute lymphoblastic leukemia (ALL; 48 patients) or chronic myelogenous leukemia in lymphoid blast crisis (LyBC; 8 patients) was recently performed with Imatinib given once daily at 400 mg or 600 mg **(6)**. Imatinib induced complete hematologic responses (CHRs) and complete marrow responses (marrow-CRs) in 29% of ALL patients (CHR, 19%; marrow-CR, 10%), which were sustained for at least 4 weeks in 6% of patients. Median estimated time to progression and overall survival for ALL patients were 2.2 and 4.9 months, respectively. CHRs were reported for 3 (38%) of the patients with LyBC (one sustained CHR). Thus, Imatinib therapy **alone** resulted in a clinically relevant hematologic response rate in relapsed or refractory Ph^+ acute lymphoid leukemia patients, but development of resistance and subsequent disease progression were rapid.

We and others have tested the combination of Imatinib with Cytarabine in patients with first chronic phase CML as well as in patients with accelerated phase CML or CML blast crisis (F. Guilhot, personal communication). The combination of Imatinib with intensified Cytarabine has proven

feasible in patients in first chronic phase CML treated with Cytarabine at a dose of 1000 mg/m^2 for 7 days concurrently with 400 mg Imatinib daily (HOVON-51). In addition, patients in accelerated phase CML treated with a similar scheme did not experience additional toxicity as compared to Cytarabine alone, especially hematological toxicity was not prolonged (F. Guilhot, personal communication).

Based on this, Imatinib mesylate is currently offered to patients with AML t(9;22). More specifically, it is proposed to add Imatinib at a dose of 600 mg once daily as from the day after (!) the bone marrow-evaluation (morphology, immunology, and RT-PCR of BCR-ABL) of the first cycle of chemotherapy in patients with proven Philadelphia-positive AML. Imatinib is given at a dose of 600 mg continuously for a maximum of 2 years (concurrently also with high dose Ara-C(in the second cycle)), but it will be discontinued at the start of conditioning for allogeneic stem cell transplantation (either sibling or unrelated donor). The time-lag between the second cycle and transplantation is covered by continued treatment with Imatinib at a daily oral dose of 600 mg. Following allogeneic stem cell transplantation, Imatinib is resumed in case of cytogenetic persistent disease or relapse. Also, Imatinib is resumed in patients who convert from PCR-negativity towards PCR-positivity.

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I. Example scheme for dosage of G-CSF in HOVON/SAKK AML - 42

The following scheme may be helpful to determine the correct dosage of G-CSF in the HOVON/SAKK AML – 42 study. However, local schemes resulting in the prescribed dosages may also be used.

Weight in kg.	G-CSF during induction 5 µg/kg/day	G-CSF for peripheral stem cell mobilisation 10 µg/kg/day
40 – 59	300 µg, once daily	300 µg, twice daily
60 – 89	480 µg, once daily	480 µg once daily and 300 µg once daily
90 – 105	300 µg, twice daily	480 µg, twice daily

J. Gene Expression Profiling

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. The samples will be taken before start of treatment (bone marrow, peripheral blood). This approach will enable us to determine gene expression profiles in AML blasts at diagnosis.

Bone marrow (5 ml) and peripheral blood (10 ml) samples for whole genome transcriptional profiling will be collected at entry at the participating centers where AML blast cells will be purified. AML blasts and mononuclear cells will purified by ficoll (e.g., Ficoll-Hypaque (Nygaard, Oslo, Norway)) centrifugation (Valk et al, N Engl J Med 350, 1617-1628, 2004).

Protocol ficoll separation:

- Count MNCs in bone marrow and peripheral blood
- Dilute blood 1:1 in PBS (max. WBC $60x10^6$/ml)/Dilute bone marrow (max. WBC $60x10^6$/ml)
- Aliquot 15 ml ficoll in 50 ml tube
- Load cell suspension carefully on ficoll
- Spin:

For example:	In a Heraeus 3,0RS:	In a Hettich P or RP
	1880 rpm (±830g)	2200 rpm (±830g)
	15 minutes, acceleration 5	15 min, acceleration 2 min
	RT	RT
	break 4	break 0

- Remove the upper plasma layer until 5 mm above interphase
- Collect interphase with 5 or 10 ml pipette in 50 ml tube
- Add PBS to 50 ml
- Spin:

For example:	In a Heraeus 3,0RS: :	In a Hettich P or RP:
	1710 rpm (±690g)	2000 rpm (±690g)
	8 minutes, acceleration 7	8 minutes
	RT	RT
	break 7	break 3

- Remove liquid
- Wash with 50 ml PBS
- Spin:

For example:	In a Heraeus 3,0RS:	In a Hettich P or RP:
	1540 rpm (±560g)	1800 rpm (±560g)

5 minutes, acceleration 7	5 minutes
RT	RT
break 9	break 9

- Resuspend cells in PBS (max. $200x10^6$/ml)
- Freeze cells

Purified cells must be stored locally at -80°C and will be collected batch-wise by the central laboratory at the Erasmus MC (Erasmus Medical Center Rotterdam, Department of Hematology, Room no. Ee1373, Dr. Molewaterplein 50 Rotterdam Z-H, 3015 GE The Netherlands, Phone: +31.10.408.7962/75, Fax: +31.10.408.9488 E-mail: p.valk@erasmusmc.nl), Rotterdam where they will be further processed and analyzed.

K. Assessment of minimal residual disease

Minimal residual disease (MRD) detection in acute myeloid leukemia (AML) using PCR based techniques is applicable only in a minority of cases. MRD detection using multiparameter flowcytometry, using aberrant phenotypes defined at diagnosis, is applicable in roughly 80% of the cases and has been shown to offer a strong prognostic factor independent of other prognostic factors in both adult and childhood AML (1-5). Both bone marrow (BM) after different courses of therapy (1-5), stem cell transplants (6) and sequential follow-up bone marrow sampling (7,8) have been applied for MRD assessment.
For the present protocol in all cases bone marrow sampling includes diagnosis, at the end of therapy, in complete remission (CR) and at relapse (see below).

Methods: as defined in detail in reference 5

Definition of MRD: malignant blasts as a percentage of the stem/progenitor compartment and as a percentage of the whole white blood cell compartment. These percentages are calculated based on the frequency of cells with an aberrant phenotype

Bone marrow sampling:

1. At diagnosis
2. Day 30 after diagnosis according to the clinical protocol (ie before the beginning of cycle II)
3. Prior to the start of cycle III or the allo/autoSCT
4. In autologous stem cell transplant
5. In cases of CR, 3 months after end of treatment.
6. At relapse (to recognize eventually occurring phenotypic shifts)

Logistics:

* Sampling conditions: all 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.

* Volume and number of cells:

Diagnosis. Volume: at least 5 ml BM. In cases of dry tap with blasts present in the peripheral blood: 10 ml PB

Before cycle 2. Volume: at least 5 ml BM.

Before cycle 3 or prior to stem cell transplant. Volume: at least 5 ml BM.

Autologous stem cell transplant Volume: 5 ml

Relapse. Volume: at least 5 ml BM. If impossible, 10 ml of PB when blasts are present

Establishment of aberrant immunophenotypes (leukaemia associated phenotypes or LAP's):

* A diagnosis panel of monoclonal antibody combinations will be used for establishment of putatively aberrant, leukemia associated phenotypes (LAP) at diagnosis.
This panel (see www.hematologie.nl/mrd/) has been validated by the MRD working group (under the auspices of HOVON/SAKK and the European Working Group on Clincial Cell Analysis, EWGCCA). Monoclonal antibodies in this panel are centrally distributed.
For confirmation of putative LAPs a second incubation will be done to directly confirm the putative LAPs. For this, other monoclonal antibody combinations will be used in order to achieve the optimal combinations for MRD follow-up (list of suitable antibodies for this purpose: see www.hematologie.nl/mrd/).
* Antibody incubations and data acquisition are SOP-based (see also www.hematologie.nl/mrd/) and will be performed at each institute.
Data analyses for diagnosis material are performed by the individual centers that intend and are capable of performing these, and otherwise by members of the MRD working group to be contacted via the VU University Medical Center (see below). In any case all results should be sent to the MRD working group for evaluation and final decision on LAPs suitable for use at follow-up.
For follow-up a similar procedure will be followed
Detailed procedures are on the website www.hematologie.nl/mrd/

For questions please contact:N Feller/GJ Schuurhuis, department of Hematology
VU University Medical Center
De Boelelaan 1117
1081 HV Amsterdam, The Netherlands
mrd@vumc.nl (please report HOVON-study number in subject), phone: (+31) 020 4443836)