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

A multicenter phase III trial

## PROTOCOL


| | | |
|---|---|---|
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| **Study drug** | : | Laromustine supplied by Vion Pharmaceuticals Inc, New Haven, CT |
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| **SAE notification** | : | **HOVON Data Center**<br>Fax +31-010-4239085 |
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| **Amendment 1** | : | December 2, 2008 |

## PRINCIPAL INVESTIGATOR SIGNATURE PAGE

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| | |
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| Printed Name of Investigator | |

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Local Investigator:

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Signature of Investigator Date

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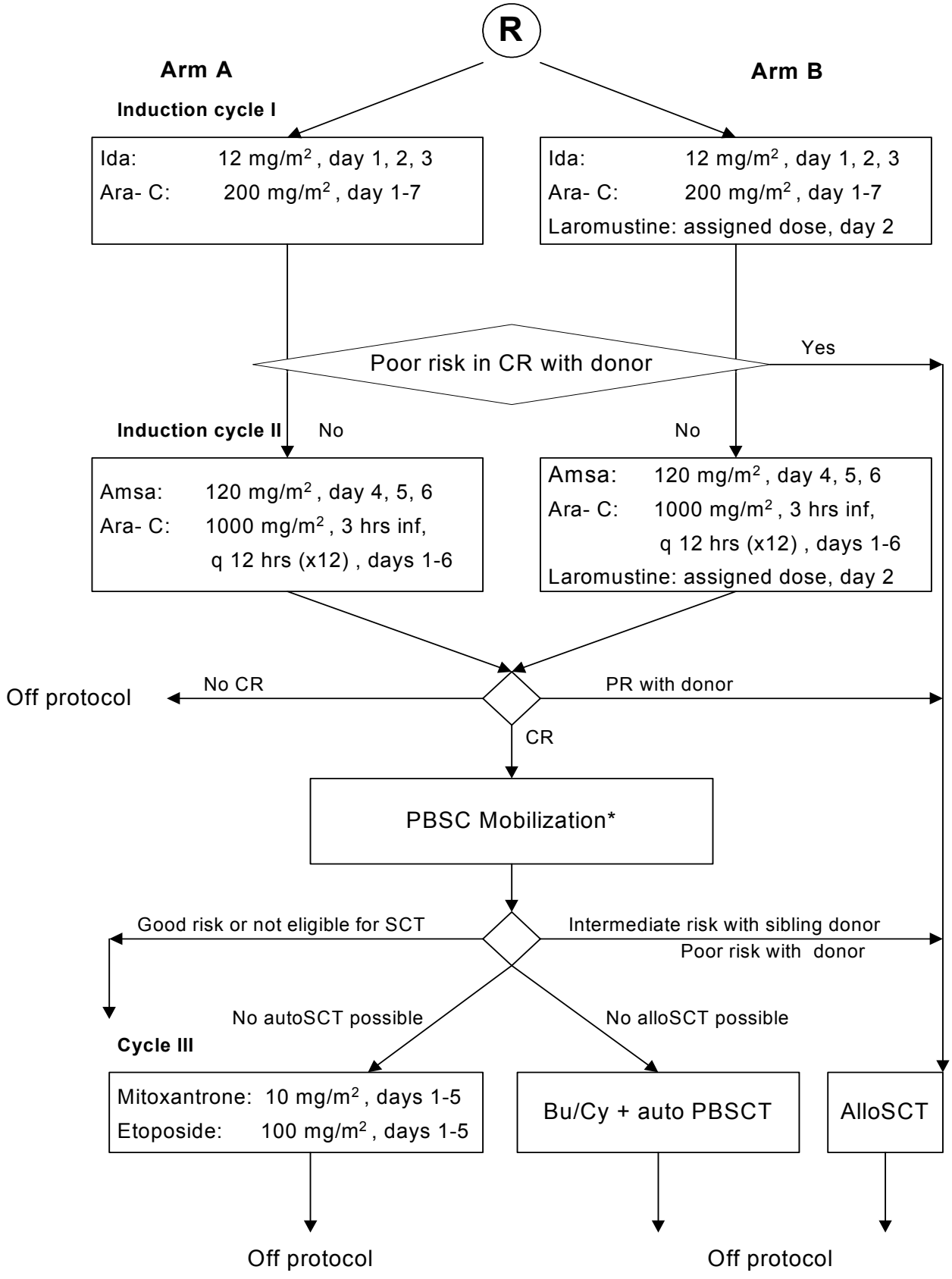
Printed Name of Investigator

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

# 1 Scheme of study




* unless known as good risk or to proceed to AlloSCT

# 2 Table of contents

Page

**1 Scheme of study ..... 4**

**2 Table of contents ..... 5**

**3 Synopsis ..... 8**

**4 Investigators and study administrative structure ..... 10**

4.1 Cytological and immunophenotype review ..... 11
4.2 Cytogenetic review ..... 12

**5 Introduction ..... 12**

5.1 Acute Myeloid Leukemia (AML) ..... 12
5.2 Laromustine (VNP40101M) ..... 14
5.2.1 Laromustine (VNP40101M) as single agent ..... 14
5.2.2 Laromustine in combination with cytarabine ..... 14
5.3 Recently completed studies by HOVON/SAKK in adults (18-60 yr age) with AML ..... 15
5.4 This new HOVON/SAKK study in adults with AML: rationale and design ..... 16
5.4.1 Remission induction therapy and rationale ..... 16
5.4.2 Post induction therapy ..... 17
5.4.3 Autologous stem cell transplantation (autoPBSCT). ..... 17
5.5 Cytogenetic prognostic markers and use of the autosomal Monosomy Index ..... 18
5.6 Molecular diagnostics and genetic markers ..... 19
5.7 Gene expression profiling on DNA microarrays and diagnostics in AML ..... 20
5.8 Risk Group Classification in Current Study ..... 20
5.9 Assessment of minimal residual disease (MRD) ..... 23

**6 Study objectives ..... 23**

6.1 Primary objectives ..... 23
6.2 Secondary objectives ..... 24

**7 Study design and rationale ..... 24**

7.1 Part A vs Part B ..... 25
7.1.1 Part A: Dose selection ..... 25
7.1.2 Part B: Efficacy ..... 26
7.2 Treatment design ..... 26
7.2.1 Remission induction treatment ..... 26
7.2.2 Post remission treatment according to risk assessment: the choice of alloSCT, autoSCT or consolidation chemotherapy ..... 26

**8 Study population ..... 28**

8.1 Eligibility criteria ..... 28
8.1.1 Inclusion criteria ..... 28
8.1.2 Exclusion criteria ..... 28

**9 Treatment ..... 29**

9.1 Dose level of Laromustine ..... 29
9.1.1 Part A ..... 29
9.1.2 Part B ..... 29
9.2 Remission induction treatment cycle I ..... 29
9.3 Remission induction treatment cycle II ..... 30
9.4 Peripheral blood stem cell mobilization and collection ..... 31
9.4.1 Peripheral blood stem cell mobilization ..... 31
9.4.2 Procedure of peripheral blood progenitor cell collection ..... 32
9.4.3 Procedure for hematopoietic cell cryopreservation ..... 32
9.5 Post induction therapy with chemotherapy cycle III ..... 32

9.6 Post-induction treatment: Busulfan-Cyclophosphamide and autologous PBSCT ..... 32
9.7 Post-induction treatment: allogeneic SCT ..... 33
9.8 Special management orders ..... 33
9.9 Forbidden concomitant medication during the study ..... 34
9.10 Study drug information ..... 34
9.10.1 Physical description of study drug and packaging ..... 34
9.10.2 Laromustine labeling ..... 35
9.10.3 Laromustine storage and handling ..... 35
9.10.4 Laromustine preparation ..... 35
9.11 Laromustine drug accountability ..... 36
9.11.1 Disposition of used Laromustine injections vials ..... 36

**10 End of protocol treatment ..... 37**

**11 Required clinical evaluations ..... 37**

11.1 Required investigations ..... 37
11.2 Observations prior to start treatment ..... 39
11.3 Observations during treatment (before start cycles and/or at evaluation previous cycle) ..... 40
11.3.1 Toxicity assessment ..... 40
11.3.2 Bone marrow and blood evaluation and response assessment during treatment ..... 41
11.4 Observations during follow up ..... 41
11.5 Pharmacokinetic Evaluation ..... 42
11.5.1 Sample Shipment ..... 43
11.5.2 Sample Analysis ..... 44

**12 Toxicities ..... 44**

**13 Reporting serious adverse events and SUSARS ..... 46**

13.1 Definitions ..... 46
13.2 Reporting of (serious) adverse events ..... 47
13.3 Processing of serious adverse event reports ..... 49

**14 Endpoints ..... 49**

14.1 Part A: of the study (Dose level selection) ..... 49
14.1.1 Primary endpoint ..... 49
14.1.2 Secondary endpoint ..... 50
14.2 Part B: of the study (Efficacy) ..... 50
14.2.1 Primary endpoint ..... 50
14.2.2 Secondary endpoints ..... 50

**15 Registration and Randomization ..... 51**

15.1 Regulatory Documentation ..... 51
15.2 Randomization ..... 51

**16 Data collection ..... 52**

16.1 CRF's ..... 52
16.2 Reporting DLT information ..... 53
16.3 Monitoring ..... 53
16.4 Data quality assurance ..... 53
16.5 On-site audits ..... 53
16.6 End of trial ..... 54

**17 Statistical considerations ..... 54**

17.1 Part A: Dose level selection ..... 54
17.2 Part B: Efficacy ..... 57
17.2.1 Patient numbers and power considerations ..... 57
17.2.2 Interim analyses and safety monitoring ..... 58
17.2.3 Efficacy analysis ..... 59
17.2.4 Toxicity analysis ..... 59
17.2.5 Additional analysis ..... 60
17.3 Data Safety Monitoring Board ..... 60

**18 Ethics ..... 60**

18.1 Accredited ethics committee or Institutional review board ..... 60
18.2 Ethical conduct of the study ..... 60
18.3 Patient information and consent ..... 61

**19 Trial insurance ..... 61**

**20 Publication policy ..... 61**

**21 Glossary of abbreviations ..... 62**

**22 References ..... 65**

**A1 WHO classification for acute myeloid leukemias ..... 70**

**A2 WHO classification for myelodysplastic syndromes ..... 72**

**A3 FAB classification of AML ..... 73**

**B International Prognostic Score System (IPSS) for MDS ..... 74**

**C Response criteria for AML and RAEB ..... 75**

**D Risk group definition ..... 77**

**E Cytogenetic and molecular analysis ..... 80**

**F Gene expression profiling ..... 83**

**G Minimal residual disease assessment ..... 85**

**H Common Terminology Criteria for Adverse Events ..... 87**

**I ZUBROD-ECOG-Performance Status Scale ..... 88**

# 3 Synopsis

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| Study phase | Phase III |
| Study objectives | Primary objectives<br>Part A: To determine the feasibility of Laromustine when given at three possible dose levels together with standard induction cycles I and II in patients with AML/ RAEB with IPSS≥1.5 in a prospective comparison to standard induction cycles I and II without Laromustine<br><br>Part B: To evaluate the effect of Laromustine at the selected feasible dose level when combined with remission induction chemotherapy cycles I and II as regards clinical outcome ("event-free survival') in comparison to remission induction cycles I and II with no addition of Laromustine in a phase III study<br><br>Secondary objectives<br>Part A:<br>♦ To evaluate the pharmacokinetics of Laromustine in the combination with cytarabine-idarubicin remission induction chemotherapy in a selection of patients at different dose levels of Laromustine as well as in a limited number of controls<br>♦ To investigate the clinical efficacy of Laromustine in combination with remission induction chemotherapy cycles I and II with regard to complete remission rate at different dose levels of Laromustine<br><br>Part B:<br>♦ To investigate the clinical efficacy of Laromustine with regard to the complete remission rate, disease free survival (DFS), risk of relapse and overall survival (OS) when combined with remission induction chemotherapy cycles I and II in all patients<br>♦ To investigate the clinical efficacy of Laromustine when combined with remission induction chemotherapy cycles I and II in molecularly and cytogenetically distinguishable subsets with regard to the complete remission rate, disease free survival (DFS), risk of relapse and overall survival (OS)<br>♦ To investigate the tolerance and toxicity of Laromustine in combination with remission induction chemotherapy cycles I and II<br>♦ To evaluate the pharmacokinetics of Laromustine and cytarabine- |

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| | idarubicine remission induction chemotherapy in a limited number of patients in both treatment arms<br>♦ To assess the effect of Laromustine on peripheral CD34 cell numbers for autologous peripheral blood transplantation<br>♦ To determine the prognostic value of molecular markers and gene expression profiles of the leukemia assessed at diagnosis<br>♦ To evaluate the treatment effects according minimal residual disease (MRD) measurements following therapy by standardized sampling of marrow/blood<br>♦ To evaluate the outcome of allogeneic sibling or unrelated donor SCT and autologous SCT in cytogenetically and molecularly defined and prognostic subgroups of patients. |
| Patient population | Patients with previously untreated AML (except acute promyelocytic leukemia) or MDS RAEB with IPSS ≥ 1.5, age 18-65 years. |
| Study design | Part A: Comparative, randomized feasibility study of remission induction chemotherapy combined with Laromustine at three possible dose levels 200, 300, 400 mg/m$^2$.<br>Part B: Multicenter, phase III study at the selected feasible dose level of Laromustine in a prospective randomized approach between Laromustine combined with two induction cycles of chemotherapy versus the same chemotherapy with no addition of Laromustine |
| Duration of treatment | Expected duration of 2 induction cycles inclusive evaluation is approximately 3 months. Consolidation treatment will take an additional 1-3 months.<br>All patients will be followed until 10 years after randomization. |
| Number of patients | 800 |
| Adverse events | Adverse events will be documented if observed and serious adverse events will be reported immediately |
| Planned start and end of recruitment | Start of recruitment: III 2008<br>End of recruitment: III 2012 |
| End of trial | at 7 months after registration and randomization of the finally enrolled patient |

# 4 Investigators and study administrative structure

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## 4.1 Cytological and immunophenotype review

Review by the Hematocytology Review Committee (HRC) will be performed at diagnosis.
At the time of registration 4 unstained blood and 6 unstained bone marrow smears should be sent together with a filled out cytology form and a copy of the report of the immunological marker analysis to

Dr. M.B. van 't Veer
Hematocytology Review Committee
Erasmus MC - Daniel den Hoed
Groene Hilledijk 301
3075 EA Rotterdam
The Netherlands.

Confirmation of diagnosis is not necessary for randomization and start of treatment.

### 4.2 Cytogenetic review

Central review will be performed for cytogenetic analysis at diagnosis.
Each cytogeneticist, responsible for the cytogenetic analysis of the patients in a hospital will be notified automatically by email of the registration of a patient from that hospital in the study. A filled out cytogenetic form together with 2 representative karyotypes and a copy of the original cytogenetic report is requested to be sent within 3 months to the HOVON Data Center for central review.
If additional FISH analysis was performed, a filled out FISH form together with a copy of the original FISH report is also requested to be sent with the cytogenetic data for central review.

## 5 Introduction

### 5.1 Acute Myeloid Leukemia (AML)

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

**Chemotherapy in AML**

The traditional approach to the patient with AML/high risk MDS has been based on treatment with a combination of an anthracyclin (daunorubicin (DNR) or idarubicin) with cytarabine (cytosine arabinoside or Ara-C). The dose of Ara-C has varied from standard dose of 100 mg/$m^2$ q day up to intensified dose of 3 g/ $m^2$ during induction treatment (1).
However, ultimately 40-50% of younger patients and 80% of elderly patients with AML do not achieve long-standing remissions and succumb from primary treatment refractory disease or more frequently from relapse. It has become clear that subsets of AML defined by cytogenetic or molecular markers carry different prognostic characteristics.

From the previous HOVON/SAKK AML studies as well as several other trials it has become clear that a certain subset of patients with AML who enter remission, have a comparatively low probability of relapse such as patients with translocation t(8;21)(q22;q22) together with a WBC of 20 x $10^9$/L or less at diagnosis and patients with inv(16)(p13;q26)/t(16;16(p13;q26) (1, 6). These patients have a probability of long-term survival of more than 60%. However, these “good-risk” patients represent only

approximately 10-15% of complete remitters. The unfavourable prognostic subgroup includes approximately 25% of patients between 15-60 years of age which are defined by the presence of unfavourable cytogenetic abnormalities involving three or more different clonal abnormalities (complex abnormalities), monosomies of chromosomes 5 or 7, deletions of the long arms of chromosomes 5 (del 5q) or 7 (del 7q), abnormalities of the long arm of chromosome 3 (abn3q26), abnormalities of the long arm of chromosome 11 (abn 11q23), translocation t(9;22) or translocation t(6;9)(p23;q34). Their relapse rate is approximately 80% and survival at 5 years is less than 25%.(6, 7, 8). Recent data have emerged that have challenged the classical cytogenetic classification as regards prognosis (see cytogenetic classification and risk assessment below)

A second subset with a high risk of relapse are the patients not classified as good risk because they reach CR only after the second cycle (8). This latter group is about 25% of the patients reaching CR. Together these two groups form about 33% of complete remitters. Finally, the third and largest group of AML patients have a disease which may be classified as intermediate risk. In this group, which includes 70% of the younger patients, the CR rate is approximately 85%. However, overall survival at 5 years is only 35%. More recently various genetic markers have been considered for prognostic value. These include for instance the somatic gene mutations in the hematopoietic growth factor fms-like tyrosine kinase III (FLT3), especially the internal tandem duplications of the FLT3 gene (designated as FLT3-ITD), which in some but not all studies carry somewhat unfavourable prognostic impact as they are associated with an increased relapse probability (9,10). FLT3-ITD are seen in frequent association with mutations in the gene nucleophosmin-1 (NPM1) which carry a contrasting comparatively more favourable prognosis (11, 12, 13). FLT3-ITD and NPM1 mutations frequently appear in the same leukemias together. Thus for instance AML's without FLT3-ITD but with NPM1 mutations can be considered of somewhat better risk. Also high expression of the oncogene EVI1 (ecotropic virus integration 1) (14, 15) (and the BAALC (brain and leukaemia C) (16, 17) and the ERG genes (18, 19) have been suggested to predict for poor outcome. 3q Cytogenetic abnormalities involves dysregulation of the EVI1 oncogene that becomes overexpressed. High EVI1 expression has also recently provided a key to cryptic abn(3q) cytogenetic anomalies, an established adverse cytogenetic factor in AML (15). On the other hand mutations in the gene of the transcription factor CCAAT binding factor alpha (CEBPA) (20, 21, 22) and the aforementioned nucleophosmin 1 (NPM1) correlate with a relatively good prognosis (reduced rate of relapse). In regards to the core binding factor leukemias the presence of additional mutations in the kinase receptor gene have been associated with an enhanced rate of relapse in some studies and survival as well (eg 23) More recently also mutations in the Wilms tumor gene (WT1) have been implicated to express negative prognostic value (24, 25) although current data between different groups are still controversial. However, some of these genetic biomarkers are not yet routinely integrated in multicenter protocols as the laboratory assays require scrutinous validation in a multicenter setting. CEBPA, FLT3-ITD and

NPM1 mutations have been reproducibly demonstrated to confer independent prognostic significance in multivariate analysis. As soon as standardization of gene expression measurements (eg EVI1) is operational they might also be introduced in a scoring system for prognostic risk in a multicenter setting.

From accumulated data it is thus clear that current modalities of chemotherapy are not sufficiently effective and that additional treatments are needed. An important approach to improving the response rates and response durations in AML is to introduce new agents with a unique mechanism of action. The treatment of AML is moving forward by integrating new drugs with different mechanisms of action in the overall treatment plan (32).

## 5.2 Laromustine (VNP40101M)

### 5.2.1 Laromustine (VNP40101M) as single agent

VNP40101M (Laromustine,101M,1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-2 -(methylamino)carbonylhydrazine) is a 1,2-bis(sulfonyl)hydrazine, a new alkylator that is related to the nitrosureas. It undergoes activation to yield an active cloroethylating species which specifically targets the O6 position of guanine (26-29). In contrast to the nitrosureas, it generates considerably more DNA cross links and it avoids the undesired N7 alkylations as well as hydroxyl alkylations at O6 of guanine. In a previous phase I study, Laromustine had demonstrated antileukemic activity in refractory AML (30,31). In a subsequent study (26) the investigators pursued the development of Laromustine at the selected dose level of 600 mg/m$^2$ by intravenous injection (iv) in previously untreated elderly patients with AML. Hydroxyurea was applied in adjunct to support leukemia cytoreduction. Eighty-nine patients with AML and fifteen patients with high-risk myelodysplasia (MDS) were enrolled. These were considered a priori of unfavourable risk because of age > 60 years. Median age of the study group was 72 years. The majority of patients had comorbidities and 46/105 patients exhibited an unfavourable cytogenetic risk profile. Twenty-nine patients achieved CR on one course of treatment. Overall CR rate in these poor risk subjects was 31% (33/105 patients) when patients with complete marrow responses but incomplete platelet recovery (CRp) and patients exposed to a second cycle of treatment are included. Considering the poor risk profile of the cohort of patients, these outcome data are interesting and promising and this agent deserves an evaluation in front line setting in young and middle aged adults.

### 5.2.2 Laromustine in combination with cytarabine

Laromustine was also evaluated in a Phase I study in combination with cytarabine (Ara-C) (24). The Laromustine/Ara-C regimen demonstrated promising activity at Laromustine dose levels ≥ 400 mg/m$^2$.

Overall, for the 31 patients treated at Laromustine doses of 400-600 mg/m$^2$, 4 CR and 6 CRp were observed for an overall response rate of 32%. Together with the results from the single-agent phase I and II trials, the data indicate that Laromustine is an active agent in patients with AML, and may contribute to Ara-C's anti-tumor activity in this malignancy.

The combination of Laromustine and Ara-C was also evaluated in a multinational, placebo-controlled, double-blind randomized Phase III study. The study was designed to determine if the combination of Laromustine and Ara-C increases the rate of CR and CRp compared to placebo and Ara-C in AML patients in first relapse. Ara-C was administered at a dose of 1.5 g/m$^2$/day as a continuous infusion over 24 hours on days 1-3. Laromustine was administered by intravenous injection over 30-60 minutes on Day 2. This study was carried out in pretreated patients and employed Laromustineat a dose level of 600 mg/m$^2$ in induction and 400 mg/m$^2$ in consolidation. Eligibility was restricted to patients with first CR durations of at least 3 months but less than 24 months.
The data safety monitoring board (DSMB) for this study reviewed interim efficacy and safety information after the first 210 patients had been randomized and were available for a response assessment. As a consequence of this review, the DSMB identified differences between the treatment groups in the number of on-study deaths as an outcome of concern, as the increase in response rate for the investigational arm would be offset by mortality. Based on the DSMB recommendation enrollment and treatment of patients was halted. Sepsis, pneumonia and infection were noted as contributing to death in approximately 67% of the patients on the Laromustine/Ara-C arm. An additional 18% of the patients receiving Laromustine/Ara-C had pulmonary complications listed as contributing to death. The key findings from the safety analysis were 1) prolonged myelosuppression with combination therapy likely predisposed patients to fatal infection and 2) the majority of deaths with combination therapy occurred in a setting of primary or intercurrent infection. The combined effects of an antimetabolite and an alkylating agent on the bone marrow, in conjunction with poor hematologic reserve suggested that the dose of Laromustine was too high. It was finally concluded that the Laromustine/Ara-C combination chemotherapy should proceed at a somewhat reduced dose level of Laromustine and with appropriate antimicrobial prophylaxis as is commonly used in the management of patients with AML in all HOVON/SAKK centers. Upon review of key safety findings and a protocol amendment, the FDA supported reinitiation of the study.

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

The most recent study of the HOVON and SAKK cooperative groups (ie, HOVON/SAKK AML 42) in previously untreated adult patients with acute myeloid leukemia (AML) has accrued approximately 1000 patients of whom 640 patients took part in the G-CSF priming study.The evaluation of G-CSF priming in induction in the earlier HOVON/SAKK AML-29 study, being the predecessor of the AML-42 study, had shown a favourable effect of G-CSF priming on the risk of relapse following complete

remission as well as an improvement of disease free survival (55). In a subgroup analysis the benefit was particularly apparent as an advantage in intermediate risk AML (ie, in 72% of patients) related to improved overall survival (OS), event free survival (EFS) as well as disease free survival (DFS). However the good-risk and poor-risk subgroups counted relatively small numbers of accrued cases prohibiting a more robust analysis as regards the value of G-CSF priming.
The randomization for induction as regards the G-CSF priming question of the HOVON 29 study has been carried over to a successor HOVON 42 study that will run until mid 2008. Therefore at this time it is not yet possible to definitely settle the value of G-CSF priming in induction but as soon as the results of the latter randomisation will become available it can be decided whether G-CSF priming will become a standard component of remission induction therapy in adults with AML. Other questions addressed by HOVON/SAKK in the adult age group of age less than 60 years involved the dose of cytarabine in induction and autologous SCT as postinduction therapy In patients of age 60+ other questions were addressed(see also 5.4.3).

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

### 5.4.1 Remission induction therapy and rationale

The backbone of 2 cycles of remission induction chemotherapy followed by a third consolidation cycle of chemotherapy (cycle III) or autologous SCT or alloSCT has been stable. Also the choice of the chemotherapeutic regimen of cycles I-III has been maintained. In the successive HOVON/SAKK AML-4, -29 and -42 a new agent has been added with the objective of improving the efficacy of the induction treatment. The approach has been to embark first on a feasibility phase when the new agent was introduced in the frontline of treatment and then secondly following confirmation of feasibility proceed towards the full prospective phase III study. A similar stepwise approach has been pursued in the parallel studies in AML in patients of older age where for instance in the recent study AML-43 two dose levels of daunomycin were compared in induction cycles I and II.
Laromustine, a promising new anti-leukemic agent (see above) will be added as a single bolus to standard induction cycles I and II. The drug is currently used at 600 mg/m$^2$ as monotherapy in patients of older age with untreated AML. The launch of the new study will begin with a feasibility part A in which the additive effect of Laromustine will be tested at three carefully selected dose levels. After selection of the feasible dose level (200 mg/m$^2$, 300 mg/m$^2$ or 400 mg/m$^2$), the part B of the study, ie the phase III of the study, will be opened. The study will be undertaken as a controlled trial. The study will directly compare the clinical value of standard remission induction chemotherapy cycles I and II with the same treatment to which Laromustine is added on day 2 of both cycles.

### 5.4.2 Post induction therapy

Allogeneic stem cell transplantation (alloSCT) will remain the consolidation therapy of choice for patients with AML of intermediate risk (in that case a matched sibling donor SCT) and for patients with AML of unfavourable prognostic risk (in that case matched sibling donor SCT or unrelated matched sibling donor SCT or umbilical cord blood SCT). In an extended analysis of alloSCT in the respective AML-4, AML-29 and AML-42 HOVON/SAKK studies the disease free survival was significantly better for alloSCT in intermediate risk and unfavourable risk subgroups in patients below 40 years of age. This finding was supported by the results of a large meta-analysis that was carried out as regards the data derived from the three large prospective studies reported by HOVON/SAKK, MRC, the French Transplant Group and EORTC (33). However, while on one hand relapse is strongly reduced following the use of allogeneic SCT, on the other hand it is evident that the increased non-leukemia mortality or transplant-related mortality associated with the alloSCT procedure may lead to excess mortality in 10-50% of patients (56,57,58). The non-leukemia mortality after alloSCT thus may largely offset the anti-leukemic benefit of alloSCT, depending on patient and donor characteristics. Therefore with respect to alloSCT consolidation in individual patients it has become important to take into account not only AML-specific risk factors but also alloSCT risk factors. For instance, in a patient with intermediate-risk AML one would be more reluctant to recommend alloSCT with a relatively high probability of transplant related mortality than in a patient with very poor risk AML. In very poor AML in an effort to take advantage of the strong antileukemic activity of allogeneicSCT one would accept a greater transplant risk. Currently available data allow for identification of transplant-related mortality after alloSCT and furnish a background for recommendations as regards alloSCT mortality risk. The recommendations of this protocol are based on AML specific risk factors (sections 5.8 and 7.2.2) and refer to allogeneic SCT specific risk factors (section 7.2.2).

### 5.4.3 Autologous stem cell transplantation (autoPBSCT).

For patients with intermediate-risk or unfavourable risk AML who have no suitable donor available or who for medical reasons will not proceed to alloSCT, autologous PBSCT following high-dose cytotoxic therapy will be the treatment of choice. This is based on the arguments that some studies have reported improved disease free survival following autoPBSCT (34,35). In this respect we note that the question as regards the clinical value of autoPBSCT in AML first remission is not definitely settled. Results from different studies do not demonstrate a consistent benefit but suggest that autoPBSCT provides better or at least as effective therapy (in terms of DFS/reduced relapse rate) than chemotherapy for consolidation. The final analysis of the large randomized study of autoPBSCT versus 3$^{rd}$ cycle of chemotherapy (within HOVON/SAKK- 29 and 42 studies) is planned for 2008. The interim analysis done in 2007 on 500 patients showed a significantly better disease-free survival in favour of the autoPBSCT arm but no significant advantage as regards overall survival. AutoSCT will be employed as a backbone consolidation therapy in the current protocol (34, 35).

A third cycle of Mitoxantrone/Etoposide chemotherapy will be applied to patients of the favourable prognostic subtype (being not proposed for up front alloSCT nor autoSCT). The third cycle of chemotherapy with Mitoxantrone/Etoposide will also be offered in the protocol to those patients who for clinical or practical reasons cannot be recommended to proceed to an allogeneic SCT (eg no donor available; medical contraindications) nor to an autoPBSCT (eg due to insufficient transplant collection, medical contraindications).

## 5.5 Cytogenetic prognostic markers and use of the autosomal Monosomy Index

Patients with the socalled core binding leukemias (ie AML with translocation t(8;21)(q22;q22) or inv(16)(p13;q26)/t(16;16(p13;q26) (1, 6) have a relatively favourable prognosis (6, 7, 8, 36). Patients with deletions of chromosomes 5 and 7 or deletions of their long arms (-5, 5q-, -7, 7q-) and patients with complex karyotypes (eg those with 3 unrelated clonal cytogenetic abnormalities) have been shown to have a poor prognosis (6, 7, 8). A recent HOVON/SAKK study has challenged the latter distinction and has proposed an improved prognostic score based upon the socalled monosomy index (MI) (37).

In the latter study cytogenetics and survival were analysed in 1,975 patients with newly diagnosed AML aged 15-60 years entered onto four successive Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON) and Swiss Group for Clinical Cancer Research Collaborative Group (SAKK) trials. Besides AML with normal cytogenetics (CN) (n=988; OS at 4-years 41%±2) and AML with core-binding-factor (CBF) abnormalities (n=254; OS 66%±3) we distinguished 733 patients with one or more cytogenetic abnormalities. Analysis of the latter subgroup showed that especially any loss of a single chromosome (n=109) confers negative prognostic impact (OS 12%±3; ‘poor outcome’). Deletions of chromosome #7 were most common (n=63, OS 13%) but outcome of AML with a single monosomy #-7 did not differ from that with the variable other single autosomal monosomies (n=46, OS 12%). When two or more autosomal chromosomal monosomies were present (n=116), the OS estimate at 4 years was 3%±2 only (‘very poor’). Hyperploidy or loss of sex chromosomes (-X,-Y) did not impact on prognosis. Structural chromosomal abnormalities added prognostic value to the poor outcome only in association with a single autosomal monosomy (OS 4%±2 versus 24%±7; ‘very poor’). These observations resulted in the proposed chromosomal 'monosomy index' for predicting prognosis of AML. The monosomy classification depends merely on the presence of two or more distinct autosomal chromosome monosomies or one single autosomal monosomy in the presence of one or more structural chromosomal abnormalities. In a direct comparison the monosomy score provides better prognostic prediction than a traditionally defined complex karyotype of at least 3 unrelated chromosomal abnormalities. The 'monosomy index' enables (in addition to CN and CBF) the prognostic classification of 2 new cytogenetic aggregates of AML with chromosome abnormalities

that includes an unfavourable-risk 'monosomy index' negative (MI-) group of AML (OS 26%±2) and a very-unfavourable risk category that is 'monosomy index' positive (MI+) (OS: 4%±1).
In addition the abn3q26 (ref 15) and t(6;9)(p23;q34) appear to confer a profound negative effect on prognosis (38).

## 5.6 Molecular diagnostics and genetic markers

Besides cytogenetics, age expresses independent prognostic value. In order to refine the prognostic predictive value of these classifications, additional parameters will need to be introduced into these models. One common prognostic parameter has been the rapidity of attaining a CR. Patients achieving a CR following the first induction cycle of chemotherapy (early CR) have a significantly better outcome than those with a CR attained after induction cycle II (late CR) especially in patients with AML with normal cytogenetics.(7) High white blood cell counts (WBC), when considered in combination with favorable cytogenetics, recognize an unfavorable subset among t(8;21) AML.(8), and also among patients without cytogenetic abnormalities. More recently, various new molecular markers have been identified that allow for dissecting these composite risk categories. For instance, FLT3 internal tandem duplications (FLT3-ITD) have been recognized as a remarkably common genetic abnormality in AML. FLT3-ITD represent activating mutations of the FMS-like tyrosine kinase 3 (FLT3), a hematopoietic receptor. AML with FLT3-ITD are seen in 15-30% of pediatric and adult patients. FLT3-ITD are associated with significantly greater risk of relapse and reduced survival in some studies, while certain other studies with large numbers of patients did not reproduce the prognostic value of FLT3-ITD for survival which makes the established value of FLT3-ITD as a prognostic marker questionable. Interestingly, FLT3 mutations are mainly seen in the largest AML category of intermediate cytogenetic risk. Hence, detection of FLT3-ITDs offers an important possible addition to recognize a new subset of poor risk AML (9,10). Another recurrent Asp835 point mutation of the FLT3 receptor, seen in approximately 5-10% of de novo AML, has not (or not yet) been correlated with prognosis. Nucleophosmin 1 (NPM1) mutations have been identified for the first time in 2004 (11,12,13) . They are prevalent in a high number of approximately 35% of cases of AML and these define a more favourable subset, especially among the subset of FLT3-ITD AMLs (eg 11, 12,13). EVI-1 (ecotropic virus integration site 1) is an oncogene overexpressed in AML with translocations of 3q26 and characterizes a notoriously poor risk AML (14). Recently it was shown that EVI-1 mRNA overexpression in AML in the absence of 3q26 cytogenetic abnormalities also predicts for notably bad prognosis (15). Some of these AML appear to carry cryptic 3q anomalies. Thus EVI-1 appears to define an intracellular pathway of poor therapy response in approximately 10% of cases. C/EBPA (CCAAT enhancer-binding protein alpha) is a transcription factor that has a key role in myelopoiesis. C/EBPA mutations have been found in patients with AML in a few percent of cases. The latter mutations define AML with relatively good risk leukemia (20, 21, 22). Point mutations of the hematopoietic receptor c-*KIT* are seen in 30% of patients with abn(16) AML and t(8;21) AML. AMLs

with abn(16) and t(8;21) represent leukemias of favourable prognosis. The presence of c-*KIT* mutations among this subgroup defines those with an enhanced risk of recurrence (23). The research area of molecular prognostic factors in AML is evolving fastly. Some other potential markers of possible interest (eg ERG, BAALC, WT1) were briefly introduced in paragraph 5.1
Many of these genetic aberrations have been investigated in retrospective analysis so that the selection of cases that has entered these analyses has not been clarified. These markers will now need to be evaluated as regards their prognostic impact in multicenter prospective studies.

## 5.7 Gene expression profiling on DNA microarrays and diagnostics in AML

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

## 5.8 Risk Group Classification in Current Study

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

For further explanation please see below and appendix D

- CN: cytogenetically normal; CA: cytogenetically abnormal; MI-: monosomy index ponegative; MI+: monosomy index positive
- If cytogenetics unknown, consider as CN
- Monosomy Index (MI) refers to AML with two or more autosomal monosomies or a single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities

- CBF refers to the core-binding factor leukemias 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 high EVI1 mRNA expression
- FLT3-ITD-/NMP1+ : Fms-like tyrosine kinase receptor internal tandem duplications (FLT3-ITD) and nucleophosmin-1 (NPM!) 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).
- *To exclude ambiguities in the classification patients should be classified in the following hierarchical order: first patients with CBF abnormalities in GR1, GR2 or IR1, of the remaining patients the MI+ patients in VPR1, followed by the abn3q26 patients in VPR2 subsequently the CEBPA+ patients in GR3 and the FLT3ITD-/NPM1+ patients in GR4, subsequently the EVI1+ patients in VPR3. The remaining patients are classified in IR2, PR1, PR2 and PR3.*

***Good risk (GR)*** .

- *GR1- t(8;21) and WBC≤20* - Cytogenetic abnormality t(8;21) or the *AML1-ETO* fusion gene together with a WBC of 20 x $10^9$/L or less at diagnosis
- *GR2 – inv(16)* - Cytogenetic abnormality inv(16)(p13q22)t(16;16)(p13;q22) or the related fusion genes *CBFB-MYH11*. AML t(8;21) and AML with inv(16)/t(16;16) are collectively designated as core-binding-factor (CBF) leukemias. (Appendix D).
- *GR3 – CEBPA-positive, MI-negative* - Leukemias with mutations in the transcription factor *CEBPA* (CCAAT binding protein alpha), provided they are monosomy index (MI) negative.
- *GR4 - FLT3ITD-/ NPM1+, MI-, and early CR* - Patients with AML who are *FLT3-ITD* mutant negative but NPM1-mutant positive and attain an early CR after cycle I provided they are monosomy index negative

*Note 1*: In the available HOVON/SAKK analysis no significant prognostic effect was apparent among core-binding-factor leukemias as regards the *KIT* mutations that are quite common in these subtypes. Also the assessment of these mutations prospectively in a prospective multicenter trial is difficult to accomplish because of the complicated assay. *KIT* mutations will not be used as a parameter in the risk group assignment.
*Note 2*: The „good-risk" patients (GR) represent approximately 29% of all diagnosed patients and 35% of complete remitters completing 2 cycles of induction therapy. Their estimated over all survival at 5 yrs from diagnosis is on average 65% and from the time of consolidation 76% (see appendix D)

***Intermediate Risk (IR)***

- *IR1- t(8;21) of AML1-ETO fusion gene and WBC> 20* - Patients with AML t(8;21) with a diagnostic white blood cell count of more than 20 x $10^9$/L
- *IR2 - CN or CN-X or CN-Y and WBC≤100 and early CR* - Patients with AML with normal cytogenetics (CN) or those with losses of chromosome X (in females) or chromosome Y (in males) as sole cytogenetic abnormalities and with a diagnostic white blood cell count of 100 x $10^9$/L or less are considered of intermediate risk provided that they attain a complete remission already after induction cycle 1 (CRe).

*Note 1:* The „IR" patients represent approximately 19% of all diagnosed patients and 23% of the complete remitters completing 2 cycles of induction therapy. Their estimated over all survival at 5 yrs from diagnosis is on average 51% and from the time of consolidation is 55%. (see Appendix D)

*Note 2*: The leukemias with normal karyotypes may involve various gene mutations or aberrant gene expression patterns. Patients with normal karyotypes and CEBPA mutations and also those with FLT3 negative/NPM1 positive combined mutations will be considered of good risk (see above). On the other hand patients with normal karyotypes and high EVI1 gene expression will be considered as very poor.

***Poor Risk (PR)***

- *PR1 – CN or CN –X or CN –Y and WBC≤100 but late CR* - AML with normal cytogenetics or those with losses of chromosome X (in females) or chromosome Y (in males) as the sole cytogenetic abnormalities and with a diagnostic white blood cell count of 100 x $10^9$/L or less are considered as poor risk in case they attain a complete remission only after induction cycle 2 (slow responders).
- *PR2 – CN or CN –X or CN –Y and WBC>100* - AML with normal cytogenetics or those with losses of chromosome X (in females) or chromosome Y (in males) as the sole cytogenetic abnormalities and with a diagnostic white blood cell count of more than 100 x $10^9$/L or less are considered as poor risk.
- *PR3 - Cytogenetic abnormalities but no CBF, no abn3q26, no MI+ and no EVI1+* - AML with non CBF cytogenetic abnormalities are classified as poor risk, except when classified in one of the very poor risk categories (see below).

*Note* : The „PR" patients represent approximately 31% of all diagnosed patients and 27% of the complete remitters completing 2 cycles of induction therapy. Their estimated overall survival at 5 years from diagnosis is on average 25% and from the time of consolidation is 33%. (see Appendix D)

***Very Poor Risk (VPR)***

- *VPR1* – *MI+ but non CBF* – non AML with cytogenetic abnormalities fulfilling the criteria of being monosomy index positive. The 'monosomy index' defines AML with a very poor prognosis as leukemia's with karyotypes with at least two autosomal monosomies or one single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities (OS at 4 years 4±1%). Patients with core binding factor leukemia (t(8;21) and inv(16)/t(16;16) are excluded
- *VPR2* – *abn3q26* – non CBF AML with 3q26 chromosomal abnormalities
- *VPR3* – *EVI1+ but CBF AML excluded* - non CBF AML with high *EVI1* gene expression.

*Note 1*: The „VPR" patients represent approximately 20% of all diagnosed patients and 15% of the complete remitters completing 2 cycles of induction therapy. Their estimated overall survival at 5 years from diagnosis is on average 7% and from the time of consolidation is 12%. (see appendix D)

*Note 2*: As patients with secondary AML, i.e., AML evolving from an antecedent hematological disorder (e.g. MDS of at least 6 months duration) or following a history of chemotherapy for a non-leukemic condition, have

often been treated on separate protocols, their prognostic value has not been independently assessed in comparison with other hematological or cytogenetic parameters in multivariate analysis. These patients will therefore be classified according to the same criteria as patients with primary AML.

### 5.9 Assessment of minimal residual disease (MRD)

The term minimal residual disease (MRD) refers to the 'occult' low amount of leukemia that may persist during remission in the absence of clinical or haematological evidence of disease. MRD detection in acute myeloid leukemia (AML) using PCR based techniques for molecular markers is applicable only in a minority of cases. Recently, the level of minimal residual disease (MRD) was established as a prognostic factor that predicts relapse. Immunophenotypical detection of MRD is based upon the presence of leukemia-associated immunophenotypes (so called LAP's,), which are unusual or aberrant immunophenotypes that distinguish leukemic cells from normal hematopoietic cells. LAP's refer to cross-lineage antigen expression (eg the expression of lymphoid markers on myeloid cells), the asynchronic antigen expression (eg the coexpression of early markers with mature myeloid markers), overexpression of antigens (eg relatively high expression levels of particular myeloid or lymphoid markers), and/or ectopic expression (eg the expression of particular antigens that normally are not expressed on hematopoietic cells). The method of detection MRD is quite easy to perform and is sensitive, with a detection ability of 1 malignant cell among 1,000 to 10,000 normal cells, but it requires detailed immunophenotypical knowledge of normal bone marrow cell differentiation. Bone marrow (BM) after different courses of therapy (44-50), stem cell transplants (51) and sequential follow-up bone marrow sampling (52) have been used for MRD assessment.
For this study, BM and blood samples will be obtained from all patients at diagnosis, at the end of the first and second cycles of induction therapy, in complete remission (CR) and at relapse (see Appendix G).

## 6 Study objectives

### 6.1 Primary objectives

For part A of the study:

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

For part B of the study:

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

in a phase III study

### 6.2 Secondary objectives

For part A of the study:

- To evaluate the pharmacokinetics of Laromustine in the combination with cytarabine-idarubicin remission induction chemotherapy in a selection of patients at different dose levels of Laromustine as well as in a limited number of controls
- To investigate the clinical efficacy of Laromustine in combination with remission induction chemotherapy cycles I and II with regard to complete remission rate at different dose levels of Laromustine

For part B of the study:

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

## 7 Study design and rationale

**Part A:** A prospective feasibility study of remission induction chemotherapy combined with Laromustine at a maximum of 3 dose levels (200, 300, 400 mg/m$^2$).

**Part B:** Subsequent to completion of the feasibility study (part A), the value of Laromustine at the selected dose level when combined with standard induction chemotherapy will be investigated in a phase III randomized study.

The choice of the chemotherapeutic regimens of remission induction cycles I and II has been maintained during the successive HOVON/SAKK AML-4, -29 and -42 studies. In each of these trials a new additional agent has been supplemented to the latter two cycles with the objective of improving the efficacy of the induction treatment (eg less relapse, better DFS). The approach that has been pursued, was to embark on an initial feasibility phase when the new agent was introduced in the frontline of treatment and following the evaluation of feasibility part of the study proceed towards the full prospective phase III study. A similar stepwise approach has been pursued in the parallel studies in AML in patients of older age where in the recent study AML-43 two dose levels of daunomycin were compared in induction cycles I and II.
Laromustine is a promising new agent that will be added as a single bolus to standard induction cycles I and II. The drug is currently used at 600 mg/m$^2$ as monotherapy in elderly patients with AML. The launch of this new study will begin with a feasibility part A in which the additive effect of Laromustine will be tested at three selected dose levels in the intermediate and poor and very poor prognostic groups. After final selection of the feasible dose level (200 mg/m$^2$, 300 mg/m$^2$ or 400 mg/m$^2$), the part B of the study (i.e. the phase III of the study) will be opened. This randomized study will directly compare the clinical effects of standard remission induction chemotherapy cycles I and II with the same treatment to which Laromustine is added on day 2 of both cycles.

## 7.1 Part A vs Part B

### 7.1.1 Part A: Dose selection

Although doses up to 600 mg/ m$^2$ of Laromustine have been administered to patients with acute leukemia and found to be feasible, it is unknown whether the combination of Laromustine and induction chemotherapy will lead to other or earlier toxicities. For this reason the drug will now need to be taken to the relevant setting of upfront treatment of newly diagnosed patients. The study will start with cohort 1 of 200 mg/m$^2$. Decisions regarding feasibility and dose escalation to the next cohort, continuation or stopping are based on the incidence of DLT (Dose Limiting Toxicity) and the duration of myelosuppression, and will be performed according to the rules defined in chapter 17. The National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events v 3.0 (CTCAE) will be used to grade toxicities.

Incidences of Dose Limiting Toxicities (DLT) in patients treated on both study arms will be compared. DLT's are defined as follows:

- Death
- Any non hematological toxicity CTCAE grade ≥ 4

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

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

### 7.1.2 Part B: Efficacy

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

## 7.2 Treatment design

### 7.2.1 Remission induction treatment

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

### 7.2.2 Post remission treatment according to risk assessment: the choice of alloSCT, autoSCT or consolidation chemotherapy

All patients will receive 2 cycles for induction and then according their prognostic risk assessment (see section 5.8) proceed to postremission therapy (chemotherapy cycle III, autologous SCT or allogeneic SCT)
- *Good risk* (GR) patients will receive a third cycle of chemotherapy (cycle III: mitoxantrone plus etoposide). In case the alloSCT risk, eg in a patient of young age, is estimated to be very low (≤ 10%), an alloSCT might in exceptional situations be considered in patients with good risk AML, but usually the option of alloSCT will be reserved for rescue in case of relapse.

- *Intermediate risk* (IR) patients with a HLA matched family donor will proceed to allogeneic SCT . If such a transplant is not a practical option (eg due to lack of donor availability or for particular medical reasons) an autologous transplantation is the second choice. If this is not possible, as the second choice patients will receive a third cycle of chemotherapy (cycle III: mitoxantrone plus etoposide). In these patients an unrelated alloSCT might also be considered if the risk of transplant related mortality of such a transplant is assessed to be 25% or less (56,57,58,59). The risk of procedural death might be assessed according a general transplantation risk score that is applied in the transplant centers and that may involve age, comorbidity score, degree of HLA matching, sex match and CMV status pretransplant.
- *Poor risk* (PR) patients with a HLA matched sibling donor, or alternatively with a phenotypically matched unrelated donor may proceed to allogeneic SCT as soon as they have entered CR provided the alloSCT mortality risk is assessed at no more than 35%. If patients are already distinguished as poor risk following cycle I and logistically there are no impediments the patient may proceed to allogeneic SCT as soon as possible after cycle I. If an allogeneic SCT is not possible, an autologous transplant is the second option and a third cycle of chemotherapy is the third option
- *Very poor risk* (VPR) patients with a HLA matched sibling donor, or alternatively with a phenotypically matched unrelated donor or if these options are not available with a cord blood transplant donor or an haploidentical donor may proceed to allogeneic SCT as soon as they have entered CR. If patients are already distinguished as very poor risk following cycle I and logistically it would be possible to immediately proceed to allogeneic SCT at that point, the alloSCT might be planned as soon as possible after cycle I. If an allogeneic SCT is not possible, an autologous transplant is a second and a third cycle of chemotherapy is a third possible option

Patients in CR, including patients who will not go on towards allogeneic stem cell transplantation, will undergo stem cell mobilization with G-CSF and stem cell collection after the second induction cycle. Patients with an adequate harvest who fullfill the eligibility criteria will be proceed to busulfan-cyclophosphamide ablation + autologous PBSCT.
All other patients (considered not eligible for an alloSCT or autoPBSCT) will be offered the chemotherapy cycle III: mitoxantrone and etoposide:

Comment 1: The choice of the preparative regimen prior to alloSCT and the schedule of immunoprophylaxis of graft-versus-host disease will follow the local institutional protocols.

Comment 2: Poor or very poor risk patients in PR after cycle II with a HLA matched family donor or with a phenotypically matched unrelated donor or an available umbilical cord transplant may proceed to allogeneic stem cell transplantation even though they have not attained a complete remission.

Comment 3: It is recommended to start a search for a donor for alloSCT as early as possible.

Comment 4: In case it is decided not to proceed to alloSCT in patients with AML/RAEB of good risk, the alloSCT will be considered as rescue in case of relapse

# 8 Study population

## 8.1 Eligibility criteria

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

### 8.1.1 Inclusion criteria

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

### 8.1.2 Exclusion criteria

- **During part A of the study** patients with a good risk AML, if already known at randomisation. These patients will be treated outside the study according to the control arm.
- Acute promyelocytic leukaemia
- Previous treatment for AML or RAEB, except hydroxyurea
- Impaired hepatic or renal function as defined by:
  - ALT and/or AST > 3 x Upper Limit of Normal (ULN), or
  - Bilirubin > 3 x ULN, or
  - Serum creatinine> 3 x ULN (after adequate hydration), unless these are most likely caused by AML organ infiltration,
- Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, infection, hypertension, pulmonary disease etcetera),

- Cardiac dysfunction as defined by:
  - Myocardial infarction within the last 6 months of study entry, or
  - Reduced left ventricular function with an ejection fraction < 50% as measured by MUGA scan or echocardiogram (another method for measuring cardiac function is acceptable), or
  - Unstable angina, or
  - Unstable cardiac arrhythmias
- Pregnant or lactating females
- Impossibility to stop Disulfiram (Antabuse) and metronidazol (Flagyl) 24 hours prior to study treatment. (Please note that this medication must be stopped 24 hours prior to study treatment.)
- Unwilling or not capable to use effective means of birth control

# 9 Treatment

## 9.1 Dose level of Laromustine

### 9.1.1 Part A

Patients will receive the induction treatment cycles as described in section 9.2 and 9.3. In part A of the study Laromustine is started at a dose level of 200 mg/m$^2$. If escalation is possible according to the decision rules as described in section 17, a dose level of 400 mg/m$^2$ is evaluated. If 400 mg/m$^2$ is not feasible, the intermediate dose level of 300 mg/m$^2$ is evaluated. If 300 mg/m$^2$ is not feasible as well,we return to 200 mg/m$^2$. If 200 mg/m$^2$ is not feasible the trial will be closed.

### 9.1.2 Part B

The Laromustine dose level for part B is selected in part A

## 9.2 Remission induction treatment cycle I

The chemotherapy is the same as used in the previous HOVON/SAKK AML studies.

**Arm A**: Idarubicin and conventional-dose Cytarabine (Ara-C)

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

**Arm B**: Idarubicin and conventional-dose Cytarabine (Ara-C) plus Laromustine.

| **Agent** | **Dose/day** | **Route of administration** | **Days** |
|---|---|---|---|
| Idarubicin | 12 mg/m$^2$ | 3 hr infusion | day 1, 2, 3 |
| Cytarabine (Ara-C) | 200 mg/m$^2$ | 24 hr infusion | day 1 thru 7 |
| Laromustine | assigned dose level from part A | 60 min iv | day 2, start 1 hr after end of idarubicin infusion |

***Cytarabine (Ara-C)*** to be dissolved in 500 mL 0.9% NaCl or 5% glucose (D5W).
***Idarubicin***, in vials of red orange lyophilized powder, containing 5 mg or 10 mg, to be dissolved in 1 ml sterile water per mg Idarubicin.
***Laromustine*** will be administered in 500 mL 5% glucose (D5W) through a freely flowing separate iv line or separate iv port lumen if a central line is in place. The admistration will begin after a 1-hour interval following completion of the idarubicin infusion. It has been noted that occasionally following the administration of Laromustine an infusion-like reaction may appear that resolves spontaneously and that does not evidently seem to improve with corticosteroids or antihistaminics. Laromustine will be withheld in cycle II in case a severe reaction appeared during first treatment.

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

## 9.3 Remission induction treatment cycle II

Cycle II will be started as soon as possible when the marrow still shows more than 15% blasts at day 17-21, and also in case of blasts of less than 15% but with hematopoietic regeneration (platelets above 100 x 109/L; ANC > 1.0 x 109/L). If the hematopoietic regeneration takes more than 56 days, Laromustine will not be given in cycle II in arm B. A bone marrow evaluation should be done before cycle II is started.
The chemotherapy is the same as used in the previous HOVON/SAKK AML studies.

**Arm A**: Amsacrine and intermediate-dose Cytarabine.

| **Agent** | **Dose/day** | **Route of administration** | **Days** |
|---|---|---|---|
| Amsacrine | 120 mg/m$^2$ | 1 hr infusion | day 4, 5, 6 |
| Cytarabine | 1000 mg/m$^2$ q 12 hrs (x12) | 3 hr infusion | day 1 thru 6 |

**Arm B** Amsacrine and intermediate-dose Cytarabine plus Laromustine.

| **Agent** | **Dose/day** | **Route of administration** | **Days** |
|---|---|---|---|
| Amsacrine | 120 mg/m$^2$ | 1 hr infusion | day 4, 5, 6 |
| Cytarabine (Ara-C) | 1000 mg/m$^2$ q 12 hrs (x12) | 3 hr infusion | day 1 thru 6 |
| Laromustine | feasible dose level from part A | 60 min iv | day 2 start 1-hr after end of cytarabine infusion |

***Cytarabine*** (Ara-C) to be dissolved in 500 ml 0.9% NaCl or 5% glucose.
***Amsacrine*** to be dissolved in 500 ml glucose 5% in glass bottles. Contact with plastic syringes or bottles should be avoided.
***Laromustine*** will be dissolved in 500 mL 5% glucose (D5W). The administration will begin after a 1-hour interval following completion of the cytarabine (Ara-C) infusion.
No dose modification should be applied. It has been noted that occasionally following the administration of Laromustine an infusion-like reaction may appear that resolves spontaneously and that does not evidently seem to respond to corticosteroids or antihistaminics.

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

## 9.4 Peripheral blood stem cell mobilization and collection

### 9.4.1 Peripheral blood stem cell mobilization

Granulocyte-colony-stimulating factor (G-CSF; (filgrastim) 5 µg/kg will be given subcutaneously twice daily to all patients after cycle II, i.e., patients being treated in both arms A and B of the study, except in patients who will certainly proceed to HLA matched allogeneic SCT, and except in patients who are already known to be good risk. G-CSF treatment will be started after cycle II chemotherapy at the onset of recovery of granulocytes of 0.5 x 10$^9$/L or more, and continued until the last day of apheresis. Patients not in CR after cycle I should first have a marrow evaluation. G-CSF for mobilization should not be started and may be terminated prematurely when marrow smears taken after cycle II show

clearly persistent leukemia (more than 15% of blasts) or when significant numbers of leukemic blasts appear in the blood. In that instance bone marrow cytology should be examined and leukopheresis for peripheral blood stem cell collection will be cancelled.

### 9.4.2 Procedure of peripheral blood progenitor cell collection

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

### 9.4.3 Procedure for hematopoietic cell cryopreservation

Procedure for hematopoietic cell cryopreservation is according to local procedures

## 9.5 Post induction therapy with chemotherapy cycle III

Patients in continued CR receiving consolidation treatment with cycle III (see section 7.2) will receive this treatment as soon as hematopoietic repopulation (platelets > 100x$10^9$/L and ANC > 1.0x$10^9$/L) has taken place.

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

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

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

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

Patients in continued CR receiving busulfan-cyclophophamide followed by autologous PBSCT (see section 7.2.2) will receive this treatment as soon as hematopoietic repopulation (platelets > 100x$10^9$/L and ANC > 1.0x$10^9$/L) has taken place.

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

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

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

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

***Infusion of stem cells (SCT)***

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

## 9.7 Post-induction treatment: allogeneic SCT

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

## 9.8 Special management orders

- Before treatment a *central venous catheter* may be placed. As a rule, patients will receive parenteral alimentation, when they have insufficient oral caloric intake.
- Extremely careful hand washing by all members of health care team is required

- Reverse barrier nursing of patients and decontamination of the GI tract will be applied according to local protocols in the various centres. *Antimicrobial prophylaxis* will be continued at least until granulocyte counts have increased to a minimum of 0.5 x $10^9$/L.
- *Menstruating premenopausal* females will be started on anovulatory drugs; for instance Orgametril (Lynestrenol) 5 mg, if necessary 10 mg, p.o. q.d. or Deproprovera (Medroxy progesterone acetate) 150 mg, if necessary 300 mg i.m., e.g. q 6 wks.
- All men and pre-menopausal women should use adequate contraception during the study. Sperm should be frozen before the start of treatment from men who wish to have children. All patients will receive two treatment cycles.
- *Hematological supportive care* will involve prophylactic platelet transfusions when counts are below 10x109/L (to prevent hemorrhage) as well as therapeutic transfusions when clinically indicated (30). In case of HLA sensitization, patients will receive HLA compatible platelet transfusions whenever necessary. Filtrated packed red blood cells will be given to keep hematocrit above 30%.
  Irradiation of blood products (25 Gy) must commence from start of cycle II. Otherwise collections of PBPC may contain viable transfused lymphocytes that may cause graft-versus-host disease. After SCT all blood products will be irradiated with 25 Gy.
- Attempts should be made, prior and during chemotherapy, to control any medical problems, such as *metabolic abnormalities and infections*. Electrolytic abnormalities should be controlled. Patients with fever should receive empirical treatment with broad-spectrum antibiotics. They should be adjusted according to the results from the sensitivity studies, whenever a pathogen has been isolated.

## 9.9 Forbidden concomitant medication during the study

Because the Laromustine formulation contains 30% alcohol, patients should discontinue disulfiram (Antabuse) and metronidazole (Flagyl) for at least 24 hours before treatment start. Antabuse and/or Flagyl should not be restarted until 24 hours after the last dose of any Laromustine dose.

## 9.10 Study drug information

### 9.10.1 Physical description of study drug and packaging

Laromustine (supplied by Vion Pharmaceuticals Inc, New Haven, CT) injection is a clear, colorless, slightly viscous, sterile, non-aqueous solution for i.v. administration. It is supplied in 10 mL clear United States Pharmacopoeia (USP) Type I glass vials, with gray butyl rubber stoppers (20 mm) and red aluminum flip-off seal. Each mL contains 10 mg of Laromustine. The ingredients and quantitative composition are listed in Table 9.10.1 (below).

**Table 9.10.1 Composition of VNP40101M (Laromustine) Injection**

| Ingredients | Unit formula | Function |
|---|---|---|
| Active:<br>VNP40101M | 100 mg/vial | Anticancer agent |
| Inactive:<br>Citric Acid, USP<br>Ethyl alcohol, anhydrous, USP | 60 mg<br>3 mL | Stabilizer<br>Vehicle |
| Polyethylene glycol 300, NF | 7 mL | Vehicle |

NF = National Formulary; USP = United States Pharmacopoeia

### 9.10.2 Laromustine labeling

Study drug labels will contain information to meet the applicable regulatory requirements.

### 9.10.3 Laromustine storage and handling

Laromustine should be stored under refrigeration, at 2˚-8˚C (36˚-46˚F), except when being prepared for injection.

Laromustine is a cytotoxic anticancer drug. All necessary precautions while handling potentially toxic compounds must be followed. Gloves and protective clothing must be worn when handling Laromustine. If the solution contacts the skin, it must be washed immediately and thoroughly with soap and water. If the solution comes in contact with mucous membranes, the membranes must be flushed thoroughly with water. Spills should be picked up with absorbent material. The spilled area must be washed at least three times with ethyl alcohol followed by water.

### 9.10.4 Laromustine preparation

Laromustine Injection should be diluted in 500 mL 5% Dextrose Injection (D5W), USP prior to i.v. administration (26, 27). Dilutions up to concentrations of 6 mg/mL may be prepared.

All dilution procedures must be performed using aseptic techniques. Attach a sterile needle to a graduated syringe. Flip off the aluminum seal from the vial. Treat septum with alcohol or other disinfectant, and then insert the syringe needle into the septum. Take up excess volume of Laromustine Injection. Expel air and excess liquid so that the syringe contains the exact volume needed. Transfer the solution into a plastic container containing sterile 5% Dextrose (D5W). Mix the content until homogeneous. The mixture should be clear with no discernible haziness or precipitate. If precipitate occurs, dilution should not be used. If haziness appears or persists after dilution, do not

use the product. Contact your clinical monitor in both instances. Please note, administration of diluted Laromustine should be completed within four (4) hours from the time of preparation; including any infusion interruptions.

The recommended final infusion volume is 500 mL administered over 60 minutes.

Diluted Laromustine Injection should be administered by i.v. infusion via a freely flowing peripheral or central venous line. Alaris® Medical Systems IVAC® System Low Sorbing Infusion Set with 15µm filters are acceptable for use; otherwise the infusion sets should not contain filters (28). Care should be taken to avoid extravasation of Laromustine Injection. No other medication may be administered concurrently via the same venous access site.

## 9.11 Laromustine drug accountability

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

### 9.11.1 Disposition of used Laromustine injections vials

Disposition of Investigational Product vials can only be performed after authorization by a representative of the HOVON Data Center or a Trialogic site monitor. Unused vials will be ordered to be destroyed at the close of the study site, or at study drug expiration. Vials that are to be destroyed may be stored at the site at room temperature, in an access-limited area, until they are verified by appropriate personnel.
Once a representative of the HOVON Data Center, or your Trialogic site monitor has verified accountability, the used and unused vials should be destroyed locally, whenever possible, per institutional standards. If not possible, the vials should be sent back to Almac Clinical Services. Return Instructions have been provided in your site Pharmacy Manual.

A Certificate of Destruction, which includes the drug name, the number of vials destroyed, the lot numbers, and the date and method of destruction, must be kept on file. Original Certificate of Destruction should be sent to Vion Pharmaceuticals, Inc., and a copy of this Certificate should be provided to the HOVON Data Center or your Trialogic site monitor. One additional copy should be maintained in your site.
If institutional guidelines do not allow for storage of used vials, sites may destroy the used vials. If this occurs, sites are to provide the HOVON Data Center or a Trialogic site monitor with a copy of this institutional procedure.

# 10 End of protocol treatment

Reasons for going off protocol treatment are:

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

# 11 Required clinical evaluations

Required investigations at entry should be no older than 14 days prior to randomization unless otherwise noted.
All investigations should be recorded in the patient's medical file .
Only required investigations marked in bold in table paragraph 1.1 will be collected on a CRF.

## 11.1 Required investigations

| | At baseline | Before start cycle 1, 2, 3 and/or SCT | Evaluation after cycle 1, 2, 3 and/or SCT | FU |
|---|---|---|---|---|
| **Medical history** | **X** | | | **X** |
| **Adverse events** | | **X** | **X** | |
| Physical examination | X | X | X | X |

| | | | | |
|---|---|---|---|---|
| **Hematology** | **X** | X | **X[1)2)]** | X[2)] |
| Blood chemistry[3)] | X | X[4)] | X | X[5)] |
| Bone marrow aspirate | | | | |
| **Morphology** | **X** | | X[6)7)] | X[7)] |
| BM immunophenotyping | X | | X[8)] | X[8)] |
| **Cytogenetics** | **X** | | X[8)] | X[8)] |
| Additional blood/marrow sampling for: | | | | |
| Molecular analysis | X | | X[8)] | X[8)] |
| Molecular profiling (Appendix.F ) | X | | X[8)] | |
| MRD assessment (Appendix G) | X | | X[8)] | X[8)] |
| **Bone marrow biopsy** | | | | |
| Histopathology | X | | | |
| **Specific investigations** | | | | |
| Coagulation tests | X | | | |
| **Chest X-ray** | **X** | o.i. | **X[9)]** | o.i. |
| **Pulse Oximetry** | **X** | o.i. | o.i. | o.i. |
| ECG | X | o.i. | o.i. | o.i. |
| Cardiac ejection fraction | o.i | | | |
| Dental examination | o.i | | | |
| Virological tests | X | | | |
| Microbiological tests | X[10)] | | | |
| Plasma storage | X | | | |

o.i. on indication

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

2) Peripheral blood will also be collected until peripheral blood recovery (three times -weekly), and thereafter according to 11.4, to record recovery of ANC and platelets on a CRF.

3) Complete blood chemistry and coagulation test results are to be collected to record as an adverse event on an Adverse Event form, if applicable.

4) - creatinine, sodium, potassium, uric acid, calcium, glucose twice weekly until discharge;
- AST, ALT, alkaline phosphatase, "γGT, bilirubin (direct and indirect), LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly

5) only creatinine, AST, ALT, Alk. Phos, γ-GT, bilirubin
Blood chemistry should be followed up until resolution of adverse events (at least once-monthly)

6) Bone marrow aspirate for response assessment from day 17 weekly during treatment

7) o.i. and if patient in first CR: at 4, 8, 12, 18, 24, 36, 48 months as long as the patients is in CR

8) Blood and bone marrow sampling at day 30 after diagnosis (ie before the beginning of cycle II). Prior to start of cycle III or the allo/auto SCT. In case of CR 3 months after end of treatment. This counts for morphology, immunophenotyping, cytogenetics and molecular analysis (for the latter two only in case of abnormalities) When relapse is suspected .

9) Chest x-ray to be performed and collected on a CRF for all patients in part A at baseline and and at the end of the first induction cycle (prior to start 2nd induction cycle) and at the end of the 2nd induction cycle (prior to off study, cycle III or allo/autoSCT).

10) according to local bacteriology guidelines

## 11.2 Observations prior to start treatment

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

- Medical history, including previous chemotherapy or radiotherapy, antecedent hematological or oncological disease, previous exposure to insecticides, prior and present other diseases, fatigue, bleedings, infections
- Physical examination including body weight, height, splenomegaly, signs of extramedullary leukemia, WHO performance status
- Hematology including hemoglobin, platelets, WBC and WBC differential within 3 days prior to randomization
- Blood chemistry, including serum creatinin, urea, sodium, potassium, uric acid, calcium, glucose, bilirubin, AST, ALT, alkaline phosphatase, gamma GT, LDH within 3 days prior to randomization
- Chest X-ray
- Pulse oximetry
- Cardiac ejection fraction, measured by MUGA or echocardiogram (another method for measuring cardiac function is acceptable) if clinically indicated
- ECG
- Dental examination and X-ortopantogram if clinically indicated
- Surveillance cultures of throat, stools and urine according to local bacteriological guidelines
- Virology including tests (PCR or serology) for cytomegalovirus (CMV) infection, HIV (human immunodeficiency virus), hepatitis A, B and C
- Coagulation studies including protrombin time (PT), partial thromboplastin time (PTT), fibrinogen
- Bone marrow aspirate for:
  - cytology and cytochemistry to establish WHO and FAB subtype of AML or MDS
  - immunological phenotyping to verify myeloid leukemia and assessment of leukemia associated phenotype (see appendix G)
  - cytogenetics (cell culture and banding analysis)
  - molecular analysis for *AML1/ETO, CBFB/MYH11, BCR/ABL, Flt3-ITD, CEBPA* and *NPM1* gene mutations and *EVI1* expression and if possible other available or emerging interesting genetic markers (mutations of *KIT*, *WT1*, *FLT3*-point mutations, gene expression markers *ERG, BAALC* and other potentially relevant markers),
  - whole genome transcriptional profiling (see Appendix F)
- Bone marrow biopsy for histopathology in case of dry tap

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

- Daily interim history and physical examination, when hospitalized; thereafter as clinically indicated: weight at start cycle, infections, medication given.,
- Blood cell count, quantitative platelets daily, and WBC count and differential at least every other day when hospitalized until discharge, thereafter once weekly
- Blood chemistry including creatinin, AST, ALT, bilirubin (direct and indirect), LDH as clinically indicated and at least twice weekly until discharge, thereafter weekly for toxicity assessment
- Chest X-ray as clinically indicated (required for all patients in part A at end of first induction cycle (prior to start 2$^{nd}$ induction cycle) and at end of 2$^{nd}$ induction cycle (prior to off study, cycle III or allo/autoSCT).
- ECG as clinically indicated
- Surveillance cultures according to local bacteriology guidelines
- Bone marrow aspirate for response assessment from day 17 weekly during treatment
  Marrow sampling for minimal residual disease assessments prior to cycle II, prior to cycle III or allo/autoSCT, in autologous transplant, in case of continuing complete response at 3 months after start treatment and at relapse

### 11.3.1 Toxicity assessment

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

- Complete history of symptoms and complaints
- Complete physical examination, with special emphasis on neurological symptoms
- Laboratory examination of hemogram, electrolytes, liver enzymes and kidney parameters twice weekly
- Chest X-ray as clinically indicated
- Electrocardiography when indicated
- DLT assessment after induction cycle I and II. (see 16.2)

Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (see Appendix H)

### 11.3.2 Bone marrow and blood evaluation and response assessment during treatment

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

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

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 E)

## 11.4 Observations during follow up

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

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

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

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

- Bone marrow aspirations for morphology will be done as clinically indicated, but at least at 4 months, 8 months, 12 months, 18 months, 24 months, 36 months and 48 months as long as the patient is in CR.
- Marrow sampling for minimal residual disease assessments prior to cycle II, prior to cycle III or allo/autoSCT, in autologous transplant, in case of continuing complete response at 3 months after start treatment and at relapse

## 11.5 Pharmacokinetic Evaluation

Blood samples as indicated in Table 11.6.1 and 11.6.2. will be collected in 5 patients during cycle I receiving the combination of Laromustine and standard chemotherapy at each of the dose levels of Laromustine in part A of the study and in 15 patients treated at the final dose of Laromustine plus chemotherapy in part A and if necessary part B. For comparison also 10 control patients will be sampled during induction cycle I. Patients receiving the combination of Laromustine and standard chemotherapy will have samples collected as indicated in Table 11.6.1 and 11.6.2, Arm B. Patients receiving standard chemotherapy alone will only have samples collected as indicated in table 11.6.2, Arm A. The samples are collected from the vein in the arm opposite of study drug infusion at the following time points beginning on day 2. Provisions should be made to record and report the actual clock times when samples are drawn.

**Table 11.6.1: Time Points for Laromustine Blood Pharmacokinetic Evaluations**

| Day | Time Point | Number of Samples |
|---|---|---|
| 2 | 30 minutes after the start of the day 2 infusion of Laromustine, 5 -10 min before end of Day 2 infusion, then 5 min, 30 min, 1 hour, and 2 hours after the end of Day 2 infusion. | 6 |

**Table 11.6.2: Time Points for Cytarabine (Ara-C) Blood Pharmacokinetic Evaluations**

| Day | Time Point | Number of Samples |
|---|---|---|
| 2 | **Arm A**<br>22 hours, 46 hours, 70 hours, and 94 hours after the end of Day 2 infusion of idarubicin.<br>**Arm B**<br>20 hours, 44 hours, 68 hours, and 92 hours after the end of Day 2 infusion of Laromustine. | 4*<br><br>4* |

*If any of the sample time points fall on a weekend, these samples may be taken beginning on the following Monday. A total of 4 samples should be drawn after the Day 2 samples have been collected. Each of these modified samples should be drawn approximately 24 hours after the previous sample.

For **Laromustine** blood pharmacokinetic evaluation, at each time point, approximately 5 mL of blood will be collected in a Vacutainer™ tube containing sodium heparin as anticoagulant. Upon collection, mix the blood with the anticoagulant by inverting 4 to 6 times, and then immediately acidify the blood by injecting 0.17 mL of a 1.5 M citric acid solution per 5 mL of blood using a syringe (blood: citric acid = 30:1, v/v) (the amount of citric acid should be reduced accordingly if less than 5 ml of blood is drawn). If it is not possible to add citric acid immediately, samples should be immediately placed on ice, and citric acid must be added within 2 hours from the time of blood collection. After the addition of citric acid, invert the tube 4 to 6 times and immediately place it on ice. Centrifuge within 2 hours after blood collection at 3,000 rpm for 10 – 20 min at room temperature. After centrifugation, transfer and split the plasma fraction into two separate labeled Nunc cryovials. The plasma samples must be immediately frozen and stored at -20°C (±10) until shipment to Vion Pharmaceuticals, Inc. on dry ice for analysis.

For **cytarabine (Ara-C)** blood pharmacokinetic evaluation, remove tetrahydrouridine from freezer approximately 1 hour prior to beginning of pharmacokinetic sampling (this will allow for the solution to sufficiently thaw). At each time point, approximately 5 mL of blood will be collected in a Vacutainer™ tube containing sodium heparin as anticoagulant. Upon collection, mix the blood with the anticoagulant by inverting 4 to 6 times, and then immediately inject 0.25 mL of 0.2% tetrahydrouridine to prevent ex-vivo deamination (blood: tetrahydrouridine = 20:1, v/v) (the amount of tetrahydrouridine should be reduced accordingly if less than 5 ml of blood is drawn). If it is not possible to add tetrahydrouridine immediately after blood collection, samples should be placed on ice, and tetrahydrouridine must be added within 30 minutes from the time of blood collection. Invert tube 4 to 6 times and immediately place it on ice. Centrifuge within 30 min after blood collection at 3,000 rpm for 10 – 20 min at room temperature. After centrifugation, transfer and split the plasma fraction into two separate labeled Nunc cryovials. The plasma samples must be immediately frozen and stored at -20°C (±10) until shipment to Vion Pharmaceuticals, Inc. on dry ice for analysis.

### 11.5.1 Sample Shipment

All plasma samples should be shipped on dry ice to Vion Pharmaceuticals, Inc. for analysis. Samples from several patients can be batched for shipment, but should be shipped within 3-4 months. The shipping address is:

Bio-analytical Laboratory
Vion Pharmaceuticals, Inc.

Four Science Park
New Haven, CT 06511

Email intent to ship and list of samples to Ala Nassar at anassar@vionpharm.com.
*Note: Please do not ship samples on Thursdays, Fridays, and weekends.*

A detailed protocol with regard to shipment of samples will be provided to the participating centers.

### 11.5.2 Sample Analysis

Plasma samples will be analyzed for Laromustine and cytarabine (Ara-C) using validated analytical methods. Analysis of their metabolites may also be included. Analysis will be conducted at the Analytical Laboratory of Vion Pharmaceuticals, Inc. or designated contract research organizations.

#### 11.5.2.1 PK Analyses

PK models and equations will be used to define the plasma disposition characteristics of Laromustine and if possible its metabolites. The following PK parameters will be computed: area under the plasma concentration-time curve (AUC), peak level ($C_{max}$), distribution half-life ($t_{1/2,\alpha}$), elimination half-life ($t_{1/2,\beta}$), volume of distribution at steady state ($V_{d,ss}$), total body clearance ($Cl_{tot}$), and other relevant parameters, if necessary. Steady-state concentrations of cytarabine (Ara-C) will be determined.

## 12 Toxicities

Laromustine

Laromustine is an investigational drug, and a detailed list of toxicities reported to date can be found in the Investigator Brochure. In addition to pancytopenia and related complications, the most common toxicity is a transient, self-limited syndrome that occurs during or shortly after the infusion of Laromustine in approximately one-third of patients. This syndrome can include hypotension, facial flushing, nausea and vomiting, dizziness or syncope, headache, leg cramps, and tachycardia. Other side effects, which are commonly grade 1-2, include fatigue, rash, diarrhea, gastro-enteritis, non-infectious pulmonary dysfunction (dyspnea, pleural effusion), elevation of hepatic enzymes, electrolyte imbalances, and neurologic toxicity (confusion, weakness). Rare (<5% patients) grade 3-4 non-hematologic toxicity include: seizure, acute respiratory distress syndrome, pneumonitis, angina, myocardial infarction, congestive heart failure, tumor lysis syndrome, and pericardial effusion.

Idarubicine

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

Cytarabine (Ara-C)

*Conventional-dose: 200 mg/m$^2$*: anorexia, nausea, vomiting, hepatic dysfunction, skin rash, pneumonitis, fever.

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

Amsacrine (AMSA)

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

Mitoxantrone

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

VP-16 (Etoposide)

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

Busulfan

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

Cyclophosphamide

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

Following conditioning and autologous PBSCT

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

G-CSF (granulocyte-colony stimulating factor)

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

# 13 Reporting serious adverse events and SUSARS

## 13.1 Definitions

**Adverse event (AE)**

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

**Adverse reaction (AR)**

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

**Serious adverse event (SAE)**

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

- death
- a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- hospitalization or prolongation of hospitalization

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

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

**Unexpected SAE**

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

**Suspected unexpected serious adverse reaction (SUSAR)**

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

## 13.2 Reporting of (serious) adverse events

**Adverse event**

All AEs of CTCAE grade 2 or higher, with the exception of alopecia, nausea/vomiting and progression of the disease under study, have to be reported on the Adverse Events CRF.
Adverse events will be reported from the first study-related procedure until 30 days following the last protocol treatment or until the start of subsequent systemic therapy for the disease under study, if earlier.
Adverse events occurring after 30 days should also be reported if considered related to study drug.
Grade 3 or 4 adverse events considered related to study drug must be followed until recovery or until 6 months after the last protocol treatment, whichever comes first.
All other adverse events must be followed until recovery or until 30 days after the last protocol treatment, whichever comes first.

**Serious Adverse Events**

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

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

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

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

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

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

### 13.3 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the prinicipal investigator, the study central datamanager and to the safety desk. The safety desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR).
Any suspected unexpected serious adverse reactions (SUSARs) arising from this trial will be reported expedited by HOVON to the investigators, to VION, and to all applicable Ethics Committees and Health Authorities within the timelines required by the EU Clinical Trial Directive.

## 14 Endpoints

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

#### 14.1.1 Primary endpoint

The assessment of DLT and duration of myelosuppression of the combination of Laromustine at three selected dose levels.

DLT is defined as

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

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

The duration of myelosuppression is defined as the median time to recovery of ANC > $0.5*10^9$/l.

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

### 14.1.2 Secondary endpoint

- The evaluation of Laromustine and cytarabine pharmacokinetics.
- Response and especially CR to chemotherapy cycles I and II

## 14.2 Part B: of the study (Efficacy)

### 14.2.1 Primary endpoint

Event-free survival (EFS) in relation to the induction treatment arms with and without Laromustine (i.e., time from registration to induction failure, death or relapse whichever occurs first).

### 14.2.2 Secondary endpoints

- EFS in the distinct prognostic subsets (AML good-risk vs AML intermediate-risk vs AML poor-risk) and cytogenetically and molecularly defined subgroups.
- Response and especially CR to chemotherapy cycles I and II
- Overall survival (OS) measured from the time of registration
- Disease-free interval (duration of the first CR) measured from the time of achievement of CR to day of relapse or death from any cause (whichever occurs first).
- Outcome of induction treatments in relation to minimal residual disease measurements
- Evaluation of Laromustine and cytarabine (Ara-C) pharmacokinetics
- Evaluation of the effect of Laromustine on peripheral CD34 cell numbers collected for autologous peripheral blood transplantation
- Evaluation of molecular prognostic markers and gene expression profiles for outcome in relation to induction and postinduction treatments
- Evaluation of toxicities and treatment related mortality (according to Appendix H)
- 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.

# 15 Registration and Randomization

## 15.1 Regulatory Documentation

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

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

By the local investigator for each investigational site:

- HDC Hospital Registration Form, signed and dated by the local investigator;
- a copy of the dated and signed (central) Ethical Committee approval of the protocol, any amendments and informed consent form for the investigational site. This approval must clearly identify the specific protocol by title, number and version date and must be signed by the chairman or authorized designee. The approval must also clearly identify the site(s) the approval applies to;
- a copy of the approved local version of the Patient Information and Informed Consent form;
- approval of participation by site's Board of Directors, if required by local regulations;
- CV of local investigator (dated and signed if not recently provided);
- signed local investigator signature page of the protocol
- site personnel signature log;
- local lab accreditation and list of local lab normal values (if not recently provided);
- any other documentation required by local regulations.

## 15.2 Randomization

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

The following information will be requested at randomization:
Protocol number
Institution name

Name of caller/responsible investigator
Patient's initials or code
Sex
Date of birth
Date of diagnosis of AML or RAEB
Date written informed consent
Eligibility criteria

All eligibility criteria will be checked with a checklist.
Patients will be randomized, stratified by center and disease (AML vs RAEB) with a minimization procedure, ensuring balance within each stratum and overall balance
Each patient will be given a unique patient study number. Patient study number and result of randomization will be given immediately by TOP or phone and confirmed by fax or email.

# 16 Data collection

## 16.1 CRF's

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

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

Each CRF page will be identified by a pre-printed trial number, and a unique combination of patient study number (assigned at registration), hospital and patient name code (as documented at registration) to be filled out before completing the form.
The CRF will be completed on site by the local investigator or an authorized staff member. Each page must be dated and signed by the local investigator upon completion. All CRF entries must be based on source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.

Copies of the CRF will be kept on site. The original CRF pages must be sent to the HOVON Data Center at the requested time points. How and when to send in forms is described in detail in the CRF header and the CRF instructions.
All data from the CRF will be entered into the study database by the HOVON Data Center.

## 16.2 Reporting DLT information

To monitor the incidence of dose limiting toxicity (DLT) and myelosuppression duration a separate CRF (DLT-form) will be used. This DLT-form must be filled out for every patient, independent of randomization result. The form should be dated, signed by the responsible investigator and returned to the HOVON Data Center by fax within 24 hours after DLT-occurrence, or weekly after start of cycle I and II if no DLT occurred, until day 30. Duration of myelosuppression must be reported until ANC recovery or until start next treatment (if not yet recovered). Investigators will weekly receive a reminder for sending in a new DLT form.

## 16.3 Monitoring

The sponsor will perform on-site monitoring visits to verify that the rights and well-being of subjects are protected, the reported trial data are accurate, complete, and verifiable from source documents and the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement(s).
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 monitoring 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 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 and associated personnel before the study, and monitoring visits by the sponsor. CRF completion guidelines will be provided. The data will be entered into the clinical study database and verified for accuracy.

## 16.5 On-site audits

The local investigator/institution 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. Subject privacy must, however, be respected.
Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study in support of a regulatory submission. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

### 16.6 End of trial

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

## 17 Statistical considerations

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

### 17.1 Part A: Dose level selection

In this study, a maximum of three dose levels of Laromustine will be considered. The study starts at a dose level of 200 mg/m$^2$, and if possible escalating to 400 mg/m$^2$. If 400 mg/m$^2$ is not feasible we return to the intermediate dose level of 300 mg/m$^2$, and we return to 200 mg/m$^2$ if 300 mg/m$^2$ is not feasible as well. At each dose level the decision to stop or escalate will be made on the basis of (a) the incidence of Dose Limiting Toxicities (DLTs) in the arm treated with Laromustine versus the incidence of DLTs in the control arm and (b) the duration of myelosuppression in the Laromustine arm compared to the control arm.

DLT is defined as

- Death
- Any non hematological toxicity CTCAE grade ≥ 4

occurring within 30 days after start of cycles I or II and before the start of the next cycle or a new treatment respectively.
The duration of myelosuppression is defined as the median time to recovery of ANC > 0.5x10$^9$/L.

Applying the criteria to the patients treated in the HOVON/SAKK-29 and 42 studies with standard dose chemotherapy, who are comparable to the patients in the control group of this trial we find:

- 20% of the patients experience DLT in cycle I
- 5% of the patients stop after cycle I with no DLT
- 14% of the patients experience DLT in cycle II
- So, overall 31% of the patients experience DLT
- the median time to recovery of ANC >0.5x$10^9$/L is 29 days following cycle I and cycle II

In the decision rules the number of DLTs in both arms and the number of days at risk for DLT are taken into account. Each patient is at risk for a maximum of 2*30= 60 days. If no early decision to stop or escalate can be made, on the moment the maximum number of 15 patients in the Laromustine arm have completed induction therapy, the active dose level is chosen as the dose level to continue Part B of the trial.
DLTs have to be reported within 24 hours and investigators weekly receive a questionnaire for patients who are still at risk. The decision rules will be checked once a week, and the trial will be closely monitored in the mean times.

The precise decision rules are presented in the table below.

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

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

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

For each row, all conditions (if given) in the first four columns should be present to take a decision to stop (and return to the previous dose level if possible) or escalate.

A patient has completed induction therapy if the patient (a) is known not to have experienced DLT 30 days after start of cycle II, (b) is known not to have experienced DLT 30 days after start of cycle I and known not receive cycle II, or (c) has experienced DLT.

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

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

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

- 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 that ANC >$0.5x10^9$/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 conventional arm with respect to the standard arm. A hazard ratio >1 implies a faster recovery in the conventional arm.

These decision rules lead to the following characteristics where we assume that the true probability of DLT in the control arm is 31% and the median recovery time for ANC > $0.5x10^9$/L is 29 days as expected; an increase of 20% in the Laromustine arm corresponds to a median recovery time of 35 days. An expected accrual of 150 patients a year (12.5/month) is used in the calculations.

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

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

If a decision to stop is implied by the decision rules we return to a lower dose level until a decision is made by the DSMB, if not possible the trial is put on hold. Until the DSMB has confirmed a decision to escalate, the trial remains open at the active dose level.

The decision rules serve as guidelines for the DSMB (see section 17.2.2). As soon as a decision can be made according to the above defined rules, a report will be generated and sent to the DSMB for final recommendations. The report contains a tabulation of the number of patients recruited, the number of evaluable patients, the number of DLTs, a specification of the DLTs and their outcome, the ANC>$0.5x10^9$/L recovery results, and the incidence and intensity of the other reported AEs, split by arm, treatment cycle and dose level.

## 17.2 Part B: Efficacy

### 17.2.1 Patient numbers and power considerations

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

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

The target number of patients for this part of the study is 800 to be accrued in approximately 4 years. After entry of the last patient an additional follow up of 1 year is planned before the first final analysis. The target number of 800 patients will give a power of 87% with 509 events with a two-sided test at 5% significance level to detect an improvement in EFS with hazard ratio HR=0.76, which corresponds to an increase of EFS at 3 years from 31% to 41%.

### 17.2.2 Interim analyses and safety monitoring

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

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

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

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

### 17.2.3 Efficacy analysis

Induction randomization

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

Evaluation of Allo SCT

The outcome of patients treated with Allo SCT will be determined by calculation of the probabilities of relapse and death in first CR after Allo SCT as competing risk and the survival probability. Estimates will be made separately by type of transplant (HLA identical sibling or MUD or umbilical cord blood or haplo-identical SCT), by age group, by diagnosis (AML versus MDS) and by risk group and molecularly defined subtypes depending on available numbers of subgroups. The results will be pooled with the data of similar patients in the previous AML studies.
The outcome of the poor risk patients treated with Allo SCT in PR will be determined by calculation of the probabilities of reaching CR, relapse and death after Allo SCT.
Since there is no randomization between Allo SCT and other consolidation treatment, the effect of Allo SCT cannot be estimated in a proper unbiased way. As an approximation an analysis will be done based on donor availability. Data will be collected for each patient (below the age of 55) in CR after cycle II with intermediate and poor risk concerning the availability of an HLA identical sibling donor or a matched unrelated donor.
A comparison will be made of the outcomes of the patients by donor availability, irrespective of the actual treatment in first CR. These data will also be submitted to the data of the AML Collaborative Group for a meta-analysis.

### 17.2.4 Toxicity analysis

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

### 17.2.5 Additional analysis

Additional analyses involve the analysis of prognostic factors, especially age, cytogenetic abnormalities and risk group with respect to CR rate, EFS, OS and DFS. Logistic and Cox regression analysis will be used for this purpose.

## 17.3 Data Safety Monitoring Board

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

# 18 Ethics

## 18.1 Accredited ethics committee or Institutional review board

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

## 18.2 Ethical conduct of the study

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

### 18.3 Patient information and consent

Written Informed Consent of patients is required before registration. The procedure, the risks and the therapy options will be explained to the patient.

## 19 Trial insurance

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

## 20 Publication policy

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

Authors of the manuscript will include the study coordinator(s), investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion), the statistician(s) and the HDC datamanager in charge of the trial, and others who have made significant scientific contributions.

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

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

# 21 Glossary of abbreviations

(in alphabetical order)

| | |
|---|---|
| AE | Adverse Event |
| ADL | Activities of daily living |
| AGT | O6 Alkylguanine alkyl transferase |
| ALT | Alanine Amino Transferase |
| AML | Acute Myelogenous Leukemia |
| ANC | Absolute Neutrophil Count |
| Ara-C | Cytarabine, cytosine arabinoside |
| ASH | The American Society of Hematology |
| AST | Aspartate Amino Transferase |
| BM | Bone Marrow |
| BMT | Bone Marrow Transplant |
| Ca | Calcium |
| CFC | Colony Forming Cells |
| CI | Continuous Infusion |
| Cl | Chloride |
| CMV | Cytomegalovirus |
| CNS | Central nervous system |
| CO2 | Carbondioxide |
| CR | Complete Remission |
| CRe | Complete Remission Early (after induction cycle 1) |
| CRi | Complete Remission with incomplete blood count recovery |
| CRp | Complete Remission without platelet recovery |
| CRF | Case Report Form |
| CT | Computerized Tomography |
| CTC | Common Terminology Criteria |
| CTCAE | Common Terminology Criteria for Adverse Events |
| DFS | Disease free Survival |
| DNR | Daunorubicin |
| DSMB | Data and Safety Monitoring Board |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| EFS | Event Free Survival |
| FAB | French American British (cytological classification) |

| | |
|---|---|
| GCP | Good Clinical Practice |
| GEP | Gene Expression Profiling |
| G-CSF | Granulocyte-Colony Stimulating Factor |
| GI | Gastro-intestinal |
| Hb | Hemoglobin |
| Ht | Hematocrit |
| HOVON | Dutch/Belgian Hematology-Oncology Cooperative Group |
| HIV | Human Immunodeficiency Virus |
| ILLN | Institutional Lower Limit of Normal |
| IPSS | International Prognostic Score System (for myelodysplastic syndromes) |
| IRB | Institutional Review Board |
| ITT | Intention to Treat |
| (I)ULN | (Institutional) Upper Limit of Normal |
| IV | Intravenous |
| K | Potassium |
| LD50 | Lethal Dose 50% |
| LDH | Lactate Dehydrogenase |
| MCV | Mean corpuscular volume |
| MCH | Mean corpuscular hemoglobin |
| MCHC | Mean corpuscular hemoglobin concentration |
| MDR | Multi Drug Resistance |
| MDR-1 | Multi Drug Resistance-1 gene |
| MDS | Myelodysplastic Syndrome |
| MTD | Maximum tolerated dose |
| MUD | Matched unrelated donor |
| MUGA | Multiple Gated Acquisition |
| Na | Sodium |
| OS | Overall Survival |
| PB | Peripheral Blood |
| PBPC | Peripheral Blood Progenitor Cells |
| PBR | Peripheral Blood Recovery |
| PK | Pharmacokinetics |
| PR | Partial Response |
| RAEB | Refractory Anemia with Excess of Blasts |
| SAE | Serious Adverse Event |
| SCT | Stem cell transplantation |

| | |
|---|---|
| WBC | White Blood Count |
| WHO | World Health Organization |

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## A1 WHO classification for acute myeloid leukemias

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

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

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

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

## A2 WHO classification for myelodysplastic syndromes

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

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

## A3 FAB classification of AML

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

| FAB subtype | |
|---|---|
| | For all AML subtypes the following criteria apply:<br>♦ Blasts ≥ 30% of bone marrow nucleated cells, except for M3<br>♦ ≥ 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<br>♦ at least one of the following myeloid markers present: CD13,CD33, CD15, CDw65<br>♦ 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)<br>♦ Maturing granulocytic cells (i.e. promyelocytes towards polymorphonuclear cells ≤ 10% of nonerythroid cells<br>♦ (pro)monocytes ≤ 10% of nonerythroid marrow cells |
| M2 | ♦ Blasts 30-89% of bone marrow nonerythroid cells<br>♦ Maturing granulocytic cells (i.e. promyelocytes to polymorphonuclear cells) > 10% of nonerythroid cells<br>♦ 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<br>• Monocytic cells (monoblasts to monocytes) ≥ 20% of nonerythroid cells<br>Or<br>• Peripheral blood monocytes ≥ 5 x $10^9$/l<br>Or<br>• 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<br>♦ Bone marrow monocytic component ≥ 80% of nonerythroid cells<br>♦ Monoblasts ≥ 80% of bone marrow monocytic component |
| M5B | ♦ Blasts ≥ 30% of bone marrow nonerythroid cells<br>♦ Bone marrow monocytic component ≥ 80% of nonerythroid cells<br>♦ Monoblasts < 80% of bone marrow monocytic component |
| M6 | ♦ Erythroblasts ≥ 50% of bone marrow nucleated cells<br>♦ Blasts ≥ 30% of bone marrow nonerythroid cells |
| M7 | ♦ > 30% of bone marrow nucleated cells are megakaryoblasts CD41 or CD61 positive or<br>♦ Platelet specific peroxidase reaction (electron microspcopy)<br>♦ < 3% of blasts positive for Sudan Black B or Myeloperoxidase |

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

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

Low : 0

Int-1 : 0.5-1.0

Int-2 : 1.5-2.0

High : ≥ 2.5

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

*Karyotype

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

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

B : all other (or not done)

**Cytopenias

Hb < 6.2 mmol/L

ANC < $1.5 \times 10^9$/L

Platelets < $100 \times 10^9$/L

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

# C Response criteria for AML and RAEB

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

## 1. Disease status and response criteria

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

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

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

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

### 1.1.a Complete hematological remission (CR):

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

(The presence of <5% blasts in the peripheral blood does not argue against and is compatible with a complete remission).

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

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

### 1.1.c Cytogenetic remission

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

### 1.2. Partial remission (PR)

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

## 2. Treatment failure

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

### 2.1. Resistant disease

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

### 2.2. Aplastic death

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

.

### 2.3. Indeterminate cause:

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

## 3. Relapse Criteria

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

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

## D Risk group definition

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

| Risk | | Definition | % pts at baseline | % pts with CR & consolidation |
|---|---|---|---|---|
| Good | GR1 | t(8;21) or *AML1-ETO*, WBC≤20 | 5 % | 7 % |
| | GR2 | inv(16)/t(16;16) or *CBFB-MYH11* gene | 6 % | 7 % |
| | GR3 | MI-, *CEBPA+* | 7 % | 8 % |
| | GR4 | MI-, *FLT3ITD-/NMP1+,* CRe | 11 % | 13 % |
| Intermediate | IR1 | t(8;21) or *AML1-ETO*, WBC>20 | 2 % | 2 % |
| | IR2 | CN –X –Y, WBC≤100, CRe | 17 % | 21 % |
| Poor | PR1 | CN –X –Y, WBC≤100, not CRe | 10 % | 8 % |
| | PR2 | CN –X –Y, WBC>100 | 5 % | 4 % |
| | PR3 | CA, non CBF, MI-, no abn3q26, EVI1- | 16 % | 15 % |
| Very Poor | VPR1 | Non CBF, MI+ | 9 % | 5 % |
| | VPR2 | Non CBF, abn3q26 | 2 % | 1 % |
| | VPR3 | Non CBF, EVI1+ | 9 % | 9 % |

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

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

The above risk classification is based on

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

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

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

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

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

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

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

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

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

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

| Risk | | | From diagnosis | | | From start consolidation | |
|---|---|---|---|---|---|---|---|
| | | | CR1 | EFS1 | OS1 | EFS2 | OS2 |
| Good | | | **94*** | **51** | **65** | **58** | **76** |
| | GR1 | t(8;21), WBC≤20 | 94 | 59 | 68 | 66 | 75 |
| | GR2 | inv(16)/t(16;16) | 93 | 44 | 68 | 50 | 77 |
| | GR3 | MI-, *CEBPA*+ | 84 | 48 | 61 | 59 | 67 |
| | GR4 | MI-, *FLT3ITD-/NMP1*+, CRe | 100* | 51 | 57 | 59 | 61 |
| Inter-mediate | | | **99*** | **42** | **51** | **48** | **55** |
| | IR1 | t(8;21), WBC>20 | 87 | 32 | 46 | 35 | 50 |
| | IR2 | CN –X –Y, WBC≤100, CRe | 100* | 43 | 51 | 48 | 55 |
| Poor | | | **75*** | **19** | **25** | **27** | **33** |
| | PR1 | CN –X –Y, WBC≤100, not CRe | 69* | 17 | 23 | 24 | 31 |
| | PR2 | CN –X –Y, WBC>100 | 74* | 23 | 27 | 32 | 37 |
| | PR3 | CA, non CBF,MI-,no abn3q26, *EVI1*- | 79 | 20 | 25 | 27 | 33 |
| Very Poor | | | **60** | **3** | **7** | **7** | **12** |
| | VPR1 | Non CBF, MI+ | 48 | 2 | 4 | 6 | 9 |
| | VPR2 | Non CBF, abn3q26 | 65 | 8 | 19 | 8 | 12 |
| | VPR3 | Non CBF, *EVI1*+ | 79 | 10 | 17 | 10 | 16 |

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

CR1 % patients reaching CR after cycle I or cycle II

EFS1 actuarial probability of event free survival 5 year from diagnosis

OS1 actuarial probability of overall survival 5 year from diagnosis

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

OS2 actuarial probability of overall survival 5 year from start consolidation

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

## E Cytogenetic and molecular analysis

**Conventional cytogenetic analysis** should be performed in all patients at diagnosis. For selected genetic abnormalities the use of molecular techniques will be required (see below).
In general, the results of the molecular/cytogenetic analysis should be known at approximately 2-3 weeks after diagnosis. This will permit the risk assessment.

**Additional FISH analysis** is recommended for the detection of abnormalities which involve 11q23 (*MLL*) or 3q26 (EVI1). RT-PCR is recommended at diagnosis for the detection of t(9;22) or *BCR/ABL*, t(8;21)(q22;q22) or *AML1/ETO*; inv(16)(p13;q26)/t(16;16(p13;q26) or *CBFB/MYH11* fusion transcripts with a preferred sensitivity (positive cells diluted in negative cells) of at least 1/102. RT-PCR for detection of t(6;9)(p23;q34) or *DEK/CAN* is recommended when available.
For diagnosis of additional abnormalities of chromosome 11q23 (such as MLL self fusions) a Southern Blot and additional PCR is required. As this will be retrospectively analyzed in this study, preparations will be made for the possibility for sending samples for central analysis.

**Molecular analysis**
A variety of molecular markers will be analysed including internal tandem repeat mutations as well as D835 mutations of the receptor of Fms-like tyrosine kinase *FLT3* (*FLT3-ITD* and *FLT3-D835*), *KIT* mutations, *NPM1* mutations, *CEBPA* mutations, *WT1* mutations, high *EVI-1* expression. Also gene expression of MGMT (O6 alkyl-guanine alkyl transferase) will be quantified by real-time PCR in up to 200 patients of the treatment arm with the study drug of part B of the study
AML blasts at diagnosis and remission marrow will be analyzed.

**Follow-up samples**
In patients with known cytogenetic and molecular markers peripheral blood and bone marrow samples will be collected for molecular analysis during follow-up in case a complete remission is obtained.

**Timing of bone marrow + blood sampling for molecular analysis:**
- At diagnosis
- Day 30 after diagnosis according to the clinical protocol (ie before the beginning of cycle II)
- Prior to the start of cycle III or the allo/autoSCT
- In cases of CR, 3 months after start of treatment.
- Every four months after completion of treatment during the first year
- Every six months during the second year.
- At relapse (to recognize eventually occurring phenotypic or genotypic shifts)

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For the HOVON centers diagnostic and follow-up samples should be sent to the local laboratory. If necessary this local lab will forward the samples to a central laboratory as agreed within the Network for Molecular Diagnostics of Hematologic Malignancies (MODHEM; www.modhem.nl) or to the laboratory in Rotterdam.

The samples for the gene expression profiling study will be transferred to the laboratory in Rotterdam (Dr P. Valk, Molecular Diagnostic lab, Dept of Hematology, ErasmusMC, Rotterdam)

A detailed protocol with regard to shipment of samples will be provided to the participating centers.

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## F Gene expression profiling

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

Whole genome transcriptional profiling with Affymetrix HGU Plus2.0 GeneChips (will be carried out to establish the expression level of over 47,000 transcripts and variants, representing 19.000 unique genes. The aim of this exploratory analysis is to further develop a molecular classification of AML, validate prognostic signatures identified in previous studies and identification of novel candidate markers that predict patient response to treatment. These genes will include *EVI1, WT1, BAALC, ERG*.
In addition, genes involved in DNA repair will be analysed as they might predict response to VNP401101M (Laromustine).These genes will include MGMT(=AG), APE, BER, ERCC1, ERCC2(XPD), XRCC1, XRCC3, AGT, TDG, ERCC3, ERCC4, FEN1, PCNA, LIG1, LIG3
The samples will be taken before start of treatment (bone marrow and peripheral blood). This approach will enable us to determine gene expression profiles in AML blasts at diagnosis and at relapse.

Bone marrow (10 mL) and peripheral blood (20 mL) samples for whole genome transcriptional profiling should be collected at entry.

A detailed protocol with regard to **shipment of samples** will be provided to the participating centers.

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## G Minimal residual disease assessment

**Introduction**

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

You can find background information regarding immunophenotypic MRD and detailed procedures on the website www.hematologie.nl/mrd and through MRD.info@vumc.nl

**Methods***:* as defined in detail in reference 5. Ideally 20 mL of bone marrow aspirate syringed in heparin coated tubes should be taken. Details concerning logistics will be distributed separately.

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

A detailed protocol with regard to **shipment of samples** will be provided to the participating centers.

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## H Common Terminology Criteria for Adverse Events

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

http://ctep.cancer.gov
http://www.hovon.nl (under Trial > General information about study)

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

# I ZUBROD-ECOG-Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

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

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

4 Unable to get out of bed