Randomized study with a run-in dose-selection phase to assess the added value of lenalidomide in combination with standard remission-induction chemotherapy and post-remission treatment in patients aged 18-65 years with previously untreated acute myeloid leukemia (AML) or high risk myelodysplasia (MDS) (IPSS-R risk score > 4.5)

A multicenter phase III trial

PROTOCOL

Principal Investigator	**:**	B. Löwenberg
Sponsor	**:**	HOVON
EudraCT number	**:**	2013-002843-26
Final protocol (version 05)	**:**	21 November 2014

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Signature of Principal Investigator

12 DEC 2014

Date

Prof. Dr. B. Löwenberg
Erasmus University Medical Center
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LOCAL INVESTIGATOR SIGNATURE PAGE

Local site name: _____

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Signature of Local Investigator Date

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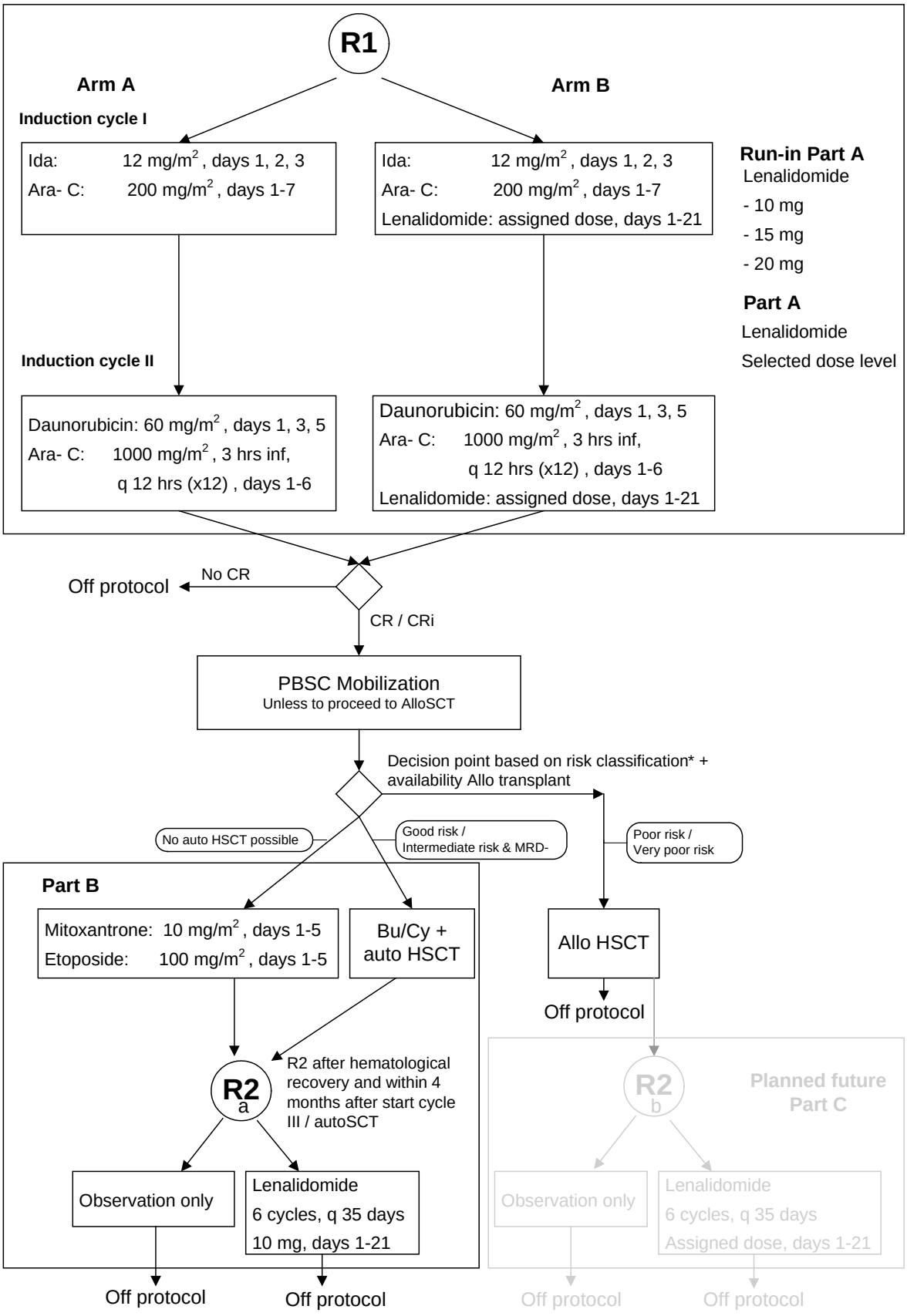
Printed Name of Local Investigator

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

1 Scheme of study



* For details see appendix D

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3 Synopsis

Rationale

Acute Myeloid Leukemia (AML) in adults continues to have a fatal prognosis in the majority of patients. The medical need for new therapies in this large group of patients is still considerable. The HOVON/SAKK Cooperative Consortium that partners with institutions in the Netherlands, Belgium, Luxembourg, Switzerland, Sweden, Norway, Finland, Germany, Austria, Lithuania and Estonia concentrates it's developmental therapeutic efforts on randomized phase III approaches introducing new treatment modalities into the backbone of standard remission-induction chemotherapy for patients with newly diagnosed AML between the age of 18-65. In single arm studies the addition of lenalidomide to chemotherapy has shown encouraging clinical response rates with acceptable side effects in AML. Lenalidomide appears a highly active anti-AML drug that has mainly been examined in relapsed AML and in patients of older age, but not yet in the first line treatment in patients of younger age, neither as part of a combination of induction treatment, nor in maintenance treatment.

Study objectives

Primary Objectives

Part A run-in:

- To select in a randomized approach the feasible dose level of lenalidomide when given orally at three variable dose levels (at 20 mg/day 1-21; at 15 mg/day 1-21 or at 10 mg/day 1-21) in combination with standard induction cycles I and II in patients with AML/ MDS with IPSS-R> 4.5

Part A:

- To evaluate the effect of lenalidomide on EFS at the selected (during Part A run-in) feasible dose level when combined with remission induction chemotherapy cycles I and II in a randomized comparison to remission induction cycles I and II without addition of lenalidomide in a phase III study

Part B:

- To evaluate the effect on the Cumulative incidence of relapse (CIR) of 6 cycles of maintenance therapy with lenalidomide treatment (10 mg/day for 21 days followed by 14 days rest) after post remission chemotherapy cycle III or autoHSCT versus observation only

Secondary Objectives

Part A :

- To investigate the clinical efficacy of lenalidomide in combination with remission induction chemotherapy cycles I and II (in comparison with the same treatment without lenalidomide) in all patients with regard to complete remission rate (CR/CRi), DFS, CIR and OS
- To investigate the clinical efficacy of lenalidomide in combination with remission induction chemotherapy cycles I and II in molecularly and cytogenetically distinguishable subsets with regard to complete remission rate (CR/CRi), DFS, CIR and OS
- To evaluate the treatment effects according to MRD measurements following therapy by standardized sampling of marrow/blood following remission induction treatment
- To determine the prognostic value of molecular markers and gene expression profiles of the leukemia cells assessed at diagnosis for both remission induction treatments
- To investigate the toxicities of lenalidomide in combination with remission induction chemotherapy cycles I and II
- To compare CIR after autoHSCT according to molecular markers and MRD measurements

	♦ To evaluate the effect of lenalidomide on the feasibility of collecting adequate autologous stem cell grafts and the probability of proceeding to autoHSCT
	Part B: ♦ To investigate the clinical efficacy of lenalidomide with regard to DFS and OS measured from 2nd randomization ♦ To assess post remission and post-transplant adverse events and need for transfusions when lenalidomide is applied after post remission chemotherapy/autoHSCT ♦ To evaluate the efficacy of lenalidomide as post-remission therapy to prevent relapse in all randomized patients, but also in relationship with the distinctive risk categories of AML (as based on cytogenetics and molecular genetics) and MRD estimates
Study design	Phase III randomized trial for remission induction as well as for the maintenance starting with a dose selection run-in phase.
Patient population	Patients aged 18-65 years with previously untreated acute myeloid leukemia (AML) or myelodysplasia (MDS) with IPSS-R > 4.5.
Intervention	First, we will establish in a randomized run-in study the dose level of lenalidomide in addition to the standard induction treatment of idarubicin/cytarabine (cycle I) and daunorubicine/cytarabine (cycle II) (part A-run-in). Following the dose-selection phase the study will continue as a randomized study for induction therapy (part A). Subsequently, we will also investigate the effect of lenalidomide maintenance treatment (10 mg/day) by randomization to be administered in first CR.
Duration of treatment	Patients will receive an induction treatment of 2-3 months. If eligible for the second part of the study, patients in the

	maintenance arm will receive maintenance therapy for 7 to 8 months. Subsequently, patients will be followed until 10 years after registration for the phase III trial
Target number of patients	860, 60 patients in Part A run-in, 800 In Part A
Expected duration of accrual	3 years
Main study endpoints	Endpoints Part A run-in: ♦ Dose Limiting Toxicity (DLT) and duration of myelosuppression after the combination of lenalidomide for each of the distinct predefined dose levels ♦ Response (CR and CRi) after chemotherapy cycles I and II Endpoints Part A: ♦ EFS after the induction treatment with and without lenalidomide (i.e., time from registration to induction failure, death or relapse whichever occurs first) ♦ EFS in the distinct prognostic subsets (AML good-risk vs. AML intermediate-risk vs. AML poor-risk vs. AML-very poor-risk) and cytogenetically and molecularly defined subgroups of AML ♦ Response (CR and CRi) to induction therapy cycles I and II ♦ Disease-free survival (DFS, measured from time of CR/CRi to day of relapse or death, whichever occurs first) ♦ OS measured from the time of registration ♦ Outcome of induction treatments in relation to MRD measurements ♦ Evaluation of molecular prognostic markers and gene expression profiles for and overexpression of defined genes (e.g. EVI1, cereblon) for outcome in relation to induction and post induction treatments ♦ Evaluation of toxicities ♦ Evaluation of MRD after induction and post-

induction treatments

- 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
- Number of platelet transfusions and last day of platelet transfusion after each cycle
- Impact of the use of lenalidomide on the effectiveness of stem cell mobilization

Endpoints Part B:

- Cumulative incidence of relapse (CIR) after the maintenance treatment with or without lenalidomide maintenance therapy
- OS and DFS (time from 2nd randomization to death from any cause or relapse (whichever occurs first)) in all patients, and also in the distinct prognostic subsets (AML good-risk vs. AML intermediate-risk vs. AML poor-risk vs. AML very poor-risk) and cytogenetically and molecularly defined subgroups of AML.
- Evaluation of toxicities
- Number of platelet transfusions and last day of platelet transfusion after each cycle.
- Number of RBC transfusions in relation to maintenance or no maintenance treatment
- Evaluation of MRD after 2nd randomization
- Time to hematopoietic recovery (ANC 0.5 and 1.0x10^9/L; platelets 50 and 100x10^9/L) after each treatment cycle

Planned interim analyses and DSMB	Interim analyses will be done as described in section 14. A DSMB will be installed. Results of the interim analyses as well as annual progress and annual safety information will be presented to the DSMB. See section 12.8 for more details.

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5 Introduction and rationale

5.1 Lenalidomide in newly diagnosed AML patients between 18-65 years of age

Acute myeloid leukemia (AML) is a clonal hematopoietic stem cell disorder characterized by an accumulation of immature progenitor cells by a block in differentiation resulting in the suppression of normal hematopoiesis. The disease is highly heterogeneous with respect to morphology, immunophenotype, cytogenetics, molecular and gene expression signatures as well as in treatment response and treatment outcome. [1, 2] Treatment for AML is intensive, consisting mostly of anthracycline-cytarabine based combination chemotherapy which in adults below 60 years of age results in complete remission (CR) rates of 70-80% and long term survival of about a 35-40%. Outcome in particular unfavorable subsets of patients is much less satisfactory with a 5-year survival of 10%. In these poor risk and very poor risk leukemia's, various adverse prognostic factors (unfavorable cytogenetics and molecular biomarkers, antecedent hematological disorders (e.g. myelodysplasia (MDS), therapy related AML)) are overrepresented. On the other hand, in patients with relatively favorable subtypes of AML, treatment outcome is much better but there is still a relapse rate of 35%-40%. The remaining majority of the patients are of intermediate prognostic risk and their survival probability is 35-40% on average. There is an apparent need to improve treatment outcome in each of these prognostic subsets. Although dose intensified therapy, by increasing the dose levels of existing drugs, will benefit some patients, there is an urgent need for new drugs to be integrated in current treatment. Recent insights in the biology of AML have revealed the dynamic clonal and sub-clonal architecture of the leukemia and has begun to explain that certain subpopulations of neoplastic cells escape the cytotoxic effect of current drugs leading to leukemia recurrence or refractory disease. These refractory sub-clones may pre-exist prior to start of treatment (including so called founder clones) or may arise during therapy. Therefore, the prevailing hypothesis states that in AML new drugs have to be combined with other effective drugs as early in treatment as possible to tackle leukemia cells at multiple targets concomitantly rather than using targeted drugs as single agents with a narrow spectrum of activity. Lenalidomide is a highly promising and active drug in AML [3, 4 and 5] that deserves evaluation in an integrated context with combination chemotherapy. It has shown marked anti-leukemic activity when employed as a single drug.

In a current study in progress by the HOVON/SAKK cooperative group, lenalidomide at various dose levels (10 mg/day; 15 mg/day and 20 mg/day) in combination with daunorubicine/cytarabine (cycle I) and intermediate dose cytarabine (cycle II) is evaluated in older patients (65 years and above) with AML. At the present time escalation to the highest planned dose level of 20 mg/day has been feasible and more than 80 patients have meanwhile been treated at this dose level. The final analysis of the study is foreseen in mid 2015. Preliminary analysis demonstrates the feasibility of the use of

lenalidomide in combination with standard chemotherapy in terms of safety and toxicity even in older patients.

5.1.1 AML heterogeneity: cytogenetic and molecular subtypes in relation to endpoints

The major clinical factors with impact on prognosis in patients with newly diagnosed AML are age, antecedent hematological disorders, therapy related leukemia, and high white blood cell counts at diagnosis.

Cytogenetic and molecular markers of prognosis are increasingly recognized: core binding factor (CBF) leukemia's [(2)], nucleophosmin-1 (NPM1) mutant without FLT3-ITD AML and bi-allelic mutant CEBPA AML' s [(6, 7, 8, 9, 10 and 11)] are characterized by a relatively favorable prognosis. AMLs with monosomal karyotypes [(12, 13)], complex karyotypes [(2)] and 3q21abn AMLs [(14)] exhibit a notoriously unfavorable prognosis. Normal cytogenetics (CN) and various cytogenetic abnormalities show intermediate prognosis.

Abnormal gene expression (e.g. EVI1, ERG, BAALC, and MN1) and particular gene mutations (e.g. CEBPA, FLT3-ITD, NPM1, ASXL1, RUNX1, TET2, IDH1, IDH2, DNMT3A, p53, EZH2 and others) determine therapeutic response rates, relapse rates and variable event free survival (EFS) and overall survival (OS) probabilities. In particular over-expression of the oncogene EVI1, which is also dysregulated and involved in the unfavorable 3q chromosomal translocation is associated with a very adverse prognosis. [(15)]

Several of the recurrent mutations that are listed in the previous paragraph have recently been shown to be consistently associated with clinical outcome. Mutations in the additional sex combs-like 1 (ASXL1) gene and in the methylcytosine dioxygenase 2 gene (TET2) were initially identified in myelodysplastic syndromes, but subsequently also found in AML. Mutations in ASXL1 were detected in approximately 10% of de novo AML, they were more frequently observed in older patients and in secondary AML, they are inversely correlated with NPM1 mutations and FLT3-ITD, and generally associated with a dismal prognosis. [(16, 17 and 18)] Specially the bi-allelic FLT3-ITD mutations (or those with high FLT3-ITD/FLT3-wild type ratios) define a distinctive subset and confer a highly unfavorable outcome in newly diagnosed patients with AML. [(61,62, 63, 64, 65)] In two studies that have investigated newly diagnosed AML patients of age less than 60 years, ASXL1 mutations appeared an independent predictor corresponding with an overall survival probability of 20% or less only. [(16, 17)]

TET2 mutations are present in approximately 20% of AML, they present more frequently at older age and with higher white blood cell counts, they are inversely associated with IDH mutations, and seem to correlate with inferior outcome in some recent publications. [(8, 9 and 10)] However, the clinical significance of TET2 mutations appeared to be most pronounced in certain molecularly defined subclasses of AML [(19, 20, 21 and 22)] and a consistent profound prognostic impact in patients below 60 years of age has yet to emerge.

RUNX1 mutations are leukemia-associated mutations that have recently been revisited for their incidence and prognostic value. [17, 23, 24, 25 and 26] Early mutation analyses of RUNX1 focused on the Runt DNA binding domain, whereas recent studies explored for mutations in the complete RUNX1 gene. RUNX1 mutations are present in up to 10% of AML, they are more frequent at older age and are seen in AML in association with ASXL1 mutations. Their prevalence appears inversely correlated with NPM1 mutations and they are consistently associated with a dismal prognosis in AML. In adults of age less than 60 years limited data are available from relatively small studies that reveal a very low overall survival probability in newly diagnosed AML with normal cytogenetics. [25, 26] Also p53 deletions correlate with a very poor prognosis.

KIT gene mutations may confer moderate prognostic value mainly in CBF AML's, but these patients also frequently present with higher white blood cell counts. IDH1 mutations were initially identified as driver mutations using parallel massive deep sequencing technology. Subsequently also IDH2 was shown to be recurrently mutated in AML. Although several clinical trial groups have investigated IDH mutations in relation to treatment outcome, the prognostic value of these mutations remains controversial and it seems apparent especially in specific genetically defined subgroups of AML or restricted to certain types of IDH mutants. At this moment the practical value of these mutations to predict treatment outcome in AML is still limited.

Generally, DNMT3A mutations appear to be associated with older age, high white blood cell counts, NPM1 mutations and they co-occur with FLT3-ITD gene mutations. [27, 28, 29, 30, 31, 32, 33 and 34] Some studies demonstrated a correlation between the presence of DNMT3A mutations and IDH mutations. Surprisingly, mutations in DNMT3A, a de novo methyltransferase, have not been correlated with any specific DNA methylation- or gene expression profile. [34] This may suggest that mutations in DNMT3A result in subtle, rather than global, deregulation of de novo methylation. A number of independent studies revealed that DNMT3A mutations are associated with inferior outcome [27, 28, 29, 30, 31, 32, 33, 34 and 35] although in patients with AML aged 60 years or less the patients with DNMT3A mutant AML have a survival probability of about 20-30% in unselected patients and 20% in normal karyotype AML. [25, 32] Furthermore, one study reported an overall survival of 23% in normal karyotype AML and one study did not reveal a significant negative effect on survival [31] or a marginally significant effect in normal karyotype AML. [34]

5.1.2 Minimal Residual Disease (MRD)

Relapses emerge from residual disease persisting after chemotherapy that can be readily detected by multicolor flow cytometry or polymerase chain reaction measurements of unique gene mutations or structural abnormalities in the majority of cases. MRD appears predictive of outcome and offers a convenient quantitative measure for sequential evaluation of treatment response.

The HOVON/SAKK cooperative group has extensive operational experience with sampling and evaluating molecular genetic markers and MRD within their prospective trials. [36, 37]
For instance, the HOVON/SAKK cooperative group has prospectively established the value of immunophenotypically assessed MRD. In adults (<60 years) with AML enrolled in a HOVON/SAKK cooperative study (with 389 of 517 patients available for analyses and a total of 471 MRD time points) MRD was evaluated in bone marrow samples in complete remission (164 after induction cycle I, 183 after cycle II, and 124 after consolidation therapy). [36] After all courses, low MRD values distinguished patients with relatively favorable outcome from those with adverse relapse free survival and overall survival. In the clinically most interesting subgroup with intermediate risk cytogenetics, MRD appears to be an independent prognostic factor. MRD measurements also identified a subgroup with poor prognosis amongst patients with favorable cytogenetic risk. Multivariate analysis after cycle II, when decisions about consolidation treatment have to be made, confirmed that high MRD values (>0.1%) were associated with a considerably higher risk of relapse even after adjustment for consolidation treatment (time dependent covariate) risk score and early or later CR. These data support the implementation of rapidly available MRD assessment for clinical decision making. Therefore, in future treatment studies, risk stratification, e.g. for allogeneic stem cell transplantation, should not only be based on risk estimation determined at diagnosis and number of cycles to complete hematological response, but also on MRD values as a therapy-dependent prognostic factor.
In the meantime also Polymerase Chain Reaction (PCR) based MRD strategies have shown promising results for monitoring MRD, e.g. based on fusion transcripts (RUNX1-RUNX1T1 or CBFB-MYH11 transcripts by real-time quantitative PCR or NPM1 gene mutations.

5.1.3 Post-remission treatment choice

Allogeneic hematopoietic stem cell transplantation (alloHSCT) [39] represents the most active therapeutic modality (in terms of preventing relapse of leukemia), but is also the most toxic intervention (in terms of transplant-related mortality and morbidity/quality of life). In addition the more favorable subgroups of patients still have a possibility for rescue with alloHSCT in case of relapse which reduces the necessity for an alloHSCT in first complete remission. Therefore patients in first complete remission are stratified according to their risk profile and then assigned to alloHSCT or non alloHSCT procedures, in particular autoHSCT (autologous hematopoietic stem cell transplantation) or an additional cycle of consolidation chemotherapy. For favorable risk patients, MRD-negative patients and patients who are not candidates for alloHSCT, autoHSCT is the preferred treatment option. [38] A large randomized study between post-remission chemotherapy versus autoHSCT revealed a better disease free survival (DFS) after autoHSCT approach. [38] For intermediate risk patients in whom MRD information could not be obtained (because no leukemia associated phenotype can be identified), an alloHSCT with a matched sibling donor or a phenotypically 10/10 HLA donor is the

preferred option. For poor risk and very poor risk patients, alloHSCT (with a matched sibling donor, a fully or partially matched unrelated donor or umbilical cord blood donor as defined below in this protocol) is considered the treatment of first choice. These transplants are increasingly applied following reduced intensity conditioning regimens, as various retrospective studies report similar overall survival compared to alloHSCT following marrow-ablative conditioning regimens. [39]
Although the probabilities of recurrence after these different post remission modalities vary, the problem of relapse remains the major therapeutic challenge in each of these treatment conditions. The risk of leukemia recurrence after post remission chemotherapy and autoHSCT (approximately 50-60% relapse) and alloHSCT (approximately 20% relapse) has remained considerable although it varies according to the baseline cytogenetic and molecular risk features of the leukemia. The relapse probability increases in poor risk and very poor risk AML. This explains the active current interest to further improve the anti-leukemic effectiveness of post-remission treatment approaches.
The current study will assess the therapeutic value of lenalidomide not only in remission induction therapy but also in first complete remission (CR1) after post-remission consolidation therapy (autoHSCT, chemotherapy) and evaluate lenalidomide, especially for its ability to prevent relapse. In a separate study linked to this protocol the feasibility of using lenalidomide after alloHSCT will be investigated.

5.2 Investigational Medicinal Product

5.2.1 Lenalidomide

Lenalidomide is a 4-amino-glutarimide analogue of thalidomide and an active agent in many hematological diseases. The chemical name is 3-(4-amino-1-oxo 1,3-dihydro-2H-isoindol-2-yl) piperidine-2,6-dione. The empirical formula for lenalidomide is C13H13N3O3, and the molecular mass is 259.3 g/mol.

Mechanism of action of Lenalidomide
Lenalidomide belongs to the class of immunomodulatory drugs (IMiDs) which are orally available compounds that modulate the immune system and other biologically important targets through multiple mechanisms of action that remain to be fully characterized. [9]
Lenalidomide possesses anti-neoplastic, immunomodulatory and anti-angiogenic properties. Lenalidomide inhibits the secretion of pro-inflammatory cytokines and increases the secretion of anti-inflammatory cytokines from peripheral blood mononuclear cells. [10] Effects both on the malignant clone and the microenvironment have been suggested. Lenalidomide inhibits cell proliferation with varying effectiveness in some but not all cell lines. Recently, cereblon part of the E3 ubiquitin ligase complex, was found to be a target of the intracellular activity of thalidomide and lenalidomide.

Lenalidomide, an approved drug for relapsed multiple myeloma and the 5q- subtype of MDS
Lenalidomide is approved in nearly 70 countries in Europe, North-America, Asia, and others for relapsed multiple myeloma and it is also approved in the USA, Canada, Australia, Switzerland and various other countries for myelodysplastic syndrome (MDS) with the 5q- chromosomal abnormality.

Clinical Experience of Lenalidomide in AML

A recent study showed that lenalidomide improves erythropoiesis in low risk MDS with 5q deletion either alone or combined with additional chromosomal abnormalities, lenalidomide is indeed FDA approved for this indication. [(11)] More recently, activity in transfusion dependent low risk MDS without deletion 5q was also described.[(12)] 75% of the patients had a normal karyotype, 13% an intermediate and 3% a poor prognostic karyotype. Patients were treated with lenalidomide 10 mg daily on days 1-21 of a 28- days cycle. In this study 26% of 214 patients achieved transfusion independence after a median of 4.8 weeks. The overall hematological improvement rate was 43%. The most common grade 3/4 side effects were neutropenia (30%) and thrombocytopenia (25%). Sekeres et al showed that cytopenia correlated with response and therefore suggested, that the development of cytopenia, be it either neutropenia or thrombocytopenia, may reflect the therapeutic effect of lenalidomide on the malignant clone. [(13)] Because lenalidomide induces morphological and cytogenetic remissions in MDS patients, including those with excess of blasts, efficacy in AML was expected and evaluated. Preliminary results of a phase II study in elderly patients with AML showed indeed activity of lenalidomide as initial treatment. [(14)] Lenalidomide was administered at high dosages (50 mg/day x 14 days, 30 days of rest, 50 mg/day x 21 days) followed by maintenance therapy. In this study 5 out of 8 patients showed clearance of circulating blasts and 9 out of 12 patients had significant reduction in bone marrow blast at day 15. In a recent manuscript, induction of sustained morphologic and cytogenetic complete remission has been observed in 2 older AML patients treated with high-dose, single-agent lenalidomide. This underlines the hypothesis that lenalidomide has clinical activity in AML even in this poor-risk cytogenetic subset. [(13)] Lenalidomide has anti-angiogenesis properties which could also be of value in AML treatment. Microvessel density (MVD) is increased in AML as compared to normal bone marrow. MVD decreases during chemotherapy induced aplasia in patients who achieve a CR but not in those who fail to therapy. [(14)] Recently it was shown by dynamic MRI that bone marrow angiogenesis is increased in AML with differences between various AML patients. Increased BM angiogenesis was highly correlated with adverse clinical outcome. [(16)] In a phase II study in older patients (median age 71 years) with newly diagnosed AML an encouraging response rate and durable remissions have been reported following single agent lenalidomide treatment at a daily dose of 50 mg in 28 day cycles. [(53)] Recently it was demonstrated that lenalidomide treatment enhances the C/EBPα-p30 protein levels (which correlates with a favorable chemotherapy response) and in turn miR-181a which appears to sensitize AML blasts to chemotherapy. [(60)]

In the current study we will evaluate the feasibility and efficacy of lenalidomide in combination with standard chemotherapy in AML in adult patients.
In our own ongoing HOVON 103 AML/SAKK 30/10 study, the addition of Lenalidomide to standard induction chemotherapy is being investigated in AML patients of older age (>65 yrs) so that the study group has meanwhile gained experience with the use of lenalidomide in remission induction in AML albeit in patients of older age and at somewhat different dose schedules. In the latter study the dose level of lenalidomide had been selected at a final dose level of 20 mg following 2 subsequent interim analyses. A recent interim analysis (ie based on the data as available at the HOVON Data Center (HDC) on 28th January, 2014) was based on comparative data in 50 pts versus 50 pts in investigational and control study arms. In the analysis among those 100 patients no apparent signals of safety issues were noted (in terms of death rates, survival, neutrophil recoveries and CR rates between the Lenalidomide 20 mg/day treatment arm and the standard treatment arm).

5.2.2 Allogeneic HSCT in AML

Allogeneic HSCT is generally recommended as the treatment of choice to consolidate remission in classically defined intermediate risk (i.e. without MRD refinements) and (very) poor risk AML in first complete remission up to 60 years of age. [40, 41] The beneficial effect of alloHSCT in terms of reduction of relapse risk applies to all categories of AML. [e.g.42] However, treatment related mortality may outbalance a beneficial effect on DFS in favorable risk AML and MRD-negative intermediate risk AML. Therefore, alloHSCT is generally not recommended in first CR in these more favorable subsets of AML. Despite its effectivity in poor-risk and very-poor risk AML, alloHSCT is still associated with a considerable risk of relapse. [43, 44] While current data underscores the importance of allogeneic HSCT, improvement is needed and should be pursued. Recent studies set out to explore the use of post-transplant chemotherapy, including e.g. azacitidine, after allogeneic HSCT. [45] These studies showed the feasibility of post-transplant chemotherapy with the aim to optimize on one hand disease control and, on the other hand, to optimize the immunotherapeutic graft-versus-leukemia (GVL) - effect. The first HOVON-study to address this policy is the HOVON-116 study, which is the current recommended option for (very) poor-risk AML CR1 patients. Lenalidomide has also been explored in the post-transplant setting, in particular in patients with multiple myeloma. While concern has been raised with respect to a possible enhancing effect on graft-versus-host-disease (GVHD) [46], encouraging anti-myeloma effects were observed due to enforced graft-versus-myeloma and/or a direct anti-myeloma effect. This has raised an interest in the utility of lenalidomide post-alloHSCT for potentiating anti-leukemic effects via GVL. [47] Thus, recent studies have set the stage for a broader exploration of lenalidomide in the post-transplant setting, whereby feasibility should be carefully addressed, preferably in a setting of intensified GVHD-prophylaxis to minimize possible GVHD exacerbating effects, while preserving GVL effects.

Reduced intensity conditioning alloHSCT

Non-myeloablative or reduced intensity conditioning (RIC) regimens have been developed in order to reduce non-relapse mortality (NRM) in elderly or medically less fit patients. Several studies have indeed shown that the morbidity and mortality following RIC alloHSCT are less than after myeloablative (MA) conditioning, whereas encouraging GVL effects are exerted. [48] Currently, most centers within our cooperative consortium use a T-cell replete allogeneic HSCT regimen with post-transplant GVHD prophylaxis consisting of cyclosporine A (CyA) and Myfortic (mycofenolic acid). With this regimen the incidence of acute GVHD II-IV is 50-60%. Recently, several groups have reported efficacy data of post-transplant GVHD prophylaxis consisting of high-dose cyclophosphamide given on days +3 and +4. [29, 30, 49, 50 and 51] The rationale for this strategy is as follows: alloreactive donor T lymphocytes are activated after the infusion into the recipient, enter a proliferative phase, and are thus sensitive to the cytotoxic effect of cyclophosphamide 72 hours later. On the other hand, non-alloreactive, non-proliferating T-cells are spared and may provide protection against infections in the short term and allow for a more robust immune reconstitution. Engraftment appears not really compromised, probably due to the fact that hematopoietic stem cells express high levels of aldehyde dehydrogenase, rendering them resistant against the cytotoxic effects of high-dose cyclophosphamide. [66] In the setting of T-cell replete haplo-identical HSCT, post-transplant high-dose cyclophosphamide was combined with conventional immunosuppression consisting of a calcineurin inhibitor plus Mycophenolate Mofetil (MMF) and resulted in an incidence of acute GVHD of 12-34%. [49, 52] In addition to these low rates of acute GVHD, the incidence of non-relapse mortality was shown to be encouraging as well (7-18%). In the setting of fully matched related or unrelated donor transplantation, post-transplant high-dose cyclophosphamide has been used as the sole form of GVHD prevention and resulted in an incidence of acute GVHD of 43% with a NRM rate of 15% at 2 years. [50] Such a strategy of a short-course prophylaxis of GVHD is specifically suited to enable the implementation of early post-transplant chemotherapy, without facing possible drug interactions. The HOVON/SAKK AML study groups and alloHSCT study groups will selectively apply the latter approach in the HOVON-116 study as well as in the alloHSCT lenalidomide study that will be planned and apply post-transplant chemotherapy and create a setting devoid of severe GVHD. This is done with the notion of providing adequate immune reconstitution to avoid excessive opportunistic infections while administering post-transplant chemotherapy.

5.3 Rationale of the study

AML in adults continues to have a fatal prognosis in the majority of patients. The medical need for new therapies in this important patient group is still considerable. The HOVON/SAKK Cooperative Consortium that partners with institutions in the Netherlands, Belgium, Luxembourg, Switzerland,

Sweden, Norway, Finland, Germany, Austria, Lithuania and Estonia concentrates its developmental therapeutic efforts on randomized phase III approaches introducing new treatment modalities into the backbone of standard remission induction chemotherapy in patients with newly diagnosed AML between 18-65 years of age. Lenalidomide appears a highly active anti-AML drug that has mainly been examined in relapsed AML and in patients of older age, but not yet as first line therapy of patients of younger age. In single arm studies the addition of lenalidomide to chemotherapy has shown encouraging clinical response rates with acceptable side effects in AML. A regimen of lenalidomide combined with standard remission induction chemotherapy potentially is of clinically value (as regards to CR and CR duration, overall survival and in prognostic subsets), but has not yet been critically evaluated for feasibility and efficacy, neither as part of combination induction treatment, nor in maintenance treatment. Besides its anti-leukemic activity, lenalidomide also shows potential to enhance the GVL activity after alloHSCT.

6 Study objectives

6.1 Primary Study objectives

6.1.1 Part A-run-in:

- To select in a randomized approach the feasible dose level of lenalidomide when given orally at three variable dose levels (at 20 mg/day 1-21; 15 mg/day 1-21 or 10 mg/day 1-21) in combination with standard induction cycles I and II in patients with AML/ MDS with IPSS-R> 4.5

6.1.2 Part A:

- To evaluate the effect of lenalidomide on EFS at the (during Part A run-in) selected feasible dose level when combined with remission induction chemotherapy cycles I and II in a randomized comparison to remission induction cycles I and II without addition of lenalidomide

6.1.3 Part B:

- To evaluate the effect on the Cumulative incidence of relapse (CIR) of 6 cycles of maintenance therapy with lenalidomide treatment (10 mg/day for 21 days followed by 14 days rest) after post remission chemotherapy cycle III or autoHSCT versus observation only

6.2 Secondary study objectives

6.2.1 Part A: First randomization

- To investigate the efficacy of lenalidomide in combination with remission induction chemotherapy cycles I and II (in comparison with the same treatment without lenalidomide) in all patients with regard to complete remission rate (CR/ CRi), DFS, CIR and OS
- To investigate the efficacy of lenalidomide in combination with remission induction chemotherapy cycles I and II in molecularly and cytogenetically distinguishable subsets with regard to complete remission rate (CR/CRi), DFS, CIR and OS
- To evaluate the treatment effects according to MRD measurements following therapy by standardized sampling of bone marrow/blood following remission induction treatment
- To determine the prognostic value of molecular markers and gene expression profiles of the leukemia cells assessed at diagnosis for both remission induction treatments
- To investigate the toxicities of lenalidomide in combination with remission induction chemotherapy cycles I and II
- To compare CIR after autoHSCT and cycle III according to molecular markers and MRD measurements
- To evaluate the effect of lenalidomide on the feasibility of collecting adequate autologous stem cell grafts and the probability of proceeding to autoHSCT

6.2.2 Part B: Second randomization

- To investigate the efficacy of lenalidomide with regard to DFS and OS measured from 2nd randomization
- To investigate post remission and post-transplant toxicities and need for transfusions when lenalidomide is applied after post remission chemotherapy/autoHSCT
- To evaluate the efficacy of lenalidomide as post-remission therapy to prevent relapse in all randomized patients, but also in relationship with the distinctive risk categories of AML (as based on cytogenetics and molecular genetics) and MRD estimates

7 Study design

Part A Run-in randomized dose-selection study

First, we will establish, according to decision rules based on DLT frequency and myelosuppression (see section 14.2.1 table 15), in a randomized run-in study the dose level of lenalidomide as an addition to standard induction chemotherapy. Treatment with and without lenalidomide added to

standard induction chemotherapy consisting of idarubicin/ cytarabine (cycle I) and daunorubicine/ cytarabine (cycle II) will be compared.

DLT is defined as:

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

Occurring after start of cycle I and before start of cycle II or going off protocol (whichever comes first) or within 42 days after start of cycle II and before the start of a new treatmentor going off protocol (whichever comes first) .
The duration of myelosuppression is defined as the median time to recovery of ANC > 0.5 $x10^9$/L.
DLT and myelosuppression both will be used in the decision process for dose escalation, dose reduction and/or dose selection (see section 14.2.1 table 15 for more details).

Part A randomized phase III study

Following the dose-selection phase the study will continue as a randomized phase III study with induction treatment with or without lenalidomide.

Part B randomized maintenance study

Subsequently, we will also investigate the effect of 6 cycles lenalidomide treatment (10 mg/day for 21 days followed by 14 days rest) after post-remission chemotherapy cycle III or autoHSCT versus observation only for patients in CR1.

8 Study population

8.1 Eligibility criteria for registration/randomization 1

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

8.1.1 Inclusion criteria

- Age 18-65 years, inclusive
- Patients with
  - a diagnosis of AML and related precursor neoplasms according to WHO 2008 classification (excluding acute promyelocytic leukemia) including secondary AML (after an antecedent hematological disease (e.g. MDS) and therapy-related AML), **or**
  - acute leukemia's of ambiguous lineage according to WHO 2008 **or**

- a diagnosis of refractory anemia with excess of blasts (MDS) and IPSS-R score > 4.5

- WHO performance status 0, 1 or 2
- Sampled bone marrow and/ blood cells at diagnosis for centralized molecular analysis and MRD evaluation, unless in case of a dry marrow tap with no possibility to collect marrow cells. In cases of marrow tap failure only blood cells will be sampled.
- Adequate renal and hepatic functions unless clearly disease related as indicated by the following laboratory values:
  - Serum creatinine ≤1.0 mg/dL (≤88.7 µmol/L); if serum creatinine >1.0 mg/dL (>88.7 µmol/L), then the estimated glomerular filtration rate (GFR) must be >60 mL/min/1.73 m^2 as calculated by the Modification of Diet in Renal Disease equation where Predicted GFR (ml/min/1.73 m^2) = 186 x (Serum Creatinine in mg/dL)$^{-1.154}$ x (age in years)$^{-0.203}$ x (0.742 if patient is female) x (1.212 if patient is black)
    NOTE: if serum creatinine is measured in umol/L, recalculate it in mg/dL according to the equation: 1 mg/dL = 88.7 umol/L) and use above mentioned formula.
  - Serum bilirubin ≤2.5 x upper limit of normal (ULN)
  - Aspartate transaminase (AST) ≤ 2.5 x ULN
  - Alanine transaminase (ALT) ≤ 2.5 x ULN
  - Alkaline phosphatase ≤ 2.5 x ULN
- Written informed consent
- Ability and willingness to adhere to the lenalidomide Pregnancy Prevention Program

8.1.2 Exclusion criteria

- Previous therapy with lenalidomide
- Acute promyelocytic leukemia
- Myeloproliferative neoplasia
- Previous treatment for AML or high risk MDS (IPSS-R > 4.5), except hydroxyurea
- Concurrent history of active malignancy in two past years prior to diagnosis except for:
  - basal and squamous cell carcinoma of the skin
  - in situ carcinoma of the cervix
- Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, infection, hypertension, pulmonary disease etcetera)
- Cardiac dysfunction as defined by:
  - Myocardial infarction within the last 6 months of study entry, **or**
  - Reduced left ventricular function with an ejection fraction < 50% as measured by MUG scan or echocardiogram **or**

- Unstable angina, **or**
- Unstable cardiac arrhythmias

- Hypersensitivity to the active substance or to any of the excipients of the drug product
- Pregnant or lactating females
- Unwilling or not capable to use effective means of birth control
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule

8.2 Eligibility criteria for randomization 2a (Part B)

Eligibility criteria for post-transplantation lenalidomide for part B (after cycle III or autoHSCT)
The second randomization ≤ 4 months after start chemotherapy cycle III or start conditioning autoHSCT.

8.2.1 Inclusion criteria

- CR or CRi
- Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L
- Platelet count ≥ 75 x 10^9/L
- Serum creatinine clearance ≥ 30 ml/min
- Total bilirubin ≤ 2.5 x ULN
- AST ≤ 2.5 x ULN
- ALT ≤ 2.5 x ULN

8.2.2 Exclusion criteria

- Severe cardiac dysfunction (NYHA classification II-IV, see appendix G)
- Severe pulmonary dysfunction (CTCAE grade III-IV, see appendix F)
- Severe neurological or psychiatric disease
- Serious active infections
- Previous serious toxicities related to the use of lenalidomide
- CMV reactivation, which is not responsive to first line valganciclovir

9 Treatment

9.1 Remission induction treatment

9.1.1 Remission induction treatment Cycle I

Table 1. Arm A (standard of care – comparator arm)

Agent	Dose/day	Route of administration	Days
Idarubicin	12 mg/m^2	3 hr infusion	1,2,3
Cytarabine (Ara-C)	200 mg/m^2	24 hr infusion on days 1 - 7	1 thru 7

Table 2. Arm B (investigational arm)

Agent	Dose/day	Route of administration	Days
Idarubicin	12 mg/m^2	3 hr infusion	1,2,3
Cytarabine (Ara-C)	200 mg/m^2	24 hr infusion on days 1 - 7	1 thru 7
Lenalidomide	20, 15 or 10 mg See below*	orally	1 thru 21 evening

Cytarabine (Ara-C) is to be dissolved in 500 mL 0.9% NaCl or 5% glucose (D5W).

Idarubicin, in vials of red orange lyophilized powder, containing 5 mg or 10 mg, is to be dissolved in 1 ml sterile water per mg Idarubicin.

Lenalidomide will be administered orally

***Dose level Lenalidomide**

The initial dose of lenalidomide in the first cohort of patients will be 20 mg/day 1-21 in cycles I and II. Decisions regarding the feasibility of 20 mg/day 1-21 of each cycle, and subsequent continuation towards dose level of 15 mg/day or perhaps the lower dose level of 10 mg/day 1-21 respectively, will be based on the incidence of DLT and myelosuppression information, according to the decision rules defined in the statistical section, chapter 14.

The marrow response will be assessed after cycle I. Patients in CR or CRi who have already been identified as belonging to the poor or very poor risk groups (see Appendix D) and for whom an allogeneic donor is available, have the option to proceed to an alloHSCT immediately after cycle I.

Cycle II will be started as soon as possible when the marrow still shows more than 15% blasts at day 17-21, and also in case of blasts of less than 15% but with hematopoietic regeneration (platelets above 50 x 10^9/L; ANC > 1.0 x 10^9/L). If the hematopoietic regeneration takes more than 50 days and is not due to residual leukemia, lenalidomide will not be given in cycle II in arm B.

Patients in CR receiving cycle II will receive this treatment as soon as possible.
A bone marrow evaluation will be done **before** cycle II is started.

9.1.2 Remission induction treatment Cycle II

Table 3. Arm A (standard treatment)

Agent	Dose/day	Route of administration	Days
Daunorubicin	60 mg/m^2	1 hr infusion	1, 3, 5
Cytarabine (Ara-C)	1000 mg/m^2 q 12 hrs (x12)	3 hr infusion	1 thru 6

Table 4. Arm B (investigational arm)

Agent	Dose/day	Route of administration	Days
Daunorubicin	60 mg/m^2	1 hr infusion	1, 3, 5
Cytarabine (Ara-C)	1000 mg/m^2 q 12 hrs (x12)	3 hr infusion	1 thru 6
Lenalidomide	20, 15 or 10 mg See section 9.1.1*	orally	1 thru 21 evening

Cytarabine (Ara-C) to be dissolved in 500 mL 0.9% NaCl or 5% glucose (D5W).

Daunorubicin to be dissolved in 100 ml 0.9% NaCl

Lenalidomide will be administered orally

The start of cycle II can be delayed in case of intercurrent infectious or metabolic complications.

All patients will be evaluated for response after cycle I and II according to appendix C. Patients in CR or CRi after cycle II will proceed to either consolidation chemotherapy, or autologous stem cell transplantation, or allogeneic stem cell transplantation depending on the risk assessment according the AML risk group classification (see appendix D).

9.1.3 Dose modification for lenalidomide

Any grade 3 non-hematological toxicity attributable to lenalidomide will require treatment with lenalidomide to be discontinued. If toxicity resolves to grade 1 within 4 weeks, treatment will be restarted (on scheduled days). No dose reductions of lenalidomide are permitted. Missed doses of lenalidomide and combination chemotherapy will not be made up. Any patient who develops any one of the following toxicities attributable to lenalidomide should not receive further lenalidomide:

- Grade 4 toxicity
- Grade 3 toxicity that does not resolve to grade 1 or less within 4 weeks
- Venous/arterial thrombolic event: permanently discontinue lenalidomide

9.1.4 Infection prophylaxis during remission induction and following consolidation

Antimicrobial prophylaxis during remission induction following cycles I and II to prevent bacterial and fungal infections is recommended and will be performed according to local guidelines and practice. A similar advice applies to consolidation treatment (cycle III).

9.2 Choosing the post-remission therapeutic options

Favorable risk patients will be planned to receive autoHSCT following busulfan-cyclophosphamide conditioning (see par 9.5). The autograft will be harvested as soon as the patient has been shown to enter CR after cycle II during the hematological recovery phase. If an autoHSCT is not possible for some reason (e.g. insufficient stem cell harvest, patient refusal), patients will proceed to chemotherapy cycle III that consists of mitoxantrone and etoposide (see par 9.3).
Intermediate risk patients with negative MRD will proceed to autoHSCT. If an autoHSCT is not possible for some reason (e.g. insufficient stem cell harvest, patient refusal), patients will proceed to chemotherapy cycle III that consists of mitoxantrone and etoposide (see par 9.3). Intermediate risk patients in whom MRD assessment information is not available will proceed to a HLA matched sibling alloHSCT and if such a donor is not available to a fully matched phenotypically HLA identical unrelated donor (10/10 molecular match).
Poor risk and very-poor risk AML patients will proceed to alloHSCT, using either sibling (preferred), or alternative donors, including unrelated donors or umbilical cord blood. If an allogeneic transplant cannot be done due to lack of a donor, patient refusal or for medical reasons, patients will be treated with autoHSCT or cycle III chemotherapy as is done in favorable risk patients and be eligible for second randomization for maintenance therapy with lenalidomide as for other patients receiving autoHSCT or chemotherapy cycle III

9.2.1 Lenalidomide randomization for maintenance treatment

Patients not proceeding to alloHSCT in CR1 and therefore undergoing autoHSCT or chemotherapy cycle III (mitoxantrone-etoposide) will be randomized for lenalidomide maintenance or observation only. Within four months after start of autoHSCT or cycle III, patients will be randomized between observation only or lenalidomide. Patients assigned to lenalidomide maintenance will receive lenalidomide at 10 mg/day for 21 days at 5 week cycles for 6 cycles.

Table 5. Maintenance therapy

Agent	Dose/day	Days – 6 cycles	Interval with no treatment (between cycles)
Lenalidomide	10 mg oral, evening	1-21 Start next cycle day 35, repeat for 6 cycles)	14 days
Control (no lenalidomide)	--	--	--

9.2.2 Dose modification for lenalidomide

Any grade 3 non-hematological toxicity attributable to lenalidomide will require treatment with lenalidomide to be discontinued. If toxicity resolves to grade 1 within 4 weeks, treatment will be restarted (on scheduled days). No dose reductions of lenalidomide are permitted. Missed doses of lenalidomide will not be made up. Any patient who develops any one of the following toxicities attributable to lenalidomide should not receive further lenalidomide:

- Grade 4 toxicity
- Grade 3 toxicity that does not resolve to grade 1 or less within 4 weeks
- Venous/arterial thrombolic event: permanently discontinue lenalidomide

9.3 Peripheral blood stem cell mobilization and collection

9.3.1 Peripheral blood stem cell mobilization

All patients with good risk cytogenetics and intermediate risk cytogenetics should have peripheral blood stem cell mobilization. Granulocyte-colony-stimulating factor (G-CSF); filgrastim 5 µg/kg will be given subcutaneously twice daily to all patients eligible for autoHSCT after remission induction cycle II, i.e., patients being treated in both arms A and B of the study, except in patients who will certainly proceed to HLA matched alloHSCT. G-CSF treatment will be started after cycle II chemotherapy at the onset of recovery of granulocytes of 0.5 x 10^9/L or more, and continued until the last day of apheresis.
Patients not in CR(i) after cycle II are not eligible for autoHSCT and therefore should not have peripheral blood stem cell mobilization.

9.3.2 Procedure of peripheral blood progenitor cell collection

Timing of apheresis; as soon as the neutrophil count begin to rise to values of 2 x 10^9/L or more and significant numbers of CD34 positive blood cells appear, peripheral blood cells will be collected in one to four leukapheresis sessions (preferably until the collection of at least 5 x 10^6 CD34+ cells/kg but minimal until 3 x 10^6 CD34+ cells/kg). G-CSF will be discontinued following completion of peripheral

blood stem cell harvest. If an insufficient total number of cells has been collected, an autologous marrow may be collected or a second peripheral blood progenitor cell (PBPC) collection may be attempted according to center policy. If no adequate PBPC or marrow graft can be obtained, post induction chemotherapy cycle III will be delivered.

9.3.3 Procedure for hematopoietic cell cryopreservation

Procedure for hematopoietic cell cryopreservation is according to local procedures.

9.4 Post induction therapy with chemotherapy cycle III

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

Table 6. Chemotherapy cycle III

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

Mitoxantrone to be dissolved in 100 mL 0.9% NaCl or 5% glucose or 5% dextrose (D5W). Mitoxantrone is supplied as blue sterile parenteral solution containing 30 mg in 15 mL vials

VP-16 (Etoposide) to be dissolved in 500 mL 0.9% NaCl immediately prior to use.

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

9.5 Post-induction treatment: Busulfan-Cyclophosphamide and autologous HSCT

Patients in continued CR receiving busulfan-cyclophosphamide followed by autologous HSCT will receive this treatment as soon as hematopoietic repopulation (platelets > $100x10^9$/L and ANC > $1.0x10^9$/L) has taken place. Patients who proceed to autoHSCT must have a confirmation of remission status within 2 weeks prior to start of autoHSCT procedure.

Table 7. Busulfan-Cyclophosphamide and autologous HSCT

Agent	Dose/day	Route of administration	Days
Busulfan *or* Busilvex	1 mg/kg q 6 hrs 0.8 mg/kg q 6 hrs	orally 2 hr infusion	-7, -6, -5, -4 -7, -6, -5, -4
Cyclophosphamide	60 mg/kg	1 hr infusion	-3, -2
Phenytoin	5 mg/kg q 6 hrs	orally	-9,-8,-7 thru -4
HSCT infusion			0

Busulfan (oral) - 4 mg/kg/day (total 16 mg/kg) divided into q 6 hours (1 mg/kg/dose oral). A 70 kg man will, for instance, receive 280 mg/day or 70 mg q 6 hrs.
Since administration of high-dose busulfan has been temporarily associated with the development of generalized seizures, prophylactic administration of Phenytoin (5 mg/kg/dose p.o. q 6 hrs beginning 2 days before the first dose of busulfan (= day -9), then 5 mg/kg/day p.o. daily through day -4) is recommended. Also Diazepam as an anticonvulsant agent may be used.
Cyclophosphamide - (60 mg/kg) will be infused in 500 mL NS (0.9% NaCl) or 5% glucose over 1 hour. Mesna 300 mg/m^2 will be administered at -10 min prior to cyclophosphamide infusion, +4 hrs, +8 hrs and +12 hrs following cyclophosphamide infusion on days -3 and -2. Patients will be hydrated with D5'NS (5% glucose, 0.45% NaCl + 20 mEq KCl/L + 5 mg furosemide/L) i.v. at 200 cc/hr for 72 hrs beginning 2 hrs before the first cyclophosphamide dose. KCl will be further supplemented in case of hypokalemia. 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 anti-emetics.
Infusion of stem cells (HSCT) 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 re-infused through a saline infusion set. Quality control of the graft prior to administration will be done according to local guidelines. 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 re-infused.

9.6 Post-induction treatment: allogeneic HSCT

Donor search

Following diagnosis of AML, meeting the upfront criteria for eligibility of the study and registration, an HLA-identical donor search must be initiated as soon as possible, first among siblings and secondly in the world donor bank for unrelated donors. In order to avoid inappropriate delay in case no suitable sibling is present, high-resolution HLA typing should be performed immediately after registration enabling a more rapid matched unrelated donor search.

9.6.1 Reduced intensity allogeneic HSCT

The allogeneic transplantation is preferably performed in a uniform, standardized way. If eligible, AML patients in CR1 may proceed to the recommended alloHSCT HOVON studies that are active concomitantly with the HO132 protocol. First, the HOVON-116 study for (very) poor-risk AML patients is the preferred protocol for the application of alloHSCT until a feasibility study with lenalidomide post alloHSCT has become available. Second, the development of a post alloHSCT study with lenalidomide is currently under discussion and will be developed in close cooperation between the HOVON AML and alloHSCT working groups.
It implies that until the HO116 is open for inclusion and thereafter for patients not qualifying for an alloHSCT-study linked to the HO132, alloHSCT is performed after either a myeloablative conditioning regimen, preferably based on the Seattle regimen consisting of low dose TBI or fludarabin. GVHD-prevention after myeloablative conditioning will be performed by the combination of methotrexate and

cyclosporine. GVHD-prevention after non-myeloablative alloHSCT will consist of the combination of cyclosporine and mycophenolate (Myfortic). Cyclosporine will be given from day +5 until day +180 and dose will be adjusted according measured trough levels of the drug in blood. Trough levels are targeted to the upper therapeutic range [250-350 µg/L using the immunoassay and 200-300 µg/L using HPLC] and monitored at day 0, day +3, day +7 and thereafter once a week.
Cyclosporine A will be given either p.o. or i.v. and dose adjustments will be based on trough levels
Myfortic will be given with a maximum of 2160 mg/day.

Table 8. AlloHSCT

Agent	Dose/day	Route	Days (see 9.1.4)
Cyclosporine A	9 mg/kg (in 2 doses) OR 3 mg/kg (in 2 doses)	p.o. i.v.	From day –3 until day 120, followed by taper
Myfortic	32 mg/kg (in 2 doses)	p.o.	From day 0 until day 90, then stop.

Duration of Immunosuppressive therapy for GVHD prophylaxis

The schedule of discontinuation of GVHD prophylaxis will depend on the stability of the CR of the leukemia and the presence or absence of GVHD as follows:

a. In patients with relapsing AML:

In case of a hematological relapse and no overt GVHD, all immunosuppression should be withdrawn immediately. In case of a cytogenetic /molecular relapse and no overt GVHD, immunosuppression will be tapered and stopped within 2-4 weeks.

b. In patients without any past history of GVHD or current GVHD:

- Myfortic stop at day +90.
- CyA taper from day +120 with 10% per week.

c. In patients with a history of GVHD:

- Myfortic stop at day +90.
- CyA taper from day +180 with 10% per week.

It is allowed to replace Cyclosporin A by tacrolimus as GVHD prophylaxis. Tacrolimus, 0.06 mg/kg, two times a day, will be given from day -3 and dose will be adjusted according measured trough levels of the drug in blood. Trough levels are targeted to the upper therapeutic range [15-20 ng/ml] until day +28. After day +28 the target range is 10-15 ng/ml until day +120 to +180. Thereafter tacrolimus is tapered and stopped.

According to local policy the Myfortic can be replaced by Cellcept, 15 mg three times a day, from day 0 until day +28 in patients with a sibling donor and until day +42 in patients with a matched unrelated donor.

Of note: as indicated in the Introduction and subsequent relating paragraphs of the current study, a feasibility study exploring lenalidomide post alloHSCT is foreseen, which study may set the stage for a future question addressing the efficacy of lenalidomide post alloHSCT. It is intended to apply lenalidomide in that setting in conjunction with a standardized non-myeloablative regimen, consisting of intravenous cyclophosphamide, fludarabine and low dose total body irradiation (TBI).

Table 9. Non-myeloablative conditioning prior to alloHSCT

Agent	Dose/day	Route of administration	Days
Fludarabine	30 mg/m^2	1 hr infusion	-4 thru -2 (3 days)
Low dose TBI	2 Gy		-1
Stem cell infusion			0
Myfortic	32 mg/kg	Oral (divided in 2 doses)	+1 until day +90
Cyclosporine A	3 mg/kg	1 hr infusion	-3 thru +120

Table 10. Myeloablative conditioning prior to alloHSCT

Agent	Dose/day	Route of administration	Days
Cyclophosphamide	60 mg/kg	1 hr infusion	-3,-2
Busulphan	1 mg/kg q 6hrs	1 hr infusion	-7, -6, -5, -4
ATG (if unrelated donor)	2mg/kg (rabbit) 5mg/kg (horse) according to local preference	Intravenous, according to local policy	-7, -6, -5, -4 -7, -6, -5, -4 Or according to local policy for ATG-treatment
Stem cell infusion			0
Methotrexate	50 mg/kg	1 hr infusion	+1, +3, +6, +11
Cyclosporine A	3 mg/kg	1 hr infusion	-3 thru +120

9.6.2 Special precautions and supportive care

Special management in conjunction with allogeneic transplantation

- Stem cell collection in related donors: donors can be mobilized according to local protocols
- HLA matching between patient and donor: HLA identical sibling donors or 10/10 allele matched unrelated donors (for HLA-A, B, C and DR) are allowed for intermediate risk patients
- HLA matching between patient and donor: HLA identical sibling donors or 9/10 and 10/10 matched unrelated donors (for HLA-A, B, C and DR) are allowed for patients with poor risk and very poor risk AML
- Stem cell source: peripheral blood mobilized or marrow stem cells are allowed

- In case of major ABO blood group incompatibility and a high load of red blood cells (>200x10^9), and/or an anti-A or anti-B titer of ≥ 1/16: pre-hydration with 1L NaCl 0.9% over 4 hrs and slow starting of stem cell graft infusion, starting with 1 ml/min, to be doubled after 30 min. and then further increased to 3 ml/min if no adverse reactions occur (or according to local protocol). In these instances washing the graft and plasma depletion to reduce the risk of antibody infusion along with the grafted cells are to be considered.
- High dose cyclophosphamide should standardly be administered along with Mesna as prophylaxis for bladder and renal toxicity

Infection prophylaxis after transplantation

Prophylaxis against bacterial and fungal infections and Pneumocystis jirovecii pneumonia will be performed according to local practice. As a minimal prophylaxis cotrimoxazole and valaciclovir is advised during the first year after transplantation. Monitoring of CMV and EBV will be performed following standard procedures and pre-emptive therapeutic intervention will be initiated when appropriate. It is advised to perform weekly CMV and EBV monitoring during the first 90 days after transplantation in particular when donor and recipient have previously been assessed to be CMV and EBV positive.

Therapy of CMV reactivation

Valganciclovir 900 mg orally twice daily for 14 days (of note: adjust dose based on renal function). In case of insufficient response further treatment with intravenous ganciclovir or foscarnet.

Therapy of EBV reactivation or post-transplant lymfoproliferative disease (PTLD)

Rituximab 375 mg/m^2 will be administered for EBV copy numbers in peripheral blood of >1000 geqs/ml (3 log EBV count) measured in 2 independent samples. Repeat if EBV copies are higher than 50% of starting level after 72 hrs.

Staging and handling of Graft versus Host Disease

Acute and chronic GVHD will be staged according to the criteria described in appendix H.
Acute GVHD grade ≥ 2 should be treated with prednisone (2 mg/kg orally or intravenously for 10 days). In case of complete GVHD response, reduce the dose with 50%. Thereafter prednisone should be tapered with 20 mg weekly until 40 mg/ day. From that moment on, prednisone should be tapered with 10 mg weekly until a dose of 20 mg/ day. Then the dose should be lowered with 5 mg weekly. CyA will be continued or restarted orally (9 mg/ kg/ day in 2 doses) or intravenously (3 mg/ kg/ day) if there is concern of GI absorption. Dosage of CyA should be based on trough levels. Steroid refractory acute GVHD is treated according to local guidelines. Extensive chronic GVHD (according to the NIH

criteria in appendix H) is treated by combination immunosuppressive therapy, preferably prednisolone and cyclosporine or according to local guidelines.

9.7 Investigational Medicinal Product Lenalidomide

Lenalidomide is approved in nearly 70 countries in Europe, North-America, Asia and others for relapsed multiple myeloma and it is also approved in Europe, USA, Canada, Australia and various other countries for myelodysplastic syndrome with the 5q-chromosomal abnormality.

9.7.1 Summary of known and potential risks

Most frequently reported adverse events during clinical studies with lenalidomide in oncologic and non-oncologic indications, regardless of presumed relationship to study medication include: anemia, neutropenia, thrombocytopenia and pancytopenia, abdominal pain, nausea, vomiting and diarrhea, fatigue, dehydration, rash, itching, infections, sepsis, pneumonia, urinary tract infection (UTI), upper respiratory infection, cellulitis, atrial fibrillation, congestive heart failure, myocardial infarction, chest pain, weakness, hypotension, hypercalcemia, hyperglycemia, back pain, bone pain, generalized pain, dizziness, mental status changes, syncope, renal failure, dyspnea, pleural effusion, pulmonary embolism, deep vein thrombosis, cerebrovascular accident (CVA), convulsions, dizziness, spinal cord compression, syncope, disease progression, death not specified and fractures. Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

Lenalidomide may increase the risk on Deep Venous Thrombosis (DVT) and Pulmonary Embolism (PE). DVT and PE are serious adverse events (SAE) and accordingly any DVT and PE must be reported to HOVON Data Center within 24 hours of the initial observation of DVT and PE (see section 12.3.1).

Patients must comply with the lenalidomide Pregnancy Prevention Program. Any suspected fetal exposure to lenalidomide must be reported within 24 hours of being made aware of the event.

Toxicities will be scored according to the NCI Common Terminology Criteria of Adverse Events, version 4.0.

9.7.2 Preparation and labeling

Lenalidomide will be shipped to trial sites in containers labeled as an Investigational Medicinal Product. Lenalidomide will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.

9.7.3 Storage and handling

See Investigators Brochure for detailed storage and handling procedures.

The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.

9.7.4 Study drug supply

The sponsor will arrange delivery of lenalidomide to trial sites. No investigational medicinal product will be shipped until the sponsor has verified that all regulatory required documents and approvals for the site are available.

9.7.5 Drug accountability

The investigator, or a pharmacist or other appropriate individual who is designated by the investigator, should maintain records of the product's delivery to the trial site, the inventory at the site, the use by each patient, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s). Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

9.7.6 Study drug return and destruction

At the end of the trial or after expiry of the product, unused investigational medicinal product should be destroyed by the trial site. Destruction should be documented.

9.8 Toxicities for non-IMP products

Idarubicin

Congestive heart failure is a major complication of anthracyclines, frequently observed after high cumulative doses. The total planned dose of Idarubicin is 36 mg/m^2. These doses are considerably lower than those associated with congestive heart failure (maximal cumulative dose is not well documented and is in the range between 150 -500 mg/m^2 and some have proposed 150 mg/m^2 as the upper limit). Cardiotoxicity has also been observed with Daunorubicin, 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 HSCT.

Other non-hematological drug toxicities of idarubicin are: hair loss, mucositis, cardiomyopathy, nausea, vomiting, colitis, infertility.

Daunorubicin

Congestive heart failure is a major complication of anthracyclines, observed after high cumulative doses. The total planned dose of Daunorubicin is 180 mg/m2. These cumulative doses are considerably lower than those associated with congestive heart failure (max cumulative dose has been postulated at about 550 mg/m^2). See Idarubicin.

Cytarabine (Ara-C)

Conventional-dose: 200 mg/m^2: anorexia, nausea, vomiting, hepatic dysfunction, skin rash, pneumonitis, fever.
Intermediate-dose: 1 g/m^2 and high-dose: 2 g/m^2 in 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 and high-dose Ara-C must be stopped immediately in case of nystagmus or dysarthria.

Mitoxantrone

Alopecia, mucositis, nausea, vomiting, diarrhea, elevations of hepatic enzymes, lethargia, peripheral neuropathy and cardiotoxicity (total dose will be 50 mg/m^2; maximal cumulative dose has been estimated at approximately 160 mg/m^2). Urine can color greenish.

VP-16 (Etoposide)

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

Busulfan

Interstitial pneumonitis, hepatic dysfunction, erythematous skin rash, myasthenia 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), diarrhea, hemorrhagic cystitis (prevented by forced diuresis and Mesna), alopecia, diffuse macropapular rash.

Following conditioning and autologous HSCT

Autologous HSCT is rarely associated with chills, fever and nausea, which can be prevented with oral antihistamines (e.g. Tavegil 2 mg iv) and solucortef (100 mg iv). Following the infusion, patients will experience a period of severe pan-cytopenia of 2-6 weeks duration and are at risks of fever, infections and/or hemorrhages, which will require transfusion and microbiological support. In addition they will

enter a 1-3 week period of gastrointestinal symptoms (nausea, diarrhea) due to the chemotherapy induced mucositis. This may also include a period of oral mucositis (stomatitis). Veno-occlusive disease may occur, but occurs in less than 10% of patients when the exclusion criteria regarding liver function abnormalities are considered.
Infertility frequently ensues following high-dose therapy and stem cell transplantation. Hair loss is a side effect, which most patients will already show due to preceding conventional anti-leukemia chemotherapy.

G-CSF (granulocyte-colony stimulating factor)
Fever, diarrhea, abdominal pain, vomiting, skin rash, headaches, bone pain and injection site reactions have been reported following the use of G-CSF.

10 Study procedures

10.1 Time of clinical evaluations

- At entry: within 14 days before start of treatment
- Before cycle II and before cycle III/autoHSCT/alloHSCT
- Before 2nd randomization
- After 2nd randomization; for patients receiving lenalidomide before start next cycle, for patients in control arm every 5 weeks.
- 6 months after second randomization
- When patient is taken off protocol
- During follow up

All patients will be followed until 10 year after registration (for phase III trial).

10.2 Required investigations

Required investigations at entry should be no older than 14 days prior to randomization unless otherwise noted. All investigations should be recorded in the patient's medical file.

Table 12. Required investigations at entry, during treatment and during follow up

	At entry (before C1)	After C1 (before C2)	After C2 (before C3 /autoHSCT	Before 2nd randomization (both arms)	After 2nd randomization (every 5 weeks or cycle)	6 months after 2nd randomization	Off protocol	Follow up	At Relapse
Medical history	X	X	X	X	X	X	X	X	X
Physical examination	X	X [1)]	X [1)]	X [1)]	X [1)]	X[1)]	X[1)]	X [1)])	X
Peripheral Blood									
- Hematology	X	X [1)]	X [1)]	X [1)]	X [1)]	X [1)]	X [1)]	X [1)]	X
- Blood chemistry [2)]	X	X [1)]	X [1)]	X [1)]	X [1)]	X [1)])	X [1)]	X [1)])	X
Bone marrow									
- Morphology [3)]	X	X	X	X		X			X
- Immunophenotyping (locally)	X								X
- Cytogenetics (local)(and molecular analysis[7])	X	X[6)]			X[9)]	X			X
- Biobank (central Rotterdam)	X		X	X		X			X
- Plasma storage (central) (appendix L)	X								X
- Saliva storage (central)(appendix L)	X								
- Molecular analysis (central Rotterdam) (appendix L)	X[7)]								X[7)]
- Molecular-MRD analysis (central Rotterdam) (appendix L)	X		X[5)]						X
- Immuno-MRD assessment and biobank (central Amsterdam (appendix L)	X		X	X		X			X
Specific investigations									
- Coagulation tests	X								
- Pregnancy test [4)]	X								
- Virus serology (according to local practice)	X								
- ECG	X								
- Pulmonary function tests (VC, FeV1)[8)]	X								
- Smears for HRC review (central) (appendix L)	X								

1) Weekly until hematological recovery

2) At entry: Creatinine, sodium, potassium, uric acid, calcium, glucose twice weekly until discharge;

AST, ALT, alkaline phosphatase, γGT, bilirubin (direct and indirect), LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly

During treatment: Creatinine, AST, ALT, bilirubin (direct and indirect), LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly

3) In case of dry tap, bone marrow biopsy for histopathology needs to be done.

4) At entry, and before and during Lenalidomide treatment according to the Pregnancy Prevention Program. Depending on the personal circumstances of the female patient a pregnancy test should be considered by the local physician. See section 12.5.

5) Only in case of NPM1 mutant AML

6) Only in case of the presence of cytogenetic abnormalities at diagnosis

7) The following markers will have to be done (on material biobank Rotterdam): AML1-ETO, CBFB-MYH11, CEBPA mono-allelic and biallelic, FLT3-ITD mono-allelic and bi-allelic, FLT3-mut, KIT mut, NPM1 mut, ASXL1 mut, RUNX1 mut, p53 mutant, EVI-1 expression. Analyses can be done locally by molecular diagnostic laboratory if approved by MODHEM. Otherwise done by ErasmusMC Rotterdam. See appendix L, section II.4 for more details

8) On indication

9) Only in case of abnormality: after cycle III or auto

Table 13. Required investigations for alloHSCT

	Before alloHSCT	**After alloHSCT**	**3, 6 months after alloHSCT**	**1, 2, 3, 4, 5 years after alloHSCT**	**At Relapse**
Medical history	X	X	X	X	X
Physical examination	X[1)]	X[1)]	X	X	X
Grading of GVHD		X[1)]	X	X	X
Comorbidity index (appendix J)	X				
EMBT-score (appendix K)	X				
Peripheral Blood					
- Hematology	X[1)]	X[1)]	X	X	X
- Chemistry[2)]	X[1)]	X[1)]	X	X	X
Bone marrow					
- Morphology[3)]	X		X		X
- Immunophenotyping (locally)					X
- Cytogenetics (local)[5)]		X[4]			X
- Molecular analysis (central) (appendix L)					X[5)]
- Immuno-MRD assessment (appendix L)	X	X	X		X
BM/PB Chimerism			X	X	
Specific investigations					
- Virus serology (according to local practice)	X				
- ECG	X				
- Pulmonary function tests (VC, FeV1)	X				

1) Weekly until hematological recovery
2) Creatinine, AST, ALT, alkaline phosphatase, γGT, bilirubin (direct and indirect), LDH
3) In case of dry tap, bone marrow biopsy for histopathology needs to be done.
4) Only in case of the presence of cytogenetic abnormalities at diagnosis
5) The following markers will have to be done (on material biobank Rotterdam): AML1-ETO, CBFB-MYH11, CEBPA mono-allelic and biallelic, FLT3-ITD mono-allelic and bi-allelic, FLT3-mut, KIT mut, NPM1 mut, ASXL1 mut, RUNX1 mut, p53 mutant, EVI-1 expression. Analyses can be done locally by molecular diagnostic laboratory if approved by MODHEM. Otherwise done by ErasmusMC Rotterdam. See appendix L: section II.4 for more details

10.2.1 Investigations prior to start treatment

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

Medical history

- WHO performance status
- previous chemotherapy or radiotherapy
- antecedent hematological or oncological disease
- previous exposure to toxic agents (e.g. insecticides)
- prior and present other diseases
- fatigue
- bleedings
- infections

Physical examination

- Body weight
- Height
- Splenomegaly
- Signs of extramedullary leukemia (e.g. gingival hypertrophy)

Hematology

- Hemoglobin
- Platelets
- WBC and differential within 3 days prior to randomization

Blood chemistry

- Serum creatinine
- Sodium

- Potassium
- Uric acid
- Calcium
- Glucose
- Bilirubin
- AST
- ALT
- Alkaline phosphatase
- Gamma GT
- LDH within 3 days prior to randomization

Bone Marrow

Bone marrow aspirate for:

- Cytology and cytochemistry to establish WHO and FAB subtype of AML or MDS (see appendix A)
- Immunological phenotyping to verify myeloid leukemia and assessment of leukemia associated phenotype (see appendix L)
- Cytogenetics (cell culture and banding analysis) according to WHO guidelines: at least 20 metaphases should be analyzed. Additional FISH analysis is recommended for the detection of abnormalities involving 11q23 (MLL), 3q26 (EVI1) or p53. In case of insufficient number of metaphases additional FISH should be performed to examine at least -7, -7q, -5 and -5q, Fish should also be performed for t(8;21) and inv(16)/t(16;16) if molecular analysis could not be performed
- molecular analysis for AML1/ETO, CBFB/MYH11, BCR/ABL, FLT3-ITD (inclusive FLT3-ITD/wt ratio), CEBPA mutations (both alleles), NPM1 mutations, ASXL mutations, RUNX1 mutations and p53 gene mutations
- EVI1 overexpression

Bone marrow biopsy for histopathology in case of dry tap

Shipping marrow specimens, peripheral blood, serum separating tubes and saliva for central banking, immunophenotypic and molecular MRD to Amsterdam and Rotterdam (Appendix L)

Specific investigations

- Virus serology: cytomegalovirus (CMV) infection, HIV (human immunodeficiency virus), hepatitis A, B, C. Follow up by PCR

- ECG
- Pulmonary function tests ;VC, FeV1 (as clinically indicated)
- Coagulation studies including prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen
- Pregnancy test according to the Pregnancy Prevention Program
- Serum and saliva (see appendix L)

10.2.2 Investigations during treatment (before start treatment and/or at evaluation previous treatment)

Medical history (daily interim o.i., when hospitalized; thereafter as clinically indicated**)**

- Infections
- Medication given

Physical examination (daily o.i., when hospitalized; thereafter as clinically indicated)

- Weight at start cycle

Hematology

- Hemoglobin
- Platelets
- WBC count and neutrophils according to local standard care

Blood chemistry

- Creatinine
- AST
- ALT
- Bilirubin
- LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly for toxicity assessment

Bone marrow

- Aspirate for response assessment from day 17-21 weekly during treatment, same timing for marrow response evaluation after cycle II if there was no CR after cycle I.
- Aspirate for response assessment prior to cycle II and prior to cycle III or autoHSCT or alloHSCT.
- Aspirate for response assessment day 100 after cycle III or autoHSCT and alloHSCT

- Aspirate for response assessment 6 months after second randomization for maintenance
- Marrow sampling for minimal residual disease assessments after cycle II, immediately before alloHSCT, in autologous transplant, after cycle III or autoHSCT (immediately before R2) or after alloHSCT, 3 months after alloHSCT, 6 months after second randomization or 6 months after alloHSCT, and at relapse

Specific Investigations

- See appendix L

Toxicity assessment

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

- Complete history of symptoms and complaints
- Complete physical examination o.i., with special emphasis on neurological symptoms
- Laboratory examination of hemogram, electrolytes, liver enzymes and kidney parameters weekly
- Chest X-ray as clinically indicated
- Electrocardiography when indicated
- DLT assessment after induction cycle I and II (see section 16.1.1).
- Rapid reporting after cycle I and II for the first 150 patients
- Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (see Appendix F). No grade 1 toxicities will be recorded.

Bone marrow and blood evaluation and response assessment during protocol treatment

After the first treatment cycle the response will be assessed between day 17-21 by bone marrow aspiration, blood evaluation and extramedullary disease status evaluation (see Appendix C). If and as long as the marrow is not conclusive before first CR has been attained a new marrow will be taken as clinically indicated, but at least at weekly intervals. If the marrow shows evidence of resistant disease after cycle I, cycle II may be started as soon as possible without waiting for peripheral blood recovery (PBR). In all other cases blood evaluation will be repeated until PBR.

After cycle II, response assessment will be done after recovery of blood counts, i.e. usually around day 28 if CR had been attained after cycle I. 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.

Patients who have undergone mobilization of peripheral blood stem cells must have a confirmation of remission status 1 week after the apheresis procedure. Of note: only patients with good and intermediate risk profile who are in CR or CRi after the induction chemotherapy are eligible for mobilization.

After consolidation the response will be assessed after recovery of blood counts. This assessment will include a bone marrow aspiration evaluation. If and as long as the marrow is not conclusive a new marrow will be taken during follow up as clinically indicated.
Immunological examination will be done if markers allow discrimination of malignant cells. These markers will be used during serial follow up during remission in order to additionally document the quality of remission.
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 L).

For a full overview of timepoints at which bone marrow and blood should be taken other then for the reponse assessment, please see appendix L.

10.2.3 Required investigations before and after alloHSCT

Medical history

Standard medical history, with special attention for:

- WHO performance status
- EMBT risk score
- Comorbidity index
- Infections

Physical examination

Standard physical examination including body weight and height, with special attention for:

- hemorrhage

Hematology

- Hemoglobin
- Leukocyte count, differential count
- ANC
- platelets

Blood chemistry

- Creatinine
- Liver enzymes
- Total bilirubin
- Albumin
- LDH

Chimerism

Chimerism of bone marrow mononuclear cells, peripheral blood mononuclear cells as in table 13.
T cell chimerism according to local practice.

Grading of GVHD

Acute and chronic Graft versus Host Disease will be scored according to the criteria defined in appendix H.

Bone marrow

- Aspirate for response assessment prior to alloHSCT
- Aspirate for response assessment day 100 after alloHSCT
- Marrow sampling for minimal residual disease assessments immediately before alloHSCT, after alloHSCT, 3 months after alloHSCT, 6 months after alloHSCT and at relapse

Specific Investigations

- Virus serology: (according to local practice)
- ECG
- Pulmonary function tests; VC, FeV1
- Serum (see appendix L)

10.2.4 Investigations during maintenance (patients receiving lenalidomide before start next cycle, for patients in control arm every 5 weeks)

Medical history (daily interim o.i., when hospitalized; thereafter as clinically indicated**)**

- Infections
- Medication given

Physical examination (daily o.i., when hospitalized; thereafter as clinically indicated)

- Weight at start cycle

Hematology

- Hemoglobin
- Platelets
- WBC count and neutrophils according to local standard care

Blood chemistry

- Creatinine
- AST
- ALT
- Bilirubin
- LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly for toxicity assessment

Bone marrow

- Bone marrow aspirations for morphology will be done as clinically indicated, but at least at 6 months after second randomization and in case of suspected relapse. Thereafter on indication.
- Marrow sampling for minimal residual disease as described in table 12 and 13.

Specific Investigations

- Plasma storage 6 months after second randomization (see appendix L).

Toxicity assessment

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

- Complete history of symptoms and complaints
- Complete physical examination o.i., with special emphasis on neurological symptoms
- Laboratory examination of hemogram, electrolytes, liver enzymes and kidney parameters weekly
- Chest X-ray as clinically indicated
- Electrocardiography when indicated

- Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 (see Appendix F). No grade 1 toxicities will be recorded.

10.2.5 Investigations during follow up

Outpatient visits to the clinic are planned twice weekly until hematological recovery. Thereafter outpatient visits are planned according to the following schedule:

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

In this schedule, time is measured from the date of completion of protocol treatment.

At each clinical visit the following examinations will be done:

- Interim history and physical examination o.i.
- Hemoglobin, WBC count and differential, platelet count,
- Creatinine, AST, ALT, alkaline phosphatase, γGT, bilirubin
- Chest X-ray when clinically indicated
- Bone marrow aspirations for morphology will be done as clinically indicated, but at least at 6 months after second randomization and in case of suspected relapse. Thereafter on indication.
- Marrow sampling for minimal residual disease as described in table 12 and 13.

In the first 6 months after the second randomization treatment evaluations have to be done every 5 weeks (after each cycle) for both arms (observation only and lenalidomide maintenance) even when patient stopped protocol treatment.

10.3 Response evaluation

The response will be evaluated after cycle I, II, III and/or HSCT and 6 months after second randomization and in case of suspected relapse. Response will be evaluated according to appendix C.

10.4 Central review

All materials sent in to central labs must be coded by HOVON 132, patient study number, date of birth and sex. Entering full patient names is prohibited.

10.4.1 Cytological and immunophenotype review

Review of bone marrow aspirate at diagnosis by the Hematology Review Committee (HRC) is required.
Send 6 unstained and not fixated bone marrow slides and 4 blood slides, identified and well packed up, together with a copy of the results of immunophenotyping, cytogenetics and molecular diagnostics to:

HOVON Hematology Review Committee (HRC)
Mrs. Trudi de Jong-Gerrits
Dept. of Hematology
Room Nc-828
P.O.Box 2040
3000 CA Rotterdam
The Netherlands

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

For questions please contact: hrc@erasmusmc.nl

Confirmation of diagnosis is not necessary for randomization and start of treatment but sending in of smears for review is required.

10.4.2 Cytogenetic review

Central review will be performed for cytogenetic analysis at diagnosis.
Each cytogeneticist, responsible for the cytogenetic analysis of the patients in a hospital will be notified automatically by email of the registration of a patient from that hospital in the study. A filled out cytogenetic and FISH form together with 2 representative karyotypes and a copy of the original cytogenetic report should be sent within 5 weeks to the HOVON Data Center for central review.

If additional Fluorescence In Situ Hybridization (FISH) analysis was performed, a filled out Cytogenetic and FISH form together with a copy of the original FISH report is also requested to be sent with the cytogenetic data for central review.

10.5 Side studies

- Relapse after autoHSCT will be evaluated according to molecular and immunophenotypic markers
- Cerebron affinity level will be attested and correlated with lenalidomide response
- Studies on leukemic new cell phenotypes in relation to treatment outcome are foreseen
- Newly emerging molecular markers will be retrospectively evaluated in relation to treatment outcome

See also Appendix L for explanations of sampling procedures.

11 Withdrawal of patients or premature termination of the study

11.1 Withdrawal of individual patients from protocol treatment

Patients should be withdrawn from protocol treatment if any of the following criteria for withdrawal are met:

- No CR(i) after cycle II
- Death
- Relapse after initial CR
- Patient not eligible in hindsight
- Patient not eligible for 2nd randomization

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a patient from the protocol treatment for urgent medical reasons. Specific criteria for withdrawal are:

- Excessive toxicity
- No compliance of the patient
- Refusal to continue protocol treatment
- Pregnancy

11.2 Follow up of patients withdrawn from treatment

Patients who are withdrawn from treatment prematurely for other reasons than death will be followed as described in 10.2.4 for follow up.
For patients who are withdrawn from treatment because in hindsight they did not fulfill the eligibility criteria (see 8.1) at time of enrolment, data will be collected until 30 days after the last protocol treatment given. SAE information will be collected as described in 12.3.
No further information will be collected for patients who have withdrawn their consent. If a patient withdraws consent please consult HOVON Data Center.
Patients who are withdrawn from protocol treatment will receive medical care according to local practice.

11.3 Premature termination of the study

The sponsor may decide to terminate the study prematurely based on the following criteria:

- One of the stopping rules has been reached (chapter 14)
- There is evidence of an unacceptable risk for study patients (i.e. safety issue)
- There is reason to conclude that it will not be possible to collect the data necessary to reach the study objectives and it is therefore not ethical to continue enrolment of more patients; for example insufficient enrolment that cannot be improved
- The DSMB recommends to end the trial based on viable arguments other than described above

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The sponsor will provide information regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.

12 Safety

12.1 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.

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, including suspected transmission of infectious agents by a medicinal product).

Suspected unexpected serious adverse reaction (SUSAR)

All **suspected** Adverse Reactions which occur in the trial and that are both **unexpected** and **serious**. Suspected adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorized medicinal product).

12.2 Adverse event

12.2.1 Reporting of adverse events

Adverse events will be reported from the first study-related procedure until 30 days following the last dose of any drug from the protocol treatment schedule or until the start of subsequent systemic therapy for the disease under study, if earlier.

Adverse events occurring after 30 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.

Adverse Events have to be reported on the Adverse Events CRF. Adverse Events will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 4.0 (see appendix F).

Pre-existing conditions will be collected on the baseline concomitant diseases CRF, i.e. active (symptomatic) diseases of CTCAE grade $\geq$ 2, diseases under treatment, chronic diseases and long term effects of past events as present at the time of baseline assessment.

All Adverse Events have to be reported, with the exception of:

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline concomitant diseases CRF
- AE's of CTCAE grade ≤1
- Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents
- Progression of the disease under study; complications as a result of disease progression remain reportable Adverse Events
- Alopecia
- Nausea/vomiting
- Hematological toxicities

12.2.2 Follow up of adverse events

All adverse events will be followed clinically until they have been resolved, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
Follow up information for grade ≥2 adverse events considered at least possibly related to the investigational medicinal product by the investigator should be reported on the AE CRF until recovery or until 6 months after the last dose of IMP, whichever comes first.
Follow up information for all other adverse events should be reported on the AE CRF until recovery or until 30 days after the last dose of any drug from the protocol treatment schedule, whichever comes first.

12.3 Serious Adverse Events

12.3.1 Reporting of serious adverse events

Serious Adverse Events (SAEs) will be reported from the first study-related procedure until 30 days following the last dose of any drug from the protocol treatment schedule or until the start of subsequent systemic therapy for the disease under study, if earlier.
Serious Adverse events occurring after 30 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.

SAEs must be reported to the HOVON Data Center by fax **within 24 hours** after the event was known to the investigator, using the SAE report form provided. This initial report should contain a minimum amount of information regarding the event, associated treatment and patient identification,

as described in the detail in the instructions for the SAE report form. Complete detailed information should be provided in a follow-up report within a further 2 business days, if necessary.

The following events should always be considered a Serious Adverse Event:

- Deep Vein Thrombosis (DVT)
- Pulmonary Embolism (PE)
- Second Primary Malignancy (SPM, see section 16.1.3)

The following events are not considered to be a Serious Adverse Event:

- Relapse/Progression of the disease under study; **death or complications as a result of disease progression remain reportable serious adverse events**
- Hospitalization for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- Hospitalization for diagnostic investigations (e.g., scans, endoscopy, sampling for laboratory tests, bone marrow sampling) that are not related to an adverse event. 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.
- Hospitalization for a procedure that was planned prior to study participation (i.e. prior to registration or randomization). This should be recorded in the source documents. Prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.

12.3.2 Causality assessment of Serious Adverse Events

The investigator will decide whether the serious adverse event is related to trial medication, i.e. any of the products from the protocol treatment schedule. The decision will be recorded on the serious adverse event report. The assessment of causality is made by the investigator using the following:

Table 14. Causality Adverse Events

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

12.3.3 Follow up of Serious Adverse Events

All serious adverse events will be followed clinically until they are resolved or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
Follow up information on SAE's should be reported monthly until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.

12.3.4 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the Principal Investigator and the manufacturer of the investigational medicinal product(s).
The HDC Safety Desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR).
The IB will be used as a reference document for expectedness assessment.
The HOVON Data Center will ensure that a six-monthly line listing of all reported SAE's is provided to the Ethics Committee(s) if this is required by national laws or regulations or by the procedures of the Ethics Committee.

12.4 Reporting Suspected Unexpected Serious Adverse Reactions

The HDC Safety Desk, on behalf of the sponsor, will ensure the reporting of any SUSAR to the Ethics Committees (EC), the Competent Authorities (CA), Celgene and the investigators in compliance with applicable laws and regulations, and in accordance with any trial specific agreements between the sponsor and a co-sponsor or Celgene.

Expedited reporting of SUSARs will occur no later than 15 days after the HOVON Data Center had first knowledge of the serious adverse event. For fatal or life-threatening cases this will be no later than 7 days for a preliminary report, with another 8 days for a complete report.
The manner of SUSAR reporting will be in compliance with the procedures of the Ethics Committees and Health Authorities involved.

12.5 Pregnancies

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

If a female patient gets pregnant while the patient is still treated with lenalidomide the investigator shall immediately:

(a) Discontinue lenalidomide treatment;

(b) Instruct the patient to return any unused portion of lenalidomide; and

(c) refer the patient to an obstetrician/gynaecologist experienced in reproductive toxicity for further evaluation and counselling.

The investigator will follow the female subject until completion of the pregnancy, and must notify the sponsor of the outcome of the pregnancy within 5 days or as specified below. The investigator will provide this information as a follow-up to the initial pregnancy report. If the outcome of the pregnancy meets the criteria for classification as a SAE (i.e., spontaneous or therapeutic abortion, stillbirth, neonatal death, or congenital anomaly - including that in an aborted fetus), the investigator should follow the procedures for reporting SAEs. In the case of a live “normal” birth, the sponsor should be

informed as soon as the information is available. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the investigator suspects is related to the in utero exposure to the investigational medicinal product(s) should also be reported.
The investigator is encouraged to provide outcome information of the pregnancy of the female partner of a male subject, if this information is available to the investigator and the female partner gives her permission.

In order to prevent pregnancies during the use of Lenalidomide, patient information, patient registration and patient counseling will occur as defined in the Pregnancy Prevention Program.

12.6 Reporting of safety issues

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of findings that could affect adversely the safety of patients, impact the conduct of the trial, increase the risk of participation or otherwise alter the EC's approval to continue the trial. In the occurrence of such an event the sponsor and the investigators will take appropriate urgent safety measures to protect the patients against any immediate hazard. The accredited Ethics Committee will suspend the study pending further review, except insofar as suspension would jeopardize the patient’s health. The local investigator will inform the patients and local ethics or review committees according to hospital policy. The sponsor will inform any other parties that are involved in the trial.

12.7 Annual safety report

The sponsor will submit once a year a safety report to the Ethics Committees and Competent Authorities of the concerned Member States. The first report is sent one year after the first approval date of the trial. The last report is sent one year after the last patient has completed protocol treatment. The content of the annual safety report will be according to the EU guidance document ‘Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use’.

12.8 Data Safety and Monitoring Board

The DSMB will advise the Principal Investigator, co-investigators and the chair of the working group in writing about the continuation of the trial. The DSMB will review the general progress and feasibility of the trial, the quality and completeness of the data, adverse events and safety, and differences in

results between the arms of a randomized trial. The DSMB will consider if there is any concern regarding the safety and well-being of trial subjects or regarding the scientific validity of the trial results. The DSMB will give recommendations about dose escalations, dose reductions, continuation at a dose level or stopping because of inefficacy on the basis of interim reports at specific time points (see section 14 for more details on the interim analyses). The DSMB will base her advice on the reports provided by the trial statistician. The DSMB is free to take into consideration external information, such as the (interim) results of other trials or literature reports.
The DSMB consists of at least three members, with at least one independent statistician and two international clinical hematologists with a broad background in AML therapeutics.
Details of the DSMB constitution and tasks are documented in the trial specific DSMB charter.
The DSMB will receive at least the following reports from the trial statistician for review:

- Interim analysis reports (as described in chapter 14)
- Annual safety data listing the incidence of (serious) adverse events, (serious) adverse reactions and SUSARs
- Annual progress data listing the number of enrolled patients and the status of data collection

13 Endpoints

13.1 Primary endpoint

Primary endpoint Part A-run-in: Lenalidomide dose level selection

- DLT and duration of myelosuppression of induction treatment with or without lenalidomide for each of the distinct predefined dose levels

Primary endpoint Part A: Induction - Efficacy

- EFS after induction treatment with or without lenalidomide (i.e., time from registration to induction failure, death from any cause or relapse whichever occurs first)

Primary endpoint Part B: Maintenance - Efficacy

- Cumulative incidence of relapse (CIR) after second randomization (maintenance treatment with lenalidomide or observation only)

13.2 Secondary endpoints

Secondary endpoints Part A Run-in : Lenalidomide dose level selection

- Response (CR and CRi) after induction therapy cycles I and II

Secondary endpoints Part A: Induction- Efficacy

- EFS in the distinct prognostic subsets (AML good-risk vs. AML intermediate-risk vs. AML poor-risk vs. AML-very poor-risk) and cytogenetically and molecularly defined subgroups of AML
- Response (CR and CRi) after induction therapy cycles I and II
- Disease-free survival (DFS, measured from time of CR/CRi to day of relapse or death from any cause, whichever occurs first)
- OS measured from the time of registration
- Outcome of induction treatments in relation to MRD measurements
- Evaluation of molecular prognostic markers and gene expression profiles for and overexpression of defined genes (e.g. EVI1, cereblon) for outcome in relation to induction and post induction treatments
- Toxicities
- Evaluation of MRD after induction and post-induction treatments
- 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
- Number of platelet transfusions and last day of platelet transfusion after each cycle
- Impact of the use of lenalidomide on the effectiveness of stem cell mobilization

Secondary endpoints Part B: Maintenance - efficacy

- OS and DFS measured from 2nd randomization, and also in the distinct prognostic subsets (AML good-risk vs. AML intermediate-risk vs. AML poor-risk vs. AML very poor-risk) and cytogenetically and molecularly defined subgroups of AML
- Toxicities
- Number of platelet transfusions and last day of platelet transfusion after each cycle
- Number of RBC transfusions in relation to maintenance or no maintenance treatment
- Evaluation of MRD after 2nd randomization
- Time to hematopoietic recovery (ANC 0.5 and 1.0x10^9/L; platelets 50 and 100x10^9/L) after each treatment cycle

14 Statistical considerations

All main analyses will be according to the intention to treat principle i.e. patients will be analyzed according to the treatment arms they were assigned to. However, patients initially registered and randomized but considered ineligible afterwards based on information that should have been

available before registration and/or randomization, whichever applicable, will be excluded from the analyses.

In part A of the study the effectiveness of addition of lenalidomide to induction therapy will be investigated. This part starts with a run-in phase in which a feasible dose level of lenalidomide will be selected.

In Part B of the study the addition of lenalidomide following consolidation with autoHSCT or a third cycle of chemotherapy will be investigated.

14.1 Patient numbers and power considerations

The primary endpoint is Event Free Survival (EFS). Based on the results of the HOVON/SAKK 42/42A trial we expect an EFS at 1, 2, 3 and 5 years in the standard arm of 50%, 40%, 38% and 33%, respectively. Based on the results of the HOVON 102/SAKK 30/09 trial we expect an accrual rate of 260-280 patients per year.

The target number of patients is 800, to be accrued in approximately 3 years. After entry of the last patient an additional follow up of 1 year is planned before the final analysis. The target number of 800 patients (corresponding to 411 events) will give power of 82% with a two-sided test at a 5% significance level to detect an improvement in EFS with hazard ratio (HR) = 0.76. This HR corresponds to an increase of 10% in EFS at 3 years (from 38% to 48%).

The final analysis will be performed using Cox regression analysis, see section 14.2.3 for more details.

14.2 Part A: Efficacy analysis of Lenalidomide in induction

In this section the statistical design of the induction randomization is presented. This section starts with the design of the preceding dose finding (Part A run-in) in section 14.2.1.

14.2.1 Part A run-in: Dose level selection

At each dose level the decision to stop or escalate will be made on the basis of (a) the incidence of DLTs in the arm treated with lenalidomide versus the incidence of DLTs in the control treatment arm and (b) the duration of myelosuppression in the lenalidomide arm compared to the control arm (after cycle II and II). See chapter 7 for the definition of DLT. Part A of the study (i.e. the phase III study) will be performed at the selected highest feasible dose level. Remark that patients registered at the

selected highest feasible dose level during the Part A-Run in, will be included in all analyses of part A and B as well.

Applying the criteria of a DLT to the patients treated in the HOVON/SAKK-42 and 42A studies with standard dose chemotherapy, who are comparable to the patients in the control group of this trial we find:

- 19% of the patients experience DLT in cycle I
- 20% of the patients experience DLT in cycle II

Overall, 34% of the patients experience DLT in cycle I and/or II

- The median time to recovery of ANC >0.5x10^9/L is 27 days following cycle I

In the decision rules the number of DLTs in both arms and the number of days at risk for DLT are taken into account. A patient is at risk during cycle I and II until day 42 after start of cycle II, date of off protocol or date of start next treatment (whichever comes first). If no early decision to stop or escalate can be made, the active dose level is chosen as the dose level to continue Part A of the trial (phase III study) at the moment when the maximum number of 15 patients in the lenalidomide arm have completed induction therapy cycle I and II.

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

The decision rules are presented in the Table 15 below.

Table 15. Decision rules

Number of patients who have completed induction therapy in the Lenalidomide arm	Excess DLTs on Lenalidomide arm		DLT Incidence Rate Ratio		ANC Recovery Hazard Ratio	Dose Escalation / Reduction
n < 10	>3	*and*	>3		(Any value)	Stop/reduce[1]
	≤3	*or*	≤3			Continue[4]
10 ≤ n < 15			>2	*or*	>2.8	Stop/reduce[1]
	(Any value)		1.3 to 2	*and*	≤2.8	Continue[4]
			≤2	*and*	2 to 2.8	
			<1.3	*and*	<2	Escalate[2]
n ≥ 15	>5		>1.6	*or*	>2.2	Stop/reduce[1]
			1.4 to 1.6	*and*	≤2.2	Continue[3]
	(Any value)		≤1.6	*and*	2 to 2.2	
			<1.4	*and*	<2	Escalate[2]

[1] At the lowest dose level, a decision to stop/reduce means that the study will be temporarily closed until an interim analysis has been performed and the DSMB has been consulted. At higher dose levels, the study will remain open at the highest feasible dose level (i.e. one dose level lower than the current one), until an interim analysis has been performed and the DSMB has been consulted.

[2] At the highest dose level no escalation is possible. So, a decision to escalate means continuation at that dose level for evaluation of efficacy in Phase III-setting (i.e. Part A of the study).

[3] A decision to continue after more than 15 patients means that no decision to stop or escalate can be made after 15 patients in the lenalidomide arm completed induction therapy (cycle I and II) at the current dose level. Then, we continue with the phase III part of the trial at the present dose level.

[4] Continue in case less than 15 patients in the lenalidomide arm are included means continuation of the active dose level. So, in that case there is no interim analysis to be performed, whereas the other decisions (to stop/reduce, escalate of continue after 15 or more patients) imply an interim analysis.

In Table 15, a patient is considered to have completed induction therapy if the patient (a) is known not to have experienced DLT 42 days after start of cycle II, (b) known not to have experienced DLT after cycle I and known not to receive cycle II, (c) has gone off protocol treatment before 42 days after start cycle II, (d) has experienced DLT in induction cycle I or II.

Excess of DLTs on the lenalidomide arm is defined as the number of DLTs in the lenalidomide arm minus the number of DLTs on the control arm.

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

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

- as observations all given cycles I and II;
- the event indicator variable = 1 when a patient recovers, i.e. ANC $>0.5x10^9$/L, after a cycle before start of the next treatment with the time to event the number of days between the start of the cycle and the date of recovery, i.e. the first date of ANC attaining a value $>0.5x10^9$/L;
- a patient is censored with event =0 if the patient dies, or starts a new treatment without previous recovery, or at date of last known ANC in case of missing ANC values after that date;
- the hazard ratio is calculated as the hazard ratio of recovery in the lenalidomide arm with respect to the standard arm. A hazard ratio >1 implies a faster recovery in the standard arm.

These decision rules lead to the following characteristics where we assume that the true probability of DLT in the control arm is 34% and the expected median recovery time for ANC > $0.5x10^9$/L is 27 days; an increase of 30% in the lenalidomide arm corresponds to a median recovery time of 35 days. An expected accrual of 150 patients a year (12.5/month) is used in the calculations. The characteristics are based on 1000 simulations for each combination of true DLT and recovery.

Table 16. Characteristics of the decision rules

Absolute increase of DLT in lenalidomide arm	Increase of duration of recovery time	Decision	Percentage	Mean (range) of number of patients entered in both arms
0%	0%	Stop/reduce	22%	34 (18-57)
		Continue	7%	42 (34-53)
		Escalate	71%	36 (24-54)
	30%	Stop/reduce	41%	35 (11-55)
		Continue	5%	43 (36-52)
		Escalate	55%	36 (20-60)
15%	0%	Stop/reduce	59%	31 (11-56)
		Continue	4%	43 (37-56)
		Escalate	37%	36 (24-52)
	30%	Stop/reduce	73%	31 (9-55)
		Continue	4%	43 (32-51)
		Escalate	23%	36 (24-56)

Thus, for example the probability of escalating in a situation in which the true incidence of DLT and true recovery time of ANC in both arms are equal and as expected, is 71%. And, the decision to stop in the situation where the incidence of DLT is increased with 15% and the duration of recovery time for ANC > $0.5x10^9$/L is increased with 30% (i.e. hazard ratio = 1.3) in the lenalidomide arm compared to the control arm is 73%.

If a decision to stop is implied by the decision rules we return to a lower dose level until a decision is made by the DSMB. At the lowest dose level, if the decision rules imply a decision to reduce/stop the trial is temporarily put on hold. Until the DSMB has confirmed a decision to escalate, the trial remains open at the active dose level.

The decision rules serve as guidelines for the DSMB. As soon as a decision can be made according to the above defined rules, an interim analysis report will be made and sent to the DSMB for final recommendations. The report contains a tabulation of the number of patients recruited, the number of evaluable patients, the number of DLTs, a specification of the DLTs and their outcome, the ANC>$0.5x10^9$/L recovery results, and the incidence and intensity of the other reported SAEs, split by arm, treatment cycle and dose level. Also information on consolidation treatment will be reported (as available at the time of interim analysis).

14.2.2 Part A: Safety interim analysis

During part A of the study, one safety interim analysis is planned after inclusion of 150 patients at the final dose level. This interim analysis focuses on the safety of the treatment of induction cycle I as well as induction cycle II. However, also results available for part B of the study, i.e. the second randomization, will be included in the report as well. The results of the interim analysis will be presented to the DSMB for advice.

The primary endpoint for the interim analysis is the number of DLTs during induction cycle I and II. The following decision rule serves as a guideline:
A higher DLT incidence rate on induction treatment in the lenalidomide arm with a P-value <0.10 is a good reason to recommend the stopping of the trial or recommendations for modifications. These decision rule serve as guideline for the DSMB, however the DSMB is free in her recommendations to the principal investigator and study coordinators.

The interim analysis report includes information on the number of patients, treatment given split by induction treatment arm and dose level and the number of DLT and specification of the DLT and their outcome split by treatment arm, cycle and dose level. Also information on the duration of hematological recovery split by treatment arm, cycle and dose level will be presented. The interim analysis report also includes the number of patient randomized for maintenance treatment, the number of maintenance treatment cycles given and (serious) adverse events during maintenance treatment.

14.2.3 Efficacy analysis

Induction treatment - primary analysis

The primary endpoint for the comparison between induction chemotherapy with or without lenalidomide is EFS (defined as time from registration to induction failure [failure to reach CR(i) on induction], relapse or death whichever occurs first). Cox regression analysis with adjustment for disease (AML vs MDS), which was a stratification factor of the induction randomization, with induction treatment arm as covariate is the primary analysis. This is in fact a multivariate analysis with induction treatment arm and disease (AML vs MDS) as covariates.

Induction treatment - other analysis

For EFS, besides the above described primary analysis, actuarial estimates of the competing risks (e.g. induction failure, relapse, or death in first CR) will be made for each treatment arm.

Secondary endpoints include CR rate (defined as the rate of CR(i)), OS and DFS. Formal tests for survival endpoints will be done by Cox regression analysis, CR rate will analysed with logistic regression analysis.

Evaluation of AlloHSCT

The outcome of patients with AlloHSCT will be determined by calculation of the probabilities of relapse and death in first CR after alloHSCT as competing risk and the survival probability. Estimates will be made separately by type of transplant, by age group, by diagnosis (AML versus MDS), by risk group and molecularly defined subtypes depending on available numbers of subgroups.
These data may also be submitted to the data of the AML Collaborative Group for meta-analyses.

14.2.4 Toxicity analyses

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events CTCAE grade 2 or more (see appendix F) by treatment arm and cycle. In the by-subject analysis, a subject having the same event more than once will be counted once. Adverse events will be summarized by maximum CTCAE grade.

Time to hematological recovery after each treatment cycle will be analyzed by actuarial methods.

14.2.5 Additional analysis

Additional analysis include the analysis of prognostic factors, especially age, cytogenetic abnormalities, molecular abnormalities, Cereblon over expression and predefined risk groups (good, intermediate, poor, very poor) with respect to EFS, CR rate, OS and DFS. Cox and logistic regression methods will be used.

14.2.6 Statistical Analysis Plan

A detailed Statistical Analysis Plan (SAP) will be made for the final analysis as well as for all interim analyses. It will be discussed with the study coordinators. However, this can only affect the exploraty analyses, but not the primary analysis on which the sample size is based.

14.3 Part B: Efficacy of Lenalidomide as maintenance following consolidation with chemotherapy cycle III or autoHSCT

Patients with a CR after induction therapy, and planned to receive an autoHSCT or a third cycle of chemotherapy are eligible for part B of the study. These patients will be randomized for consolidation treatment with or without lenalidomide. All randomized patients randomized for or against the highest

feasible dose level of the induction therapy for the first randomisation will be included in the analysis of the efficacy of lenalidomide after consolidation with an autoHSCT or a Cycle III on the intention to treat principle. Endpoints for Part B of the study are DFS, and cumulative incidence or relapse and death in CR (the competing risks of DFS).

Based on the results of the HOVON/SAKK 42(A) study the following assumptions are used:

Accrual rate (based on Ho102)	250 patients per year registered for ind. th.
CR rate on cycle I/II	72%
Patients without treatment on protocol after CR	20%
Number of patients consolidation	144 patients per year
DFS at 1 and 3 years after 2nd rand.	60%, 45% respectively

In contrary to the HOVON/SAKK-42(A) protocols, in this study intermediate risk patients which are MRD negative will receive an autoHSCT (instead of an alloHSCT). In the HOVON/SAKK 42(A) about 40% of the receiving consolidation treatment after CR received an autoHSCT or third cycle of chemotherapy. About 25% of the patients in CR are intermediate risk from which about 70% are MRD negative (See Appendix D). This results in the following expectation on consolidation therapy:

AlloHSCT as consolidation	45%
AutoHSCT or Cycle III as consolidation	55%

Based on the assumptions as given above, about 253 patients (of the 800 randomized for induction treatment) per year are eligible for treatment with or without lenalidomide after autoHSCT or cycle III. The resulted number of 253 patients will give a power of (only) 48% with a two-sided test at a 5% significance level to detect an improvement in DFS with hazard ratio (HR) = 0.70. This HR corresponds to an increase of 12% in DFS at three years (from 45% to 57%).

Of course, a larger benefit would increase the power (14.5% difference in DFS at 3 years would imply a HR = .65 and a power of 62%). It is not likely that we will have sufficient power to detect superiority of lenalidomide after consolidation with cycle III/autoHSCT. However, this analysis will give an estimate (HR and 95% confidence interval) of the possible benefit of lenalidomide after Cycle III/autoHSCT.

15 Registration and Randomization

15.1 Regulatory Documentation

Required regulatory and administrative documents must be provided to the HOVON Data Center before shipment of study drug and before enrolment of the first patient. This will always include an Ethics Committee approval for the investigational site. The HOVON Data Center will provide each investigator with an overview of the required documents. Each investigational site will be notified when all requirements are met and enrolment can start.

15.2 Registration and Randomization

Eligible patients should be registered before start of treatment. Patients need to be registered at the HOVON Data Center by one of the following options:

- Trial Online Process (TOP, https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.
- By faxing the completed registration/randomization CRF +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET
- By phone +31.10.7041560 Monday through Friday, from 09:00 to 17:00 CET

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Sex
- Date of birth
- Date written informed consent
- Specific items patient gives consent for (see ICF)
- Eligibility criteria
- Stratification factors

All eligibility criteria will be checked with a checklist.

At registration, patients will be randomized (1:1) for induction treatment with or without lenalidomide. Patients will be randomized stratified by center and disease (AML vs. MDS) with a minimization procedure, ensuring balance within each stratum and overall balance.

Each patient will be given a unique patient study number (a sequence number by order of enrolment in the trial). Patient study number and result of randomization will be given immediately by TOP or phone and confirmed by fax or email.

Local Patient Code is a code assigned to the patient by the investigational site for local administrative purposes. The code may be up to 8 characters long (letters and numbers allowed). The code should be in compliance with privacy regulations. It should not contain identifying data, such as patient initials or the complete hospital record number. The local code will be visible in the confirmation messages sent by TOP to local participants after registration of the patient. The key to this local patient code should only be accessible by the local investigator and the local trial staff. Using or entering a local patient code is not obligatory.

15.3 Second Randomization for part B of the study

A second randomization will take place after cycle III or autoHSCT for patients eligible for maintenance treatment with or without lenalidomide (see par 8.2). Eligible patients should be registered before start of maintenance treatment. Patients need to be registered at the HOVON Data Center by one of the following options:

- Trial Online Process (TOP, https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.
- By faxing the completed 2nd randomization CRF +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET
- By phone +31.10.7041560 Monday through Friday, from 09:00 to 17:00 CET

The following information will be requested at registration for 2nd randomization:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Patient study number
- Date start last treatment
- Eligibility criteria
- Stratification factors

Patients will be randomized (1:1) and stratified by center, disease, treatment arm of the induction randomization and type of consolidation treatment (cycle III vs. autoHSCT) with a minimization procedure ensuring balance within each stratum and overall balance.

16 Data collection and quality assurance

16.1 Case Report Forms

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

- Inclusion and exclusion criteria;
- Baseline status of patient including medical history and stage of disease;
- Timing and dosage of protocol treatment;
- Baseline concomitant diseases and adverse events;
- Parameters for response evaluation;
- Any other parameters necessary to evaluate the study endpoints;
- Survival status of patient;
- Reason for end of protocol treatment.

Each CRF page will be identified by a pre-printed trial number, and a combination of patient study number (assigned at registration) and hospital name to be filled out before completing the form.
The CRF will be completed on site by the local investigator or an authorized staff member. All CRF entries must be based on source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.
The CRF pages must be made available to the HOVON Data Center at the requested time points as specified in the CRF instructions.
All data will be collected in the study database by the HOVON Data Center.

16.1.1 DLT data collection

To monitor the incidence of dose limiting toxicity (DLT) and myelosuppression duration a separate CRF (DLT-form) will be used. This DLT-form must be filled out for every patient, independent of randomization result for the induction therapy. The form should be dated, signed by the responsible investigator and returned to the HOVON Data Center by fax within 24 hours after DLT-occurrence, or weekly after start of cycle I if no DLT occurred. DLTs should be reported until day 42 after start of cycle II, or until start next treatment, or until off protocol (whichever comes first). Duration of myelosuppression must be reported on the DLT form until ANC recovery or until start next treatment (if not yet recovered). Investigators will receive a weekly reminder for sending in a new DLT form.

16.1.2 Rapid Reporting

In this trial the occurrence of DLT and myelosuppression during induction treatment are considered of special interest. During cycle I of the Run-in part A, these events will be reported on the DLT form as described above.
After inclusion of 150 patients, a safety interim analyses will be performed, to closely monitor safety during induction cycle I and II (see section 14.2.2). To monitor safety for the first 150 patients a separate CRF (Rapid Reporting Form) will be used during cycles I and II for those patients who did not already report this information on the DLT form. The form should be filled out, dated and signed by the responsible investigator and returned to the HOVON Data Center by fax after a DLT or at day 42 of cycle II if an event did not occur. If there was no ANC recovery at that time, a second form for the applicable cycle needs to be filled out on date of recovery or start of new treatment (whatever comes first). Investigators will receive a reminder to fill out a rapid reporting form after each cycle.

16.1.3 Reporting of Second Primary Malignancies

Second Primary Malignancies (SPM) should be reported as SAE during treatment and during the Follow Up period. The SAE form together with the Second Primary Malignancy CRF must be reported to the HOVON Data Center by fax **within 24 hours of the initial observation of the SPM.**
For each case of SPM occurring during treatment, contact the Principal Investigator to discuss if treatment needs to be discontinued.

16.2 Data quality assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers, review of protocol procedures with the investigator before the study, and site visits by the sponsor.
Data collected on the CRF will be verified for accuracy. If necessary, queries will be sent to the investigational site to clarify the data on the CRF. The investigator should answer data queries within the specified time line.

16.3 Monitoring

This trial is part of the HOVON Site Evaluation Visit program. Site evaluation visits will be performed for HOVON trials to review the quality of the site and not specifically the quality of a certain trial. It will enable HOVON to collect quality data and facilitate improvement of the participating sites. Data cleaning or monitoring of the performance of specific trials is not the goal of the site evaluation visits. Site evaluation visits will be performed according to the site evaluation visit plan.

The HOVON site evaluation visit plan applies to sites in the Netherlands and Belgium only. Monitoring of the quality of trial conduct in participating sites from other countries will be organized by the coordinating investigator or co-sponsor. The frequency and content of the site visits in other countries will be at least equal to the specifications of the site evaluation visit plan, and are described in a monitoring plan provided by HOVON.
Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that during site visits the relevant investigational staff will be available, the source documentation will be available and a suitable environment will be provided for review of study-related documents.

16.4 Audits and inspections

The investigator will permit site-visits to carry out an audit of the study in compliance with regulatory guidelines. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Patient privacy must, however, be respected.
Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

17 Ethics

17.1 Accredited ethics committee

An accredited Ethics Committee will approve the study protocol and any substantial amendment.

17.2 Ethical conduct of the study

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

17.3 Patient information and consent

Written informed consent of patients is required before enrollment in the trial and before any study related procedure takes place.
The investigator will follow ICH-GCP and other applicable regulations in informing the patient and obtaining consent. The investigator should take into consideration if the patient is capable of giving informed consent. Before informed consent may be obtained, the investigator should provide the

patient ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the patient.
There is no set time limit for the patient to make a decision. The investigator should inform each patient if there is a specific reason why he/she must decide within a limited time frame, for example if patients condition necessitates start of treatment or if the trial is scheduled to close for enrollment.

The content of the patient information letter, informed consent form and any other written information to be provided to patients will be in compliance with ICH-GCP and other applicable regulations and should be approved by the Ethics Committee in advance of use.
The patient information letter, informed consent form and any other written information to be provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent. Any revised informed consent form and written information should be approved by the Ethics Committee in advance of use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient's willingness to continue participation in the trial. The communication of this information should be documented.

17.4 Trial insurance

Prior to the start of the trial, the sponsor will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted. The sponsor will take out an insurance policy or delegate this responsibility to a national co-sponsor. Proof of insurance will be submitted to the Ethics Committee.
In addition, the sponsor will ensure that adequate insurance is in place for both investigator(s) and sponsor to cover liability pertaining to death or injury resulting from the trial.

18 Administrative aspects and publication

18.1 Handling and storage of data and documents

18.1.1 Patient confidentiality

Each patient is assigned a unique patient study number at enrollment. In trial documents the patient's identity is coded by patient study number as assigned at enrolment. In some cases date of birth is also listed.
The local investigator will keep a subject enrollment and identification log that contains the key to the code, i.e. a record of the personal identification data linked to each patient study number. This record

is filed at the investigational site and should only be accessed by the investigator and the supporting site staff, and by representatives of the sponsor or a regulatory agency for the purpose of monitoring visits or audits and inspections.

18.1.2 Filing of essential documents

Essential Documents are those documents that permit evaluation of the conduct of a trial and the quality of the data produced. The essential documents may be subject to, and should be available for, audit by the sponsor's auditor and inspection by the regulatory authority(ies)
The investigator should file all essential documents relevant to the conduct of the trial on site. The sponsor will file all essential documents relevant to the overall conduct of the trial. Essential documents should be filed in such a manner that they are protected from accidental loss and can be easily retrieved for review.

18.1.3 Record retention

Essential documents should be retained for a minimum of 15 years, according to the local procedures, after the end of the trial. They should be destroyed after this time.
Source documents (i.e. medical records) of patients should be retained for at least 15 years after the end of the trial. Record retention and destruction after this time is subject to the site's guidelines regarding medical records.

18.1.4 Storage of samples

Biological samples should only be stored for the purpose of additional research if the patient has given consent. If no informed consent was obtained, samples should be destroyed after the patient has completed all protocol treatment and procedures.
Storage of biological samples on site is subject to the site's guidelines; samples may be labeled with the patients identifying information (e.g. name, hospital record number).
Samples that are shipped to another facility (e.g. a central laboratory) for a purpose as described in this protocol or for additional scientific research, should be stripped from any identifying information and labeled with a code (trial name or number and patient study number as assigned at enrollment).

18.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the Ethics Committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the patients of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be submitted to the Ethics Committee and to the Competent Authority.

Non-substantial amendments will not be submitted, but will be recorded and filed by the sponsor.

18.3 Annual progress report

The sponsor will submit a summary of the progress of the trial to the accredited Ethics Committee once a year. The first report is sent one year after the first approval date of the trial. The last report is sent one year after the last patient has completed protocol treatment. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

18.4 End of study report

The sponsor will notify the accredited Ethics Committee and the Competent Authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the accredited Ethics Committee and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the primary endpoint analysis of the trial, the sponsor will submit an end of study report with the results of the study, including any publications/abstracts of the study, to the accredited Ethics Committee and the Competent Authority. Upon request of the accredited Ethics Committee or the Competent Authority the sponsor will submit an updated version of the end of study report within one year after the last patient's last visit.

18.5 Publication policy

Final publication of trial results

Trial results will always be submitted for publication in a peer reviewed scientific journal regardless of the outcome of the trial – unless the trial was terminated prematurely and did not yield sufficient data for a publication.

The final publication of the trial results will be written by the Principal Investigator, the Co-investigators and the trial statistician on the basis of the statistical analysis performed by the trial statistician. A draft manuscript will be submitted for review to:

- All co-authors
- The chair of the relevant HOVON working group, who is entitled to share and discuss the manuscript with working group members
- An industry partner if so agreed in the contract between HOVON and company

After revision the final manuscript is submitted to the HOVON secretary for review of compliance with this policy. After approval by the HOVON board the manuscript will be sent to a peer reviewed scientific journal.

Authorship

Authors of the main manuscript will include the Principal Investigator, the Co-investigators, investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion rate), the trial statistician and the trial manager. If a substantial part of the publication is based on centrally reviewed data (e.g. cytogenetics or pathology), the central reviewer will be included as author. Others who have made a significant contribution to the trial may also be included as author, or otherwise will be included in the acknowledgement.

Authors of correlative manuscripts (e.g. results of side studies) will include the Principal Investigator, the Co-investigators, and those persons who have made a significant contribution to the published results.

The Principal Investigator should discuss and decide on the matter of authorship of the main manuscript prior to the start of the trial – with the exception of authors included on account of inclusion rate. The Principal Investigator is urged to use the maximum number of authors allowed by the journal to the full extent.

Interim and partial publications

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

Investigators participating in the trial have a right to publish results from data they collected for the study. The Principal Investigator, the Co-investigator(s) and the trial statistician must approve any such publication, abstract or presentation based on patients included in this study. This is applicable to any individual patient or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study endpoints unless the final results of the trial have already been published.

Abstracts and presentations

Abstracts and presentations at public meetings will represent the trial as a project under HOVON affiliation. The abstract or presentation should not be represented under affiliation of the working group or a specific hospital.

Slides will be designed using the HOVON style template and any other presentation materials will show the HOVON logo.

If the trial is conducted in partnership with a co-sponsor (e.g. intergroup trial), the abstract and presentation should represent the co-sponsor contribution and slides may show the co-sponsor logo in addition to the HOVON logo.

Prior to it’s public use, the abstract or presentation is submitted to the HOVON secretary for review of compliance with this policy.

Glossary of abbreviations

(in alphabetical order)

AE	Adverse Event
alloHSCT	Allogenetic hematopoietic stem cell transplantation
ALT	Alanine transaminase
AML	Acute myeloid leukemia
ANC	Absolute Neutrophil Count
AraC	Cytarabine
AST	Aspartate transaminase
ASXL1	Additional sex combs-like 1
autoHSCT	Autologous hematopoietic stem cell transplantation
BAALC	Brain and Acute Leukemia, Cytoplasmic
BM	Bone Marrow
BPDC	Blastic plasmacytoid dendritic cell neoplasm
CBF	Core binding factor
CEBPA	CCAAT/enhancer-binding protein alpha
CIR	Cumulative incidence of relapse
CN	Normal cytogenetics
CR	Complete Remission
CR1	First complete remission
CRe	Early CR
CRF	Case Report Form
CRi	Complete Remission with incomplete platelets recovery (< 100x 10^9/L) and/or incomplete ANC recovery (< 1.0 x 10^9/L)
CTCAE	Common Terminology Criteria for Adverse Events
CTD	Connective tissue disease
CVA	Cerebrovascular accident
CVD	Cerebrovascular disease
CyA	Cyclosporine
DFS	Disease Free Survival
DLco	Diffusion capacity of carbon monoxide
DLT	Dose Limiting Toxicity
DNMT3A	DNA (cytosine-5)-methyltransferase 3A
DS	Down syndrome
DFS	Disease Fee Survival
DSMB	Data Safety and Monitoring Board

DVT	Deep Venous Thrombosis
EBMT	European Group for Blood and Marrow Transplantation
ECG	Electrocardiogram
EF	Ejection fraction
EFS	Event Free Survival
ERG	ETS related gene
EVI1	Ectopic virus expression site 1
FAB	French-American-British
FEV1	forced expiratory volume in one second
FISH	Fluorescence In Situ Hybridisation
FLT3	Fms-like tyrosine kinase receptor
FLT3-ITD	Fms-like tyrosine kinase receptor internal tandem duplications
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor
GFR	Glomerular filtration rate
GMP	Good Manufacturing Practice
GR	Good risk
GVHD	Graft-versus-host-disease
GVL	Graft-versus-leukemia
Hb	Hemoglobin
HCT-CI	AlloHSCT-Comorbidity Index
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte histocompatibility Antigen
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group
HR	Hazard ratio
HRC	Hematology Review Committee
HSCT	Hematopoietic Stem Cell Transplantation
IBD	Inflammatory bowel disease
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IDH	Isocitrate dehydrogenase
IDH1	Isocitrate dehydrogenase 1
IDH2	Isocitrate dehydrogenase 2
IMP	Investigational Medicinal Product
IMiDs	Immunomodulatory Drugs
IPSS	International Prognostic Score System

IR	Intermediate risk
ITD	Internal tandem duplications
KCl	Potassium chloride
LAP	Leukemia-associated immunophenotype
LDH	Lactate Dehydrogenase
MA	Myeloablative
MDS	Myelodysplasia
MDS-U	Myelodysplastic syndrome-unclassified
MI	Monosomy index
MK	Monosomal karyotype
MMF	Mycophenolate mofetil
MK-	Monosomal karytope negative
MK+	Monosomal karytope positive
MN1	Meningioma (disrupted in balanced translocation) 1
MPAL	Mixed phenotype acute leukemia
MRD	Minimal Residual Disease
MVD	Microvessel density
NaCl	Sodium Chloride
NCI	National Cancer Institute
NIH	National Institutes of Health
NK	Normal karyotypes
NMP1	Nucleophosmin
NRM	Non-relapse mortality
NYHA	New York Heart Association
OS	Overall Survival
PB	Peripheral Blood
PBPC	Peripheral Blood Progenitor Cell
PCR	Polymerase Chain Reaction
PE	Pulmonary Embolism
PR	Partial Response
PR	Poor risk
RA	Rheumatoid arthritis
RA	Refractory anemia
RAEB-1	Refractory anemia with excess blasts-1
RARS	Refractory anemia with ring sideroblasts
RCMD	Refractory cytopenia with multilineage dysplasia

RCUD	Refractory cytopenias with unilineage dysplasia
RIC	Reduced intensity conditioning
RN	Refractory neutropenia
RT	Refractory thrombocytopenia
RUNX1	Runt-related transcription factor 1
SAE	Serious Adverse Event
SC	Subcutaneous
SLE	Systemic lupus erythmatosis
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBI	Total body irradiation
TET2	Methylcytosine dioxygenase 2 gene
ULN	Upper limit of normal
VPR	Very poor risk
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

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A1. WHO 2008 classification for Acute Myeloid Leukemias (AML) and related precursor neoplasms

- Definition AML: ≥ 20% myeloblasts in blood or bone marrow
- Abnormal promyelocytes in acute promyelocytic leukemia, promonocytes in AML with monocytic differentiation and megakaryoblasts in acute megakaryoblastic leukemia are considered blast equivalents

Table 17. WHO 2008 classification for AML and related precursor neoplasm

WHO code	Category	Subcategory and short description
9896	AML with recurrent genetic abnormalities	AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1**
9871		AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFß-MYH11**
9866		Acute promyelocytic leukemia; AML with t(15;17)(q22;q12); *PML-RARA* and cytogenetic variants
9897		AML with t(9;11)(p22;q23); *MLLT3-MLL*
9865		AML with t(6;9)(p23;q34);*DEK-NUP214*
9869		AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
9911		AML (megakaryoblastic) with t(1;22)(p13;q13);*RBM15-MKL1*
9861		Provisional entity: AML with mutated *NPM1*
9861		Provisional entity: AML with mutated *CEBPA*
9895	AML with myelodysplasia related changes	Previous history of myelodysplastic syndrome **or** Myelodysplastic syndrome-related cytogenetic abnormality **or** Dysplasia present in > 50% of 2 or more cell lineages **Absence of both** Prior cytotoxic therapy for an unrelated disease Recurring cytogenetic abnormality as described in AML with recurrent genetic abnormalities
9920	Therapy-related myeloid neoplasms	Includes t-AML, t-MDS and t-MDS/MPN
9861	**Acute myeloid leukemia, NOS**	
9872	AML with minimal differentiation	**<**3% of blasts positive for Sudan Black B or MPO. Blasts usually express CD13 and/or CD117, with or without CD33 in absence of lymphoid markers cCD3, cCD22 and cCD79a
9873	AML without maturation	Blasts ≥90% of bone marrow non-erythroid cells (i.e. excluding also lymphocytes, plasmacells, macrophages and mast cells) ≥3% of blasts positive for Sudan Black B or MPO Blasts express MPO and one or more of myeloid-associated antigens such as CD13, CD33 or CD117
9874	AML with maturation	≥10% maturing cells of neutrophil lineage <20% bone marrow monocytes
9867	Acute myelomonocytic leukemia	>20% neutrophils and precursors of marrow cells >20% monocytes and precursors of marrow cells
9891	Acute monoblastic and	≥80% of the leukemic cells are monoblasts,

	monocytic leukemia	promonocytes and monocytes
9840	Acute erytroid leukemia	Erythroleukemia (erythroid/myeloid) Presence of medium to large size erythroblasts: ≥ 50% of bone marrow cells Blasts: ≥ 20% of the bone marrow nonerythroid cells
		Pure erythroid leukemia Presence of medium to large size erythroblasts
9910	Acute megakaryoblastic leukemia	>50% of the blasts are of megakaryocytic lineage Blasts express CD41 and/or CD61
9870	Acute basophilic leukemia	Primary differentiation to basophils; mature basophils are usually sparse
9931	Acute panmyelosis with myelofibrosis	Acute panmyeloid proliferation with accompanying fibrosis
9930	Myeloid sarcoma	Tumor mass of myeloblasts or immature myeloid cells occurring in an anatomical site other than the bone marrow
Myeloid proliferations related to Down syndrome (DS)		
9898	Transient abnormal myelopoiesis (TAM)	Morphologic and immunophemotypic features are similar to the blasts in most cases of DS AML
9898	Myeloid leukemia associated with Down syndrome	Usually an acute megakaryoblastic leukemia
9727	**Blastic plasmacytoid dendritic cell neoplasm (BPDC)**	
		Blastic NK-cell lymphoma

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

A2. WHO 2008 classification for myelodysplastic syndromes

Table 18. WHO 2008 classification for myelodysplastic syndromes

WHO code	Disease	Blood findings	Bone marrow findings
9980 *9991* *9992*	Refractory cytopenias with unilineage dysplasia (RCUD) Refractory anemia (RA); Refractory neutropenia (RN); Refractory thrombocytopenia (RT)	Unicytopenia or bicytopenia[1] No or rare blasts (<1%)[2]	Unilineage dysplasia: ≥ 10% of the cells in one myeloid lineage <5% blasts <15% of erythroid precursors are ring sideroblasts
9982	Refractory anemia with ring sideroblasts (RARS)	Anemia No blasts	≥15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts
9985	Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1%)[2] No Auer rods <1 x 10^9/l monocytes	Dysplasia in ≥10% of the cells in ≥ two myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) <5% blasts in marrow No Auer Rods ±15% ring sideroblasts
9983	Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts[2] No Auer rods <1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 5-9% blasts[2] No Auer rods
9983	Refractory anemia with excess blasts-1 (RAEB-2)	Cytopenia(s) 5-19% blast Auer rods ± <1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 10-19% blasts[2] Auer rods ±
9989	Myelodysplastic syndrome-unclassified (MDS-U)	Cytopenias ≤1% blasts[2]	Unequivocal dysplasia in less than 10% of the cells in one or more myeloid cell lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS < 5% blasts
9986	MDS associated with isolated del(5q)	Anemia Usually normal or increased platelet count No or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei < 5% blasts No Auer rods Isolated del(5q) cytogenetic abnormality
9985	Refractory cytopenia of childhood		

[1]Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U
[2]If the marrow myeloblast percentage is <5% but there are 2-4% myeloblasts in the blood, the diagnostic classification is RAEB 1. Cases of RCUD and RCMD with 1% myeloblasts in the blood should be classified as MDS-U.
[3] Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB-2

A3. WHO 2008 Acute leukemia's of ambiguous lineage

Table 19.WHO 2008 Acute leukemia's of ambiguous lineage

WHO code	Category	Short description
9801	Acute undifferentiated leukemia	Expresses no markers considered specific for either lymphoid or myeloid lineage
9806	MPAL, with t(9;22)(q34;q11.2); *BCR-ABL1*	
9807	MPAL, with t(v;11q23); *MLL* rearranged	
9808	MPAL, B/myeloid, NOS	
9809	MPAL, T/myeloid, NOS	
	MPAL, NOS-rare types	
	Other ambiguous lineage leukemia's	A combination of markers is expressed that does not allow classification as either AUL or MPAL

MPAL= mixed phenotype acute leukemia

Table 20. Requirements for assessing more than one lineage to a single blast population (mixed phenotype)

Myeloid lineage Myeloperoxidase (flow cytometry, immunohistochemistry or cytochemistry) Or Mononcytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme)
T lineage Cytoplasmic CD3 (flow cytometry with antibodies to CD3 epsilon chain; immunohistochemistry using polyclonal anti-CD3 antibody may detect CD3 zeta chain, which is not T-cell specific) Or Surface CD3 (rare in mixed phenotype acute leukemia's)
B lineage (multiple antigens required) Strong CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10 Or Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10

A4. FAB classification of AML

Table 21. 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

B. Revised International Prognostic Score System (IPSS-R) for MDS

Table 22. Risk categories

Risk category	IPSS-R score
Very low	≤ 1.5
Low	> 1.5-3
Intermediate	> 3-4.5
High	> 4.5-6
Very high	> 6

Table 23. IPSS-R prognostic score values

Prognostic score value	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	-	Good	-	Intermediate	Poor	Very poor
BM blast, %	≤2	-	>2- <5	-	5-10	>10	-
Hemoglobin mmol/l	≥6.2	-	5.0- <6.2	<5.0	-	-	-
Platelets $x10^9$/l	≥100	50- 100	<50	-	-	-	-
ANC $x10^9$/l	≥0.8	<0.8	-	-	-	-	-

-indicates not applicable

Table 24. Cytogenetic scoring system for IPSS-R

Prognostic subgroups	Cytogenetic abnormalities
Very good	-Y,del(11q)
Good	Normal, del(5q),del(12p),del(20q), double including del(5q)
Intermediate	del(7q),+8,+19,i(17q), any other single or double independent clones
Poor	-7, inv(3)/t(3q),del(3q), double including -7/del(7q), complex: 3 abnormalities
Very poor	Complex: >3 abnormalities

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C. AML Response criteria

These response criteria were published in the 2009 paper, "Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet" [(67)], and are based on International Working Group recommendations published in 2003 [(68)].

Table 25 Response Criteria

CATEGORY	DEFINITION
Complete remission (CR) [1]	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 10^9/L; platelet count >100 x 10^9/L; independence of red cell transfusions
CR with incomplete recovery (CRi) [2]	All CR criteria except for residual neutropenia (<1.0 x 10^9/L) or thrombocytopenia (<100 x 10^9/L)
Morphologic leukemia-free state [3]	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	Relevant in the setting of phase I and II clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%
Cytogenetic CR (CRc) [4]	Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow
Molecular CR (CRm) [5]	No standard definition; depends on molecular target
Resistant disease (RD)	Failure to achieve CR or CRi (general practice; phase II/III trials), or failure to achieve CR, CRi or PR (phase I trials); only includes patients surviving > 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring > 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring > 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available
Relapse [6]	Bone marrow blasts > 5%; or reappearance of blasts in the blood; or development of extramedullary disease

[1] All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5-7 days; flow cytometric evaluation may help to distinguish between

persistent leukemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

[2] The criterion of CRi is of value in protocols using intensified induction or double induction strategies, in which hematologic recovery is not awaited, but intensive therapy will be continued. In such protocols, CR may even not be achieved in the course of the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients. Some patients may not achieve complete hematologic recovery upon longer observation times.

[3] This category may be useful in the clinical development of novel agents within phase I clinical trials, in which a transient morphologic leukemia-free state may be achieved at the time of early response assessment.

[4] Four studies showed that failure to convert to a normal karyotype at the time of CR predicts inferior outcome.

[5] As an example, in CBF AML low-level PCR-positivity can be detected in patients even in long-term remission. Normalizing to 104 copies of ABL1 in accordance with standardized criteria, transcript levels below 12 to 10 copies appear to be predictive for long-term remission.

[6] In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of hematopoiesis. Cytogenetics should be tested to distinguish true relapse from therapy-related MDS/AML.

D. Risk Group Classification in Current Study Based upon Combined Baseline determinants, time to CR (early/late) and MRD evaluation after cycle II

Table 26 Risk Group Classification in Current Study

Risk Group	Criteria at diagnosis and early/late CR	Plus criteria after cycle II based on MRD
Good (autoHSCT consolidation*)	t(8;21) or *AML1-ETO*, WBC≤20	irrespective of MRD- or MRD+
	inv16/t(16;16) or *CBFB-MYH11*	irrespective of MRD- or MRD+
	CEBPA-biallelic mutant+	irrespective of MRD- or MRD+
	FLT3ITD-/NMP1+,	irrespective of MRD- or MRD+
Intermediate (autoHSCT consolidation*&**)		*Intermediate risk features at baseline (see category in left section) and also MRD-:*
	CN –X –Y, WBC≤100, CRe	also MRD-
	t(8;21) or *AML1-ETO*, plus WBC>20 or mutant KIT	also MRD-
		(if no MRD information available, see legend below)
Poor (alloHSCT consolidation***)		*Intermediate risk features as above but MRD pos:*
	CN –X –Y, WBC≤100, CRe	but MRD+
	t(8;21) or *AML1-ETO*, WBC>20 and/or mutant KIT	but MRD+
	CN –X –Y, WBC≤100, not CRe	irrespective of MRD- or MRD+
	CN –X –Y, WBC>100,	but MRD-
	CA, but non-CBF, MK-, no abn3q26	but MRD-
Very Poor (alloHSCT consolidation***)		*Poor risk features as above but MRD pos:*
	CN –X –Y, WBC>100	but MRD+
	CA, but non CBF, MK-, no abn3q26, EVI1-neg	but MRD+
		Very poor risk features at diagnosis, *independent of MRD assessment:*
	MK+	irrespective of MRD- or MRD+
	abn3q26	irrespective of MRD- or MRD+
	Non CBF with EVI1+	irrespective of MRD- or MRD+
	Non CBF with mutant p53, or mutant RUNX1, or mutant ASXL1 or	irrespective of MRD- or MRD++
	bi-allelic FLT3-ITD with FLT3-ITD/FLT3wt ratio of >0.6	irrespective of MRD- or MRD+

MRD + = MRD positive after cycle II either by flow cytometry or molecular(NPM1)

CBF, core binding factor; MK, monosomal karyotype

CRe – early CR (ie CR after cycle I)

High EVI1 expression is defined as EVI1 expression above 0.1x EVI1 expression in the cell line SKOV3 (reference gene normalized (Groschel et al., JCO 2010, 12 (28) p. 2101-07))

MRD is considered positive whenever residual disease is demonstrated by any assay, whether it is by flow cytometry or molecular analysis (ie NPM1mutant)

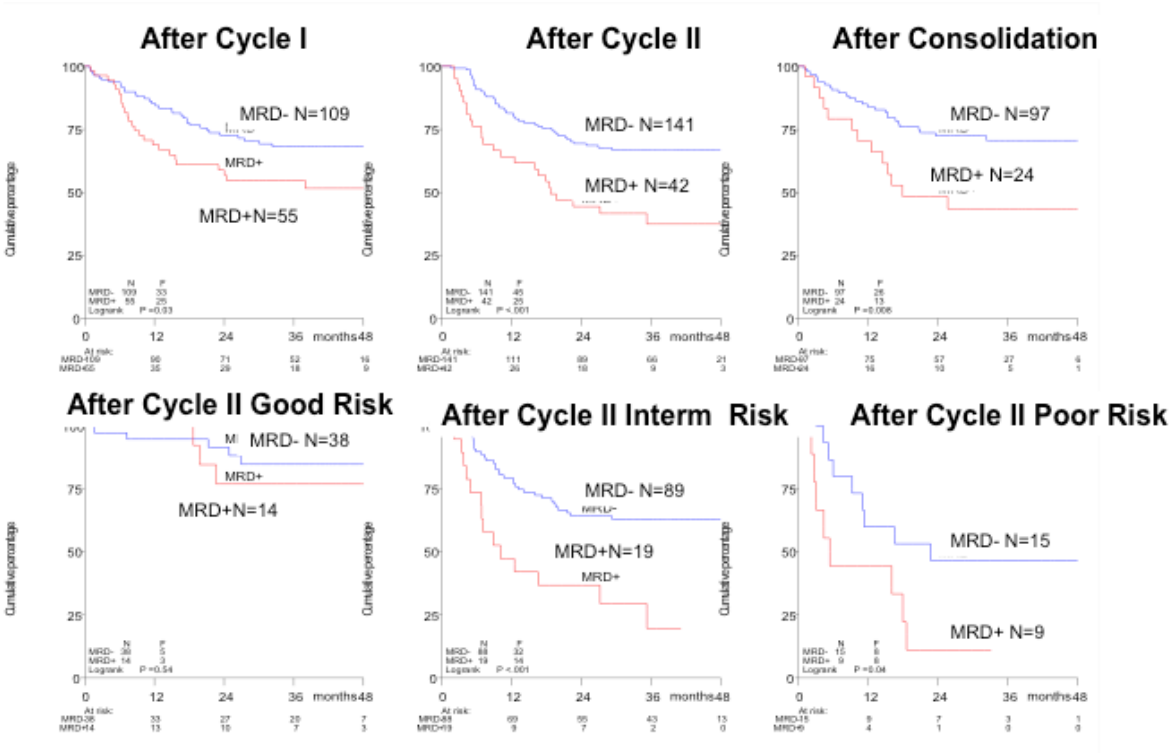
*** Patients with Good/Favorable Risk AML and Intermediate Risk AML (based on available MRD negativity) are recommended for post-remission treatment with autoHSCT according protocol**

**** Patients with Intermediate Risk disease in whom no MRD data have been obtained and/or for whom no MRD information is available are recommended for HLA identical sibling alloHSCT or phenotypically 10/10 HLA-matched unrelated donor alloHSCT.**
***** Unfavorable (Poor) Risk AML and Very Unfavorable (Very Poor) Risk AML patients are recommended for post-remission treatment with alloHSCT according to protocol**

For further explanation please see below.

- If cytogenetics unknown, consider as CN
- MK refers to AML with two or more autosomal monosomies or a single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities, excluding marker and ring chromosomes
- CBF refers to the core-binding factor leukemia's which include AML's with cytogenetic abnormality t(8;21)(q22;q22) or the *AML1-ETO* fusion gene and the cytogenetic abnormalities inv(16)(p13q22) or t(16;16)(p13;q22) or the related fusion gene *CBFB-MYH11*
- CRe: attainment of early CR, ie after cycle I
- EVI1+ refers to EVI1 mRNA over expression (> 1.0 EV1 expression in the cell line SKOV3)
- FLT3-ITD-/NPM1+: Fms-like tyrosine kinase receptor internal tandem duplications (FLT3-ITD) and nucleophosmin-1 (NPM1) mutations often go together as dual genetic anomalies in the same AML. AMLs being FLT3-ITD mutant negative (FLT3ITD-) but NPM1-mutant positive (NPM1+) are considered good risk (GR)

Post-remission treatment is based on MRD data from HOVON/SAKK-42 AML study together with data from the literature:



See appendix L for molecular biomarker diagnostics and minimal residual disease assessments.

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

5 Death

F. Common Terminology Criteria for Adverse Events

The grading of adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 4.0. A complete document may be downloaded from the HOVON website:

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

G. NYHA scoring list

The New York Heart Association functional and therapeutic classification applied to dyspnea

Grade 1	No breathlessness
Grade 2	Breathlessness on severe exertion
Grade 3	Breathlessness on mild exertion
Grade 4	Breathlessness at rest

H. Diagnosis, staging and grading of GVHD

Diagnosis

Acute and chronic GVHD is defined according to the proposal of the recent National Institutes of Health (NIH) Consensus Conference, which recognizes 2 categories of GVHD 1:

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

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

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

(2) Chronic GVHD, comprising:

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

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

Staging and grading of acute GVHD

For staging and grading the Glucksberg classification updated according to Przepiorka et al [2, 3] is used:

Table 27. Staging of acute GVDH

Stage	Skin Rash	Liver Total bilirubin (μmol/L)	Intestinal tract Diarrhea (ml/day)
1	<25%	34-50	500 –1000 or persistent nausea without diarrhea*
2	25-50%	50-102	1000-1500
3	> 50%	102-255	>1500
4	generalized erythroderma with bullous formation	>255	severe pain/ileus

Table 28. Grading of acute GVDH

Grade	
I	Skin: stage 1-2 and Liver: stage 0 and Gut: stage 0
II	Skin: stage 3 or Liver: stage 1 or Gut: stage 1
III	Liver: stage 2-3 or Gut: stage 2-4
IV	Skin or Liver: stage 4

Table 29. Signs and symptoms of chronic GVHD according to the National Institutes of Health (NIH) Consensus Conference

Organ or Site	Diagnostic (Sufficient to Establish the Diagnosis of Chronic GVHD)	Distinctive (Seen in Chronic GvHD, but Insufficient Alone to Establish a Diagnosis of Chronic GVHD)	Other Features*	Common (Seen with Both Acute and Chronic GVHD)
Skin	Poikiloderma Lichen planus-like features Sclerotic features Morphea-like features Lichen sclerosus-like features	Depigmentation	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritus
Nails		Dystrophy Longitudinal ridging, splitting, or brittle features Onycholysis Pterygium unguis Nail loss (usually symmetric; affects most nails)		
Scalp and body hair		New onset of scarring or non-scarring scalp alopecia (after recovery from chemo-radiotherapy) Scaling, papulosquamous lesions	Thinning scalp hair, typically patchy, coarse, or dull (not explained by endocrine or other causes) Premature gray hair	
Mouth	Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseudomembranes Ulcers		Gingivitis Mucositis Erythema Pain
Eyes		New onset dry, gritty, or painful eyes Cicatricial conjunctivitis Keratoconjunctivitis sicca Confluent areas of punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus-like features Vaginal scarring or stenosis	Erosions Fissures Ulcers		
GI tract	Esophageal web Strictures or stenosis in the upper to mid third of the esophagus		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss Failure to thrive (infants and children)
Liver				Total bilirubin, alkaline phosphatase >2 × upper limit of normal ALT or AST >2 × upper limit of normal
Lung	Bronchiolitis obliterans diagnosed with lung biopsy	Bronchiolitis obliterans diagnosed with PFTs and radiology		BOOP

Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis or polymyositis	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hypergammaglobulinemia Autoantibodies (AIHA and ITP)	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality or cardiomyopathy	

* Can be acknowledged as part of the chronic GVHD symptomatology if the diagnosis is confirmed
# Diagnosis of chronic GVHD requires biopsy or radiology confirmation (or Schirmer test for eyes).

I. Acute GVHD response definition

Complete response:	the return of acute GVHD to grade 0
Partial response:	improvement of at least 1 organ, with no worsening in other organs
Mixed response:	improvement of at least 1 organ, with worsening in at least 1 other organ
Stable disease:	no significant change in any organ system
Progressive disease:	progression in at least 1 organ system without improvement in any other

J. Risk of TRM after alloHSCT – Comorbidity index

Table 30. AlloHSCT-Comorbidity Index (HCT-CI) as developed in Seattle

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

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

K. Risk of TRM after AlloHSCT – EBMT score

EBMT risk score

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

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

TRM by alloHSCT-EBMT score

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

Table 31. TRM by allo HSCT-EBMT score

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

(Gratwohl et al Cancer 2009)

L. INSTRUCTIONS FOR SPECIALIZED LABORATORY INVESTIGATIONS

Table of Content

I. Table 1 with summary of sampling time points for diagnostics and biobanking

Discipline	Local or Central	Diag-nosis	After cycle I	After cycle II	Before allo-HSCT	After cycle 3 / Auto HSCT immediat-ely before R2 or after alloHSCT	Sample of auto HSCT graft	3 Months after allo HSCT	6 Months after 2nd Randomizat ion or 6 Months after AlloHSCT	At relapse
Hematocyto-logy	Local	X	X	X	X	X	X	X	X	X
Smears in HRC review	Central	X								
Immunophe-notyping	Local	X								X
Cytogenetics	Local	X	X$			X$			X$	X
Immuno MRD Biobanking	Central Amster-dam	X		X	X	X	X	X	X	X
Molecular MRD Novel molecular markers* Biobanking	Central Rotter-dam	X#		X	X	X	X	X	X	X

*: AML1-ETO, CBFB-MYH11, CEBPA mono-allelic and biallelic, FLT3-ITD mono-allelic and bi-allelic, FLT3-mut, NPM1 mut, ASXL1 mut, RUNX1 mut, p53 mutant, EVI-1 expression

#: Also saliva at diagnosis only

$: Cytogenetics only in case of abnormality

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

II.1.a Hematocytological analysis (local)

Hematocytology examination of bone marrow and peripheral blood should be performed at diagnosis and the disease should be classified according to WHO and FAB classification.

This is performed at the local institute according to local procedures using smears of BM and PB. Time points for hematocytologic analysis at diagnosis and response evaluation during treatment and follow-up are summarized in Table 1.

II.1.b Hematocytological analysis (central)

The HOVON Haematology Review Committee (HRC) will review diagnostic slides (after shipment to Rotterdam), to confirm diagnosis and classification. For CRF see: http://www.hovon.nl/working-groups/technical-commissions/hrc.html.

Guideline:

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

II.2. Immunophenotyping (local)

This is performed at diagnosis at the local institute according to local procedures using flowcytometry performed on BM or PB.

Recommended Guideline:

Sampling moment	BM Sampling	PB Sampling
At diagnosis	7 ml heparin BM	7 ml heparin PB
At relapse	7 ml heparin BM	7 ml heparin PB

II.3. Cytogenetic analysis (local)

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

Conventional cytogenetic analysis should be performed in all patients at diagnosis and following treatment in case of an abnormality (time points summarized in Table 1). For particular cytogenetic abnormalities the use of molecular techniques will be required. In general, the results of the cytogenetic analysis should be available within maximally 2 weeks after diagnosis. This will enable appropriate leukemia risk assessment.

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

Recommended Guideline:

Sampling moment	BM Sampling	PB Sampling
At diagnosis	7 ml heparin BM	7 ml heparin PB
Only in case of abnormality at diagnosis: After cycle I	7 ml heparin BM	7 ml heparin PB
Only in case of abnormality at diagnosis: after cycle III or auto/allo	7 ml heparin BM	7 ml heparin PB
Only in case of abnormality at diagnosis: after 6 cycles maintenance / observation	7 ml heparin BM	7 ml heparin PB
In case of relapse	7 ml heparin BM	7 ml heparin PB

II.4. Molecular diagnostics (local)

(RT-)PCR is obligatory for the detection of *AML1/ETO* (t(8;21)) and *CBFB/MYH11* inv(16), t(16;16)) fusion transcripts, the *FLT3* internal tandem duplication (ITD), mutations in *NPM1,* biallelic mutations in *CEBPA* and increased expression of *EVI1*. These analyses are carried out centrally at the ErasmusMC Rotterdam but according to local preferences can be done locally by the molecular diagnostic laboratories of the participating centers approved by the MODHEM. Assays for novel molecular biomarkers may be introduced during the study. Assays of comparatively more recently introduced assays for biomarkers such as *RUNX1* mutations, *ASXL1* mutations, *p53* mutations and *FLT3* ITD/*FLT3* wild-type ratios, and whenever applicable new emerging markers will be assessed centrally at the ErasmusMC in Rotterdam on the material shipped for the central biobanking.

Molecular diagnostics is necessary and should be performed in all patients at diagnosis. In general, the results of the molecular/cytogenetic analysis should be known within maximally 3 weeks after diagnosis. This will enable appropriate leukemia risk assessment according protocol.

The results of the local molecular diagnostics assays should be uploaded by employees of the local molecular diagnostic laboratory on the MODHEM website (www.modhem.nl).

Log on to the website (In case you need a username and password please contact Peter J.M. Valk (p.valk@erasmusmc.nl)).
Go to ‘options’
Go to ‘HOVON’
Select ‘HOVON132 AML’
Fill out the electronic form

Guideline:

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

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

II.5. Assessment of minimal residual disease (MRD) by immunophenotyping (immunoMRD) and molecular markers (molecularMRD)

The MRD assessment in the HOVON-SAKK AML132 protocol involves both the immunophenotypic MRD as well as the molecular MRD approaches. The term minimal residual disease (MRD) refers to the ‘occult’ low amount of leukemia that may persist during remission in the absence of clinical or

hematological evidence of disease. Recently, the level of MRD was established in various confirmatory studies as a prognostic factor that predicts for relapse. In the current protocol the MRD level (immunophenotypically and/or molecularly defined) after the second cycle of chemotherapy treatment will guide treatment decisions in a defined subgroup of study patients (see risk assessment (Appendix D)). For these purposes, material at diagnosis (as reference for follow up), as well as at follow up, including relapse, should be sent to the VU University Medical Center (VUMC) for immunophenotypic MRD evaluation and to Erasmus Medical Center for the mutant NPM1 MRD evaluation.

II.5.1. Immunophenotyping MRD detection (immunoMRD) and biobanking (Amsterdam)

Immunophenotypic detection of MRD is based upon the presence of leukemia-associated immunophenotypes (so called LAP's). These unusual or aberrant immunophenotypes distinguish leukemic cells from normal hematopoietic cells. LAP's refer to cross-lineage antigen expression (e.g. the expression of lymphoid markers on myeloid cells), a-synchronic antigen expression (e.g. the co-expression of early markers with mature myeloid markers), overexpression of antigens (e.g. relatively high expression levels of particular myeloid or lymphoid markers), lack of antigens (e.g. the lack of a myeloid marker) and/or ectopic expression (e.g. the expression of particular antigens that normally are not expressed on hematopoietic cells). The method to detect MRD has a sensitivity of 1 malignant cell among 1,000 to 10,000 normal cells, but it requires detailed immunophenotypical expertise and knowledge of normal bone marrow cell differentiation. MRD levels will be assessed in both BM and PB, to explore the potency of PB MRD. Immunophenotypic MRD will be carried out centrally at the VUMC Amsterdam on material shipped for biobanking purposes.

Background information regarding immunophenotypic MRD and detailed procedures can be found on the website www.vumc.nl/afdelingen/hematologie/behandelaars/onderzoek/3663436

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

Guideline sampling:

Sampling moment*	BM Sampling	PB Sampling
At diagnosis•	10 ml heparin BM or (in case of dry tap) 10 ml heparin PB	10 ml heparin PB
After cycle II (before auto/alloHSCT or cycle III)	10 ml heparin BM	10 ml heparin PB
Before alloHSCT	10 ml heparin BM	10 ml heparin PB
Sample of auto HSCT graft	1 ml of HPC, Apheresis product in tube	
After cycle III or autoHSCT: Immediately before R2 or after alloHSCT	10 ml heparin BM	10 ml heparin PB
3 months after alloHSCT	10 ml heparin BM	10 ml heparin PB
6 months after 2nd randomization or 6 months after alloHSCT	10 ml heparin BM	10 ml heparin PB
In case of relapse#	10 ml heparin BM or (in case of dry tap) 10 ml heparin PB	10 ml heparin PB

* The indicated volumes are the minimal volumes required for adequate immunoMRD

• Send the sample immediately after aspiration, even if the patient is not yet included! (if the patient will not be included the samples will be destroyed)

Relapse material is important to retrospectively identify false negative MRD time points that result from immunophenotype shifts

II.5.2 Novel molecular biomarkers, molecular MRD detection (molecularMRD) and biobanking (Rotterdam)

RUNX1, ASXL1, p53 mutation and *FLT3* ITD/*FLT3* wild type ratio analysis and molecularMRD will be carried out centrally at the Erasmus MC in Rotterdam on material shipped for biobanking purposes.

The timing of the mutation detection assays is scheduled at diagnosis. The required sensitivity (positive cells diluted in negative cells) of all these assays is at least $1/10^2$.

MRD detection in acute myeloid leukemia (AML) using PCR based techniques for molecular markers will initially been done centrally only in AML patients with an *NPM1* mutation when demonstrated at diagnosis. The assays are carried out during follow-up and the required sensitivity (positive cells diluted in negative cells) of all these assays is at least $1/10^4$ (OCI-AML3 diluted HL60).

For biobanking purposes a serum-separating tube (‘stol buis’) and saliva of each AML patient should be shipped to Rotterdam at diagnosis and a serum-separating tube (‘stol buis’) at relapse. Saliva should be collected using DNAgenotek Oragene DNA (OG-500) collection kits (http://www.dnagenotek.com/ROW/products/OG500.html). These samples will be stored in Rotterdam for retrospective analysis.

BM, PB, saliva and serum separating tube of all AML patients should be sent directly to the Erasmus MC in Rotterdam (where the patient material is further processed for use in biobanking, molecular diagnostics (novel assays) and molMRD).
For additional questions please contact:
Peter Valk (p.valk@erasmusmc.nl) (+31)(0)10-7043975
Mojca Jongen-Lavrencic (m.lavrencic@erasmusmc.nl) (+31)(0)10-7041367

Guideline sampling:

Sampling moment*	**BM Sampling**	**PB Sampling**	**Saliva**
At diagnosis	10 ml heparin BM or (in case of dry tap) 10 ml heparin PB	10 ml heparin PB 7 ml serum separating tube ('stolbuis')	Saliva[%]
After cycle II (before auto/alloHSCT or cycle III)	10 ml heparin BM	10 ml heparin PB	
Before alloHSCT	10 ml heparin BM	10 ml heparin PB	
Sample of auto HSCT graft	1 ml of HPC, Apheresis product in tube		
After cycle III or auto: Immediately before R2 or after alloSCT	10 ml heparin BM	10 ml heparin PB	
3 months after alloHSCT	10 ml heparin BM	10 ml heparin PB	
6 month after 2nd randomization or 6 months after alloHSCT	10 ml heparin BM	10 ml heparin PB	
In case of relapse[#]	10 ml heparin BM or (in case of dry tap) 10 ml heparin PB	10 ml heparin PB 7 ml serum separating tube ('stolbuis ')	

* The indicated volumes are the minimal volumes required for adequate immunoMRD

[%] DNAgenotek Oragene DNA (OG-500) collection kits (http://www.dnagenotek.com/ROW/products/OG500.html)

[#] At relapse is important to retrospectively identify false negative MRD time points due to immunophenotype shifts

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

III. Contact and shipping details

III.1. Hematocytology (review)

For review by the HOVON Hematology Review Committee (HRC), please directly send 6 unstained and not fixated bone marrow slides and 4 blood slides, well packed up, together with a copy of the results of immunophenotyping, cytogenetics and molecular diagnostics to:

HOVON Hematology Review Committee (HRC)
Mw. T. de Jong, room Nc-828
Department of Hematology
Erasmus MC
's Gravendijkwal 230
3015 CE Rotterdam
Tel: (+31)(0)10-7033741 / (+31)(0)10-7041797 (Priscilla van Hilst, secretary HRC)
Fax: (+31)(0)10-7041004 (Priscilla van Hilst, secretary HRC)

Samples should be accompanied by a completely filled out registration form that can be downloaded from: http://www.hovon.nl/working-groups/technical-commissions/hrc.html

For questions please contact: hrc@erasmusmc.nl

III.2. Molecular diagnostics, immunoMRD, molecularMRD and Biobanking

Sampling conditions

All BM samples should be gathered in heparin-coated tubes and kept at room temperature. With the large amounts of material needed, BM samples should be obtained by aspiration from different sites thereby replacing the needle. Please avoid aspiration from the first tap after obtaining diagnostic sample since the BM material will not be of high quality. In any case it should be indicated whether the sample is from the first or second tap.

Make sure you obtain the volumes BM and PB indicated. These are the minimal volumes required for adequate immunoMRD, molMRD and biobanking!

Informing VUMC and ErasmusMC about incoming samples:

- Inform VUMC by email **(MRD.info@vumc.nl)** with at least 1 day advance notice, preferably before 11:00pm, prior to bone marrow aspiration.

- Make sure the email contains the following information:

- Time point of aspiration: diagnosis or follow-up
- The place where the samples can be collected by the courier
- The expected time for the samples to be ready for transport
- VUMC will subsequently take care of informing ErasmusMC and the courier
- The courier will send the necessary shipment papers by email.
- The samples should contain the date of birth and HOVON number (if known)!
- The samples have to be packed up (otherwise the courier is not allowed to transport it) together with the shipment papers and labeled with the addresses:

At diagnosis and follow-up

VU medical Center
Department of Hematology
To: A. Kelder/ MRD
CCA 4.12
De Boelelaan 1117
1081 HV Amsterdam
The Netherlands
(Tel: +31) (0) 20-4443836)

Samples should be accompanied by a HO132 transmittal form that can be downloaded from: www.hovon.nl <log in> > Studies > AML > HO132 (HO132 transmittal.pdf).

At diagnosis and follow-up

Erasmus MC
Department of hematology
To: Dr. P.Valk/ Molecular Diagnostics
Faculteitsgebouw, kamer Ee1363
Dr.Molewaterplein 50
3015 GE Rotterdam
The Netherlands
(Tel: +31 (0) 10-7043936)

Samples should be accompanied by a HO132 transmittal form that can be downloaded from: www.hovon.nl <log in> > Studies > AML > HO132 (HO132 transmittal.pdf).

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

There are no financial responsibilities for the participating center as regards sample shipping, centralized molecular diagnostics and centralized MRD assessments since all invoices will go to HOVON.

For additional questions please contact:

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

ErasmusMC - Rotterdam:
Peter Valk (p.valk@erasmusmc.nl) (+31)(0)10-7043975
Mojca Lavrencic-Jongen (m.lavrencic@erasmusmc.nl) (+31)(0)10-7041367

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

The courier involved is:

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