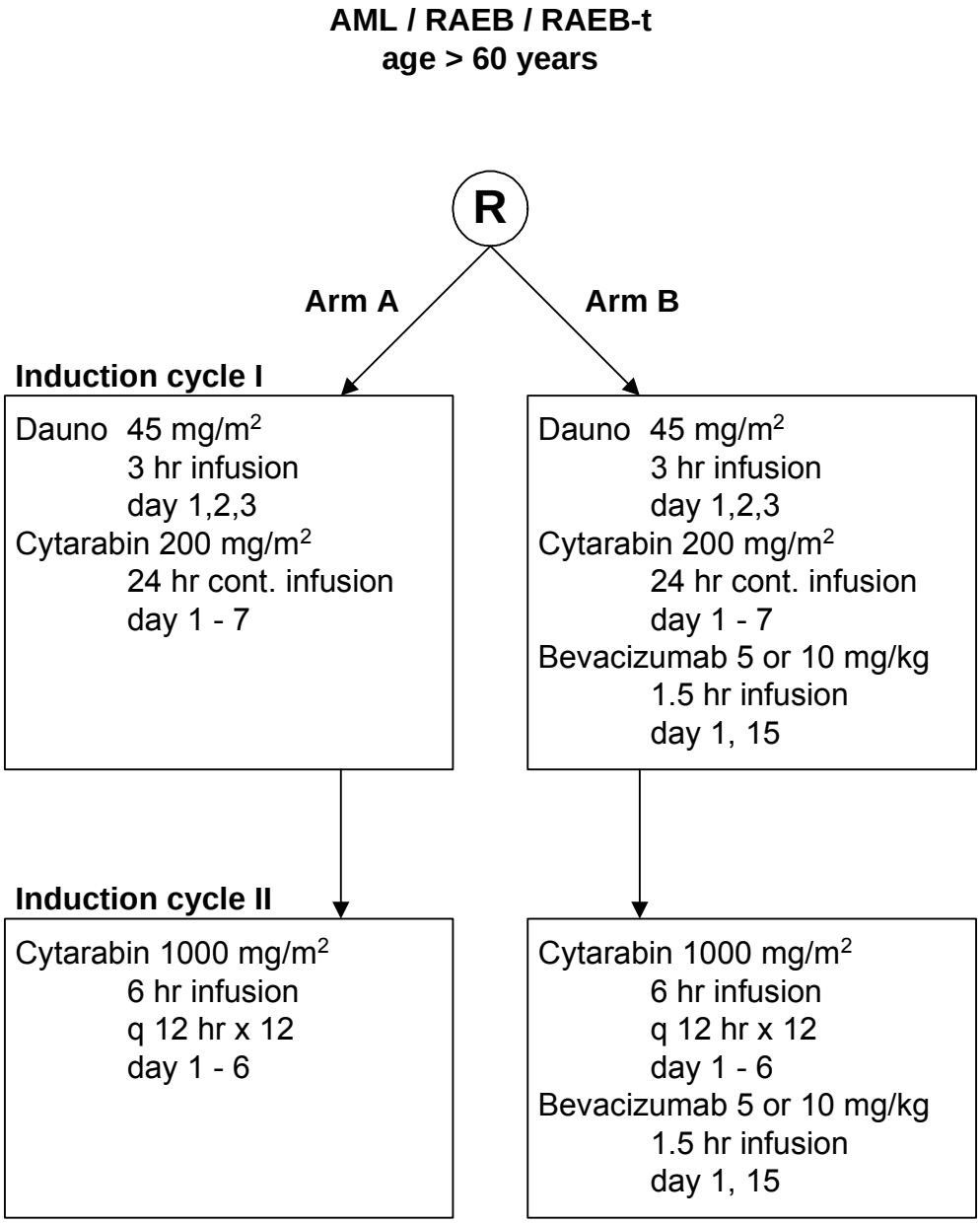
A Phase II multicenter study to assess the tolerability and efficacy of the addition of Bevacizumab to standard induction therapy in AML and high risk MDS above 60 years.

A randomized phase II study

PROTOCOL

Study Coordinators	:	G.J. Ossenkoppele B. Löwenberg
Statistician	:	W.J.L. van Putten
Datamanager	:	C.M.C. van Hooije
Registration	:	HOVON Data Center Erasmus MC – Daniel den Hoed P.O. Box 5201 3008 AE ROTTERDAM The Netherlands Tel. +31.10.4391568 Fax +31.10.4391028 https://www.hdc.hovon.nl/top
EudraCT number	:	2006-001777-19
First version	:	February 20, 2006
Final version	:	July 20, 2006
Date of activation	:	
Approved	:	CKTO 2006-10, July 12, 2006 METC VUMC 2006/215, December 7, 2006

1 Scheme of study



2 Table of contents

3 Synopsis

Study phase	Phase II
Study objectives	Evaluation of the safety and tolerability of Bevacizumab added to standard induction chemotherapy. Evaluation of the effect of Bevacizumab on the CR rate
Patient population	Patients with AML (except FAB M3), RAEB or RAEB-t with IPSS ≥ 1.5, previously untreated, age > 60 yrs.
Study design	Prospective, multicenter, open-label, with randomization between standard induction chemotherapy with or without Bevacizumab. The initial Bevacizumab dose is 5 mg/kg i.v. on day 1+15 of each cycle. Decisions regarding dose escalation to 10 mg/kg, continuation with dose level 5 mg/kg, or stopping, are based on the incidence of DLT (dose limiting toxicity: death within 30 days of start cycle I and before start cycle II)
Duration of treatment	Expected duration of 2 cycles of induction chemotherapy with of without Bevacizumab including evaluation is about 3 months.
Number of patients	200 patients registered and randomized
Planned start of recruitment	Start of recruitment: III 2006
Planned end of recruitment	End of recruitment: II 2008

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Study Coordinators	G.J. Ossenkoppele	VU University Medical Center, Amsterdam
	B. Löwenberg	Erasmus MC, Rotterdam
Writing Committee	G.J. Ossenkoppele	VU University Medical Center, Amsterdam
	B. Löwenberg	Erasmus MC, Rotterdam
	W.L.J. van Putten	HOVON Data Center, Rotterdam
	E. Vellenga	University Medical Center, Groningen
	A. Gratwohl	Kantonspital Basel, Basel
Cytogenetics review	B. Beverloo	Erasmus MC, Rotterdam
	E. van den Berg-de Ruiter	University Medical Center, Groningen
Cytological and immunophenotype review	M.B. van 't Veer	Hematocytology Review Committee, Erasmus MC, Rotterdam
Statistician	W.L.J. van Putten	HOVON Data Center, Rotterdam
Datamanagement	C.M.C. van Hooije	HOVON Data Center, Rotterdam
Serious Adverse Events (SAEs) notification	HOVON Data Center	Fax: +31.10.4391028

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 AML

Acute myeloid leukaemia is a clonal hematopoietic stem cell disorder characterised by an accumulation of immature progenitor cells by a block in differentation resulting in the suppression of normal hematopoiesis**.** The disease is heterogeneous with respect to morphology, immunophenotype cytogenetics and molecular and gene expression signature as well as in outcome[1]. Treatment for AML is intensive consisting mostly on 3-4 courses of Anthracycline and Cytosine-arabinoside based cytostatic therapy resulting in a CR of 70-80% in patients below 60 years and about a 40% long term survival[2]. Outcome in older patients is much less satisfactory falling to a 2 years survival of 10% due to overrepresentation of prognostic factors determining poorer outcome and the unability to deliver very intensive therapy in these older patients[3]. Relapses are emerging from residual disease present after chemotherapy that easily can be detected by multicolour flowcytometry in the majority of cases and is correlated with outcome[4]. Although therapy has been intensified in elderly patients by increasing the doses of existing drugs there is no evidence of improvement of survival[5]. So there is an urgent need for new treatment modalities in this elderly patient group. The improvement in understanding better the biology of AML has identified new targets which have been used for new drug development. There are now a wide range of these new molecular targeted treatments waiting for clinical application[6].

5.2 VEGF and AML

Vascular endothelial growth factor (VEGF) is an essential regulator of physiologic and pathologic angiogenesis but it also triggers growth, survival and migration of leukaemia cells. VEGF is a heparin-binding cytokine that stimulates the proliferation and survival of endothelial cells and also multipotential haematopoietic progenitor cells[7]. Microvessel density (MVD) is increased in AML as compared to normal bone marrow. MVD decreases during chemotherapy induced aplasia in

patients who achieve a CR but not in those who fail to therapy[8]. Recently it was shown by dynamic MRI that bone marrow angiogenesis is increased in AML with differences between various AML patients. Increased BM angiogenesis was highly correlated with adverse clinical outcome[8a]. VEGF and VEGF receptor expression are reported in AML[9]. Co-expression of VEGF and VEGF receptors in AML coupled with the direct effects on cell survival, migration and proliferation confirms the pivotal role for autocrine loops in the pathogenesis of AML[10]. A significant proportion of cultured AML blasts secrete VEGF in amounts exceeding cultured normal bone marrow counterparts[11]. Some AML blasts overexpress VEGF receptors. The MD Anderson group found that the amount of VEGF produced by AML cells is inversely related to the duration of CR and survival[12]. Apart from autocrine stimulation, secreted VEGF stimulate stromal cytokine production that drive leukemic and endothelial cell proliferation in a paracrine fashion[13]. VEGF –C production by stromal cells, via interaction with VEGF receptor-3, protects AML cells from chemotherapy induced apoptosis[14]. Given this comprehensive data it is reasonable to postulate that VEGF may act as growth and survival factor in AML contributing to drug resistance suggesting that targeting this growth factor could be a new treatment modality in AML.

5.3 Bevacizumab

Bevacizumab is a recombinant humanized IgG monoclonal antibody directed against all biological active forms of VEGF because it recognizes the binding sites for its cognate receptors[15]. The use of anti-VEGF antibodies have been extensively studied in preclinical in vivo models and has demonstrated an inhibition of tumor growth in a dose dependent matter[16]. Phase I clinical trials have shown that rhuMAb VEGF (Bevacizumab, Avastin®) can be safely administered without dose limiting toxicity at doses ranging up to 15mg/kg[17]. Linear pharmacokinetics have been shown for doses >1mg/kg with a $t_{1/2}$ of about 15 days. No anti-bevacizumab antibodies are detected in various clinical studies. In phase II and III it has been shown that the antibody can be safely combined with cytotoxic therapy (CT) without influence on pharmacokinetics[18-21]. The toxicity profile consists of hemorrhagic and trombotic events, proteinuria and hypertension. In a phase III study in colorectal cancer only grade 3 hypertension was more common during treatment with CT plus bevacizumab as compared to CT plus placebo and could be handled easily[22].
Phase II and Phase III studies in combining bevacizumab with chemotherapy in colon cancer, breast cancer, renal cancer and non small cell lung cancer resulted in increased response rates and prolonged time to progression for the CT plus bevacizumab treatment arms. The positive results found in a randomized phase III trial combining bevacizumab with a regimen consisting of fluoruracil and leucovorin in metastatic colorectal cancer resulted in the approvement of bevacizumab by the US FDA in February 2004. In various studies bevacizumab is applied in a dosage of 10mg/kg/2-3weeks.

5.4 Clinical Experience of Bevacizumab in AML

In a single-arm trial in AML investigators combined bevacizumab with 1-β-D-arabinofuranosylcytosine (ara-C)- based treatment using timed sequential therapy (TST) [23]. The theory behind TST is to exploit drug-induced changes in residual leukaemia cell cycle kinetics to increase their sensitivity to cycle-dependent antileukaemic drugs. Patients who entered the study had refractory or relapsed AML and were unlikely to be cured by existing therapies.
The first treatment cycle consisted of ara-C 2g/m^2 given as a 72-hour continuous infusion followed by mitoxantrone 40mg/m^2 as a single i.v. bolus over 30–60 minutes on day 4; bevacizumab 10mg/kg was given on day 8 by 90-minute i.v. infusion.
Marrow aspirates and biopsies on day 8 showed that though some patients had responded to TST alone, 38 out of the 48 (79%) still had residual leukaemia. On day 15 after treatment with bevacizumab, bone marrow examination revealed that 28 patients (58%) had complete tumour clearance.
There was complete response in 16 patients (33%; 95% confidence interval [CI] 20–48%) and partial response in 7 patients (15%; 95% CI 6–28%). So overall, 23 patients responded to treatment with bevacizumab plus TST, or 48% (95% CI 33–63%). 18 of the patients who responded went on to a second cycle of bevacizumab plus TST. Median overall survival was 8.4 months (95% CI 4.1–11.2 months), with 35% of patients still alive after 1 year (95% CI 19.5–38.4%) and 18% still alive after 2 years (95% CI 4.1–32.1%).
The toxicity profile of bevacizumab in this trial was somewhat different to that seen in patients with colorectal cancer (CRC). There was no thrombosis – this may be because of the greater predisposition of CRC patients to thrombotic events, or because patients in this study were protected by thrombocytopenia. The most common events were hypertension that easily could be treated and cardiovascular events. 3 patients developed cardiac failure in cycle 1 (grade 4 in one), and two additional cases developed CHF in cycle 2, although whether this was directly related to bevacizumab or to mitoxantrone is unclear. The overall safety profile of bevacizumab is shown in Appendix E

5.5 Rationale for this study

AML in the elderly has a worse prognosis. Intensifying therapy have not brought any improvement in previous clinical trials. The need for new targeted therapies in this patient group is emerging. VEGF could be theoretically such a target. In a one armed study addition of bevacizumab to chemotherapy showed clear clinical responses with acceptable side effects. So we will investigate in a randomised study whether the addition of anti-VEGF monoclonal antibody to standard remission induction therapy will improve outcome in elderly patients with AML. Bevacizumab in a dosage of 5 and eventually 10 mg/kg will be delivered in addition to standard chemotherapy at day 1 and 15 of Cycle I and II as a consequence of the pharmacokinetic profile resulting in through levels of 100pg/ml,

considered as therapeutic in in vitro experiments. Thus this dosing regimen guarantees that at day 15 at the time of MRD and the possible start of regrowth of blasts a therapeutic level of bevacizumab will be present to hopefully prevent regrowth of the malignant population.

6 Study objective

Primary objectives:

1. To assess the safety and tolerability of bevacizumab added to standard induction chemotherapy for AML (frequency and severity of toxicities and the durations of neutropenia and thrombocytopenia)
2. To assess in a randomized comparison the effect of bevacizumab on the CR rate.

Secondary objectives:

1. To determine the efficacy profile (event free survival and disease free survival) associated with the two therapy regimens.
2. To measure MRD by immunophenotyping in relation to clinical response parameters.
3. To determine micro vascular density after the first course of therapy in both treatment arms.
4. To measure VEGF levels in plasma and VEGF receptors on leukemic blasts in relation to clinical parameters.

In addition, exploratory proteomic and genomic analysis are planned to identify potential biomarkers predictive of response and progression free survival.

7 Study design

This is a multicenter, open-label, randomised phase II trial. In this trial, elderly patients with AML (except those with FAB M3) and RAEB or RAEB-t (with IPSS ≥ 1.5) fit for standard chemotherapy will be randomized on entry to two cycles of chemotherapy with or without bevacizumab.

Arm A: Cycle I: Daunorubicin/cytarabine-arabinoside(3/7 days)
Cycle II: Intermediate dose cytarabine-arabinoside

Arm B: Cycle I: Daunorubicin/cytarabine-arabinoside(3/7 days)
Cycle II: Intermediate dose cytarabine-arabinoside
Bevacizumab 5 or 10mg/kg i.v. day 1+15 of each cycle

The initial dose of bevacizumab in the first cohort of patients will be 5 mg/kg i.v. day 1+15 of each cycle. Decisions regarding dose escalation to 10 mg/kg i.v. day 1+15 of each cycle, continuation

with dose level 5 mg/kg, or stopping, is based on the incidence of DLT (Dose Limiting Toxicity: death within 30 days of start cycle I and before start cycle II), and will be performed according the rules defined in chapter 17.
All patients will be evaluated for response after cycle I and II. Patients in CR after cycle II with an HLA identical sibling donor can be offered an allograft with non-myeloablative conditioning.

8 Study population

8.1 Eligibility for registration

8.1.1 Inclusion criteria

- Patients > 60 years.
- Patients eligible for standard chemotherapy.
- Patients with a confirmed diagnosis of
    - AML FAB M0-M2 or M4-M7 (see appendix A) or
    - with refractory anemia with excess of blasts (RAEB) or refractory anemia with excess of blasts in transformation (RAEB-T) with an IPSS score ≥ 1.5 (see Appendix B)
- Subjects with secondary AML progressing from antecedent (at least 4 months duration) myelodysplasia are also eligible.
- SGOT (AST) and SGPT (ALT) ≤ 1.5 x the upper limit of the normal range (ULN) at the laboratory where the analyses were performed.
- Total serum bilirubin level ≤ 1.5 x the ULN at the laboratory where the analysis was performed.
- Serum creatinine concentration ≤ 1.5 x the ULN at the laboratory where the analysis was performed.
- Proteinuria at baseline: Urine dipstick of proteinuria <2+. Patients discovered to have ≥ 2+ proteinuria on dipstick urinalysis at baseline, should undergo a 24-hour urine collection and must demonstrate ≤ 1 g of protein/24 hr.
- WHO performance status ≤ 2 (see Appendix G)
- Written informed consent.

8.1.2 Exclusion criteria

- Patients previously treated for AML (any antileukemic therapy including investigational agents)
- Past or current history (within the last 2 years prior to randomization) of malignancies except for the indication under this study and curatively treated:

- Basal and squamous cell carcinoma of the skin
- in situ carcinoma of the cervix

- Clinically significant (i.e. active) cardiovascular disease, for example cerebrovascular accidents (≤ 6 months prior to randomization), myocardial infarction (≤ 6 months prior to randomization), unstable angina, New York Heart Association (NYHA) grade II or greater congestive heart failure, serious cardiac arrhythmia requiring medication, reduced left ventricular ejection fraction of < 50% as evaluated by echocardiogram or MUGA scan.
- Uncontrolled hypertension
- Patients with a history of non-compliance to medical regimens or who are considered unreliable with respect to compliance
- Patients with any serious concomitant medical condition which could, in the opinion of the investigator, compromise participation in the study.
- Patients who have senile dementia, mental impairment or any other psychiatric disorder that prohibits the patient from understanding and giving informed consent.
- Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to study treatment start, or anticipation of the need for major surgical procedure during the course of the study
- Serious, non-healing wound, ulcer, or bone fracture
- Patients with bleeding diathesis or coagulopathy (unless related to AML)
- Patients with known allergy to Chinese hamster ovary cell proteins or other recombinant human or humanized antibodies or to any excipients of bevacizumab formulation; or to any other study drugs.

9 Treatments

9.1 Treatment schedules

Remission induction treatment Cycle I

Arm A :

Agent	Dose/Day	Route	Days
Daunorubicin	45mg/m^2	3hr infusion	1,2,3
Cytarabin	200mg/m^2	continuous infusion (24hrs)	1 thru 7

ArmB :

Agent	Dose/Day	Route	Days
Daunorubicin	45mg/m^2	3hr infusion	1,2,3
Cytarabin	200mg/m^2	continuous infusion (24hrs)	1 thru 7
Bevacizumab	5-10mg/kg	1.5 hr infusion	1,15

Remission induction treatment Cycle II:

Arm A:

Agent	Dose/Day	Route	Days
Cytarabin	1000mg/m^2 q 12 hrs	6 hr infusion	1 thru 6 (12 doses)

Arm B:

Agent	Dose/Day	Route	Days
Cytarabin	1000mg/m^2 q 12 hrs	6 hr infusion	1 thru 6 (12 doses)
Bevacizumab	5-10mg/kg	1.5 hr infusion	1,15

Cycle II will be given as soon as possible after Cycle I but at least within 8 weeks after start of Cycle I. If after Cycle I the bone marrow shows persistence of leukaemia it is recommended that patients proceed to Cycle II immediately. Otherwise Cycle II will be started as soon as there is evidence of haematological regeneration. No dose modification is allowed.

9.2 Method of administration of bevacizumab

All patients will receive an infusion of study drug in a total volume of 100 ml of 0.9% NaCl. Once bevacizumab has been added to a bag of sterile saline, the solution must be administered within 8 hrs. **Bevacizumab infusions should not be administered or mixed with dextrose or glucose solutions.** The study drug will be delivered over 90 minutes as a continous i.v. infusion. If the first infusion is tolerated without infusion-associated adverse events the second infusion may be delivered over 60 minutes. If the 60 minutes delivery is tolerated, the subsequent infusions may be delivered over 30 minutes. In clinical studies, infusion related reactions with the first dose of bevacizumab were uncommon(<3%) and severe reactions occurred in 0.2% of the patients. If mild infusion-related reactions(e.g. fever, chills, headache, nausea) occur, pre-medications should be given with the next dose. For patients with grade 3 infusion-related reactions, the bevacizumab infusion should be stopped and not restarted that day. At the physicians discretion, bevacizumab may be re-instituted with pre-medications at a rate of 90 per minute. For patients with grade 4 infusion-related reactions, bevacizumab should be permanently discontinued. Patients with grade 3 and 4 allergic reactions should permanently discontinue bevacizumab.

9.3 Dose modification for bevacizumab

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

- Grade 4 toxicity
- Grade 3 toxicity that does not resolve to grade 1 or less within 4 weeks
- Arterial thromboembolic events
- Gastrointestinal perforation

Specific:

- Venous/arterial thrombolic event: Permanently discontinue bevacizumab
- Proteinuria (Dipstick3+/4+): do 24 hour urine collection:
    - if <2g protein/24hours: give next dose bevacizumab as scheduled
    - if >2g protein/24hours omit bevacizumab dose and do 24-hour urine collection before next scheduled dose. Readminister only once protein <2 g protein/24 hour.
- Hypertension:
    - Grade 1 hypertension: Asymptomatic, transient (<24 hrs) increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal range. Intervention not indicated.
    - Grade 2 hypertension: Recurrent or persistent (>24 hr) or symptomatic increase by >20 mmHg (diastolic) or to >150/100 mmHg if previously within normal range. Monotherapy of anti-hypertensive may be indicated. Once controlled to <150/100 mmHg, patients may continue bevacizumab therapy.
    - Grade 3 hypertension: Requiring more than one anti-hypertensive or more intensive therapy than previously. Bevacizumab should be withheld for persistent or symptomatic hypertension and should be permanently discontinued if blood pressure is not controlled.
    - Grade 4 hypertension: Life-threatening consequences. Occurrence of grade 4 hypertension should lead to permanent discontinuation of bevacizumab. All doses of anti-hypertensive medicines should be recorded at all visits.
- Infusion related or allergic reactions: see 9.2
- Livertoxicity: Applies only in cases when grade 3 or 4 liver function test (ALAT, ASAT, ALP) increase is due to bevacizumab: grade 3 or 4 first occurrence: withhold bevacizumab until toxicity has improved to grade<2; grade 3 or 4 second occurrence: Permanently discontinue bevacizumab.

9.4 Provision of study medication

Roche will supply bevacizumab. Medication labels will comply with the legal requirements of each country and will be printed in the local language. The storage conditions for study drug will be described on the medication label. Bottles must be stored in a safe, secure location. The study drug will be (prepared and) dispensed by the investigator. The investigator must maintain an overall drug accountability log for the study, as well as individual records for each patient. The drug formulation, dose, number of bottles/capsules dispensed, received, and returned must be recorded for each patient.

10 End of protocol treatment

A patient may discontinue participation in the study for any one of the following reasons categorized on the Off Treatment Form of the CRF as one of the following:

1. Normal completion of protocol treatment (i.e. cycles I + II)
2. Excessive extramedullary drug toxicity preventing continuation of treatment
3. Hypoplastic bone marrow abnormalities preventing continuation of treatment
4. Relapse
5. Adverse event preventing further treatment
6. Refusal of further treatment by the patient
7. Death
8. Major protocol violation

All relevant information related to the reason for treatment discontinuation, including contributory factors, must be included in the Off Treatment Form of the CRF and recorded in the patient medical records.

11 Required clinical evaluations

11.1 Required investigations

	At entry	After Cycle I	After Cycle II	FU
Medical history	X	daily until discharge	daily until discharge	X
Physical examination	X	daily until discharge	daily until discharge	X
Hematology	X	every other day until PBR	every other day until PBR	X
Blood chemistry	X	X[1)]	X[1)]	X[4)]
Bone marrow aspirate				
Morphology	X	X	X	X[5)]
BM immunophenotyping	X			
Cytogenetics	X	X[3)]	X[3)]	X[3)]
Molecular analysis	X	X[3)]	X[3)]	X[3)]
Molecular profiling	X			
MRD assessment	define LAP(s)	day 28-35	day 28-35	at relapse
Bone marrow biopsy				
Histopathology	X			
MVD (MicroVasculature Density)	X	day 28-35		
Specific investigations				
Coagulation tests	X	only INR, weekly until discharge	only INR, weekly until discharge	
Chest X-ray	X	o.i.	o.i.	
ECG	X	o.i.	o.i.	
Cardiac ejection fraction	X			o.i.
Dental examination	X			
Proteinuria-test[6)]	X	weekly	weekly	
Virological tests	X			
Microbiological tests	X	X[2)]	X[2)]	
Plasma storage	X			

o.i. on indication

1) - every other day until discharge: Creatinine, Na, K, Glucose.
- weekly until discharge: Ca, P, Mg, Cl, AST, ALT, Alk. Phos., γ-GT, bilirubine (direct+indirect), LDH, albumin

2) according to local bacteriology guidelines

3) only when cytogenetic abnormalities were evident: to document remission after protocol treatment or when relapse is supected

4) only creatinine, AST, ALT, Alk. Phos., γ-GT, bilirubin

5) o.i. And if patient in first CR: at 4, 8, 12, 18, 24, 36, 48 months

6) dipstick urinalysis for proteinuria. If > 1+, protein measurement in 24 hours urine collection.

11.1 Observations prior to start of treatment

- History, including exposure to insecticides, previous chemotherapy or radiotherapy, antecedent hematological or oncological diseases
- Physical examination including body weight, height, **blood pressure**, performance status (see Appendix G), splenomegaly, hepatomegaly, signs of extramedullary leukemia

- Hemoglobin, hematocrit, reticulocytes, platelets, WBC and WBC differential
- Blood chemistry, including serum creatinin, urea, Na, K, Mg, Cl, Ca, uric acid, glucose, albumin, bilirubin, AST, ALT, alkaline phosphatase, gamma GT, LDH
- Dipstick urinalysis for proteinuria (if >1+, protein measurement in 24 hours urine collection)
- Surveillance cultures of throat, stools and urine
- Chest X-ray
- Cardiac ejection fraction, measured by MUGA or echocardiogram .
- ECG
- Dental examination
- Serology for cytomegalovirus (CMV) infection, HIV (human immunodeficiency virus), hepatitis A, B and C
- Coagulation studies including fibrinogen, APTT, INR
- Bone marrow aspiration for:
    - cytology and cytochemistry to establish FAB subtype of AML or RAEB or RAEB-t
    - cytogenetics (cell culture and banding analysis)
    - immunological phenotyping to verify myeloid leukemia or RAEB/RAEB-t , determination of leukemia associated phenotype(LAP), VEGF receptor 1-2
    - molecular analysis for t(8;21)(q22;q22), inv/del(16)(p12;q22) and 11q23 abnormalities is recommended
    - molecular profiling
- Bone marrow biopsy for histopathology and micro vasculature density
- Store Plasma for proteomics and VEGF level.

11.2 Observations during and following induction treatment cycle I and II

- Daily interim history and physical examination, **including blood pressure measurement**, when hospitalized; thereafter as clinically indicated
- Blood cell count, quantitative platelets, WBC count and differential at least every other day when hospitalized until PBR, thereafter as clinically indicated.
- X-chest, ECG as clinically indicated
- Creatinin, Na, K, glucose every other day until discharge
- Ca, Mg, Cl, phosphate, AST, ALT, alkaline phosphatase, gamma GT, bilirubin (direct and indirect), LDH , albumin, INR as clinically indicated and at least weekly until discharge
- Surveillance cultures according to local bacteriology guidelines
- Weekly dipstick urinalysis for proteinuria (if >1+, protein measurement in 24 hours urine collection)

11.2.1 Response assessment after Cycle I and Cycle II

Following each cycle, at day 18-21, the response will be assessed by bone marrow aspiration, blood evaluation and extramedullary disease status evaluation (see Appendix C). If and as long as the marrow is not conclusive a new marrow will be taken as clinically indicated, but at least at weekly intervals. If the marrow shows evidence of resistant disease after Cycle I, Cycle II may be started as soon as possible without waiting for PBR. In all other cases blood evaluation will be repeated until PBR.

Cytogenetic or molecular analysis may be used in patients when karyotypic or molecular markers exist to document remission.

A trephine biopsy to determine MVD will be performed at respons evaluation between day 28-35 after cycle I

11.3 Observations during follow up.

Outpatient visits to the clinic during first CR are planned according to the following schedule, the 1st year every month; 2nd and 3rd year at least at 3 months intervals; 4th and 5th year at 4-6 months intervals and thereafter according to the local scheme of the institute. This time is measured from the date of completion of protocol treatment. At each clinical visit the following examinations will be done:

- Interim history and physical examination
- Hemoglobin, WBC count and differential, quantitative platelet count, erythrocyte count, reticulocyte count
- Creatinin, AST, ALT, alkaline phosphatase, gamma-GT, bilirubin
- Bone marrow aspirations for morphology will be done as clinically indicated, but at least at 4 months, 8 months, 12 months, 18 months, 24 months, 36 months and 48 months as long as the patient is in first CR.
- Immunological examination if markers allow discrimination of malignant cells.
- Cytogenetic analysis or molecular analysis will be included when cytogenetic abnormalities were evident and when leukemic relapse is suggested.
- Cardiac ejection fraction, measured by MUGA or echocardiogram, if indicated

11.4 MRD assessment

Immunological examination for MRD detection at diagnosis, at day 28-35 after cycle I as well as after cycle II, and at relapse (see Appendix D)

12 Toxicities

Daunorubicin

Congestive heart failure is a major complication of anthracyclins, frequently observed after high cumulative doses. The total dose of daunorubicin in this study is 135 mg/m². This dose is below the levels associated with congestive heart failure. Daunorubicin causes pancytopenia and can induce septic or hemorrhagic complications. Other side effects are hair loss, mucositis, cardiomyopathy, nausea, vomiting, colitis.

Cytarabine (Ara-C)

Cytarabine causes pancytopenia and can induce septic or hemorrhagic complications.
Cytarabine at a dose of 200 mg/m² may cause anorexia, nausea, vomiting, hepatic dysfunction, skin rash, pneumonitis, fever.
Intermediate dose cytarabine (1000 mg/m^2) may cause nausea, vomiting, stomatitis, skin rash, fever, conjunctivitis (prevented by the use of methylcellulose or steroid eye drops), somnolence, and in few cases cerebellar toxicity. Ara-C must be stopped immediately in case of nystagmus or dysarthria.

Bevacizumab

In a recent phase II and III studies Bevacizumab was generally well tolerated. The most frequently reported adverse reactions with suspected relationship to treatment were: hypertension, proteinuria, mostly tumour associated hemorrhage, tumour associated gastro-intestinal perforation, thrombosis/embolism.
For the safety profile of bevacizumab, see Appendix E.

Toxicities will be scored according to the NCI Common Terminology Criteria of Adverse Events, version 3.0 (Appendix F)

13 Safety evaluations and adverse advents reporting

13.1 Definitions

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory

finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Adverse reaction (AR)

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

Serious adverse event (SAE)

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

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

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

Unexpected SAE

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

Suspected unexpected serious adverse reaction (SUSAR)

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

Protocol treatment period

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

13.2 Reporting of (serious) adverse events

Adverse event

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

SAE and Unexpected serious adverse event

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

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

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

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

The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be

recorded on the serious adverse event form. The assessment of causality is made by the investigator using the following:

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

13.3 Processing of serious adverse event reports

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

Suspected unexpected serious adverse reactions

Any suspected unexpected serious adverse reactions (SUSARs), from any source, will be reported by HOVON Data Center to the investigators, the Ethics Committee which approved the study, and to all applicable Health Authorities within required timelines.

14 Endpoints

14.1 Primary endpoint

- Incidence of DLT
- The effect of Bevacizumab on the CR rate.

14.2 Secondary endpoints

- Overall survival (time from registration till the death of the patient.)
- Event free survival (i.e., time from registration to induction failure, death or relapse whichever occurs first)
- MRD %

15 Data collection

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

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

Each CRF page will be identified by a pre-printed trial number, and a unique combination of patient study number (assigned at registration), hospital and patient namecode (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 authorised 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 timepoints. 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.

15.1 Reporting DLT information

To monitor the incidence of dose limiting toxicity (DLT) 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 48 hours after DLT-occurrence, or 30 days after start of cycle I if no DLT occurred. An automatic reminder will be sent to the local investigator 30 days after randomization.

16 Registration/Randomisation

Eligible patients who have given written informed consent should be registered and randomised before start of treatment. Patients can be registered and randomised at the HOVON Data Center of the Erasmus MC - Daniel den Hoed by phone call: +31.10.4391568 or fax +31.10.4391028 Monday through Friday, from 09:00 to 17:00, or via the Internet through TOP (Trial Online Process; https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Patient's initials or code
- Patient's hospital record number (optional)
- Sex
- Date of birth
- Eligibility criteria

All eligibility criteria will be checked with a checklist.
Each patient will be given a unique patient study number. Patients will be randomized, stratified by center. Patient study number, result of randomization, and doselevel of Bevacizumab (if applicable) will be given immediately by TOP or phone and confirmed by fax or email.

17 Statistical considerations

The aim of this study is to decide whether the addition of Bevacizumab to standard induction treatment could be sufficiently effective to warrant a continuation as a Phase III study. The target number of patients for this Phase II study is 200 (100 in each arm).

17.1 Toxicity

Decisions regarding dose escalation, continuation with the initial dose of 5 mg/kg or stopping will be based on the incidence of dose limiting toxicity (DLT) in the Bevacizumab arm only. DLT is defined as death within 30 days of start of cycle I and before initiation of start of cycle II, irrespective of the cause of death. In the HOVON/SAKK AML 43 the incidence of DLT defined in this was 13%. A patient is evaluable for toxicity if the patient has experienced a DLT or if the patient is still alive at day 30 after start of cycle I. The following decision rules will be applied.

Abbreviations:

P the (unknown) true probability of DLT; and for this probability :

P1(D | N, P) the probability that ≥ D DLT's are observed in N patients

P2(D | N, P) the probability that ≤ D DLT's are observed in N patients

Dose level [mg/kg]	Number of evaluable Bev. patients	Criterium	Decision
5	N ≤ 20 N > 20	D ≥ 3 and P1(D | N,.13) <10% P1(D | N, .13)<5%	Trial on hold
5	N ≥ 15 and ≤ 30 N ≥ 25 and ≤ 30	P2(D | N, .26) <10% D ≤ 0.13*N +1	Escalate
10	N ≤ 20 N > 20	D ≥ 3 and P1(D | N,.13) <10% P(D | N, .13) <5%	Go back to dose level 5

In all other situations the recruitment continues at the same dose level until the target number of patients has been reached or if a criterium for another decision will be reached.

Trial on hold means that the recruitment in the study will be stopped and decisions to stop or amend the trial need to be made. The stopping and escalation boundaries till 30 evaluable patients according to these rules are shown in the figure below.

The trial will be put on hold when the number of DLT's exceeds the upper boundary of X-'s, including the boundary, e.g. after 3 DLT's / 3 patients or 8/30. Escalation is possible when the number of DLT's is below the lower boundary of +signs, i.e. if ≤ 1/15, 2/19, 3/24 or 4 DLT's /25 patients.
The characteristics of these decision rules have been evaluated with 1000 simulations for true probabilities of DLT 13% and 26% and with target number of patients treated with Bevacizumab equal to 100 patients at dose level 5 mg/kg and 80 patients at 10/mg/kg.

	Dose level 5 mg/kg			Dose level 10 mg/kg		
Decision	perc	npat	pdlt	perc	npat	pdlt
	[%]	[n]	[%]	[%]	[n]	[%]
True probability of DLT=13%						
Stop/reduce dose	19.3	15.6	38.2	20.7	19.2	36.6
Continue	4.3	100.0	13.7	79.3	80.0	12.0
Escalate	76.4	18.3	8.3	-	-	-
True probability of DLT=26%						
Stop/reduce dose	79.1	15.9	39.2	95.2	20.5	37.4
Continue	0.3	100.0	17.0	4.8	80.8	16.5
Escalate	20.6	19.9	10.0	-	-	-

perc:	% times this decision is made in 1000 simulated trials
npat:	mean # evaluable pts (at time of the decision)
pdlt:	mean % pts with DLT (at time of the decision)

Thus if the true probability of DLT is 13% the decision to escalate to the higher dose level will be made in 76% of the trials after on average 18 patients. In 19% of the trials the trial will be put on hold after on average 16 patients. This is because at the moment this decision is made the observed incidence of DLT is high (on average 38%).

If the true probability of DLT is 26% the (inappropriate) decision to escalate to the higher dose level will be made in 21% of the trials after on average 20 patients. This is because at the moment this decision is made the observed incidence of DLT low (on average 10%). In 79% of the trials the proper decision to put the trial on hold will be made.

A teleconference will be organized after 30 evaluable patients with the members of the writing committee together with responsible medical leaders of Roche to discuss meticulously the toxicity data.

17.2 Efficacy

Only if the trial will not be stopped because of too much DLT in the bevacizumab arm an analyis of efficacy is relevant. Primary endpoint for efficacy is the CR rate on protocol after induction treatment. The observed difference Dcr in CR rates between arm B (Bevacizumab) at the final dose level and arm A (standard) will be used as criterion in decision rules. Two interim analyses regarding efficacy will be performed after 1/3 and 2/3 of the target number of patients at the final dose level will be evaluable for CR. The following decision rules will be used regarding efficacy.

Let LLCI(nn) and ULCI(nn) be the lower and upper limit of the nominal nn% twosided confidence interval for the estimate Dcr of the true (unobserved) difference Dcr in CR rates between both arms.

Decisions	Conditions
At interim analysis	
Stop because of inefficacy	ULCI(80) <10%
Continue as Phase III	LLCI(95) >0
Continue as Phase II	Otherwise
At final analysis (if not stopped before)	
Stop because of inefficacy	D<=0 or ULCI(80) <10%
Continue as Phase III	Otherwise

Assuming that the true CR rate in the standard arm is around 50% (as in the AML 43), and that a total of 80 patients will be treated in arm B at the final dose level then these decision rules lead to the following characteristics.

True difference Dcr in CR rates arm B minus arm A	Probability to continue as Phase III	Expected number of patients per arm at decision
-10%	6%	41
0%	39%	56
10%	82%	63
20%	96%	52

Thus if arm B would in truth be not more effective than arm A (true Dcr=0%), there is still a 39% probability that the study will continue as Phase III (type I error), while if arm B would be more effective (true Dcr=10%) the probability to continue as Phase III (power) is 82%.

Note that above considerations apply if the decisions would be purely based on the primary endpoint only. In reality the actual decisions at the final analysis will also take into account outcomes with respect to secondary endpoint and information from other studies

18 Ethics

18.1 Independent ethics committee or Institutional review board

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

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki and the ICH-GCP Guidelines. 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, randomization and any other study specific procedure.

19 Trial insurance

The HOVON insurance program covers all patients from participating centres in The Netherlands according to Dutch law (WMO). The WMO insurance statement can be viewed on the HOVON Web site www.hovon.nl.
Individual participating centers from outside the Netherlands have to inform the HOVON about the risk insurance of patients participating in a study.

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

20 Publication policy

The final publication of the trial results will be written by the Study Coordinator(s) on the basis of the statistical analysis performed at the HOVON Data Center. A draft manuscript will be submitted to the Data Center and all co-authors (and the sponsor, where applicable) for review. A report of the analysis of the data and a draft manuscript with the study results will be sent to Roche 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), the lead investigators of the major groups (in case of intergroup studies), investigators who have included more than 5% of the evaluable patients in the trial (by order of number of patients included), the statistician(s) and the HOVON datamanager in charge of the trial, and others who have made significant scientific contributions.

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

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

21 Glossary of abbreviations

(in alphabetical order)

AE	Adverse Event
ALT	Alanine Amino Transferase (SGPT)
ANC	Absolute Neutrophil Count
Ara-C	Cytarabine, cytosine arabinoside
AST	Aspartate Amino Transferase (SGOT)
BM	Bone Marrow
CBC	Complete blood count
CHF	Congestive Heart Failure
CI	Confidence interval
CMV	Cytomegalo virus
CNS	Central nervous system
CR	Complete Remission
CRC	Colorectal cancer
CRF	Case Report Form
CT	Cytotoxic therapy
CTCAE	Common Terminology Criteria for Adverse Events
DFS	Disease free Survival
DLT	Dose Limiting Toxicity
ECG	Electrocardiogram
EFS	Event Free Survival
EMD	Extra medullary disease
FISH	Fluorescent In Situ Hybridization
FU	Follow up
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HOVON	Dutch/Belgian Hemato-Oncology Cooperative Group
HRC	Hematocytology Review Committee
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IM	Intramuscular
IPSS	International Prognostic Score System (for MDS)
IV	Intravenous
LAP	Leukemia Associated Phenotype
METC	Medical Ethical review committee
MRD	Minimal Residual Disease

MVD	MicroVessel Density
OS	Overall Survival
PB	Peripheral Blood
PBR	Peripheral Blood Recovery
PR	Partial Response
SAE	Serious Adverse Event
SCT	Stem cell transplantation
SUSAR	Suspected Unexpected Serious Adverse Reaction
TST	Timed sequential therapy
ULN	Upper Limit of the Normal range
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen
WBC	White Blood Count

22 References

1. Appelbaum F, Rowe J, Radich J, Dick J. Acute myeloid leukemia. Hematology (Am Soc Hematol Educ Program). 2001: 62-86.
2. Lowenberg B, Downing JR and Burnett AAcute myeloid leukemia. N Engl J Med. 1999 Sep 30;341(14):1051-62.
3. Goldwin JE, Smith SE Acute myeloid leukemia in the older patient. Crit Rev Oncol Hematol. 2003 Oct 15;48(Suppl):S17-26
4. Feller N, van der Pol MA, van Stijn A, Weijers GW, Westra AH, Evertse BW, Ossenkoppele GJ, Schuurhuis GJ. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukaemia.Leukemia. 2004 Aug;18(8):1380-90.
5. Buchner T, Berdel WE, Wormann B, Schoch C, Haferlach T, Schnittger S, Kern W, Aul C, Lengfelder E, Schumacher A, Reichle A, Staib P, Balleisen L, Eimermacher H, Gruneisen A, Rasche H, Sauerland MC, Heinecke A, Mesters RM, Serve HL, Kienast J, Hiddemann W. Treatment of older patients with AML Crit Rev Oncol Hematol. 2005 Nov;56(2):247-59.
6. Martin S. Tallman, D. Gary Gilliland, and Jacob M. Rowe Drug therapy for acute myeloid leukemia Blood 2005 106: 1154-1163
7. Ferrara N. Vascular endothelial growth factor and the regulation of angiogenesis. Recent Prog Horm Res, *55:* 15-35, 2000
8. Padro T, Ruiz S, Bieker H, et al Increased angiogenesis in the bone marrow of patients with acute myeloid leukemia. Blood, *95:* 2637-44, 2000

8a. Shih TT-F, Tien H-F, Liu C-Y, Su W-P, Chan W-K and Yang P-C. Functional MR imaging of tumor angiogenesis predicts outcome of patients with acute myeloid leukaemia. Leukemia2006;20,357-362

9. Padro T, Bieker R, Ruiz S, et al Overexpression of vascular endothelial growth factor (VEGF) and its cellular receptor KDR (VEGFR-2) in the bone marrow of patients with acute myeloid leukemia. Leukemia, *16:* 1302-10, 2002.
10. Dias S, Hattori K, Zhu Z, et al. Autocrine stimulation of VEGFR-2 activates human leukemic cell growth and migration. J Clin Invest. 2000;106: 511-521
11. Bellamy WT, Richter L, Frutiger Y, Grogan TM. Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. Cancer Res. 1999;59: 728-733
12. Aguayo A, Estey E, Kantarjian H, et al Cellular vascular endothelial growth factor is a predictor of outcome in patients with acute myeloid leukemia. Blood, *94:* 3717-21, 1999
13. Fiedler W, Graeven U, Ergun S, et al Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. Blood, *89:* 1870-5, 1997

14. Dias S, Choy M, Alitalo K, Rafii S. Vascular endothelial growth factor (VEGF)-C signaling through FLT-4 (VEGFR-3) mediates leukemia cell proliferation, survival and resistance to chemotherapy. Blood, *99:* 2179-84, 2002.
15. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov. 2004;3: 391-400.
16. Fidler IJAngigenesis and cancer metastasis Cancer J 6:S134-141
17. Gordon MS Margolin K Talpaz M et al :Phase I safety and pharmacokinetic study of recombinant human anti-vascular endothelial growth factor in patients with advanced cancer. J Clin Oncol 19:843-850, 2001
18. Phase II, randomized trial comparing bevacizumab plus fluorouracil(FU)/leukovorin (LV) with FU/LV alone in patients with metastatic colon cancer. J Clin Oncol, *21:* 60-5, 2003
19. D. R. D'Adamo, S. E. Anderson, K. Albritton, J. Yamada, E. Riedel, K. Scheu, G. K. Schwartz, H. Chen, and R. G. Maki Phase II Study of Doxorubicin and Bevacizumab for Patients With Metastatic Soft-Tissue Sarcomas
J. Clin. Oncol., October 1, 2005; 23(28): 7135 - 7142.
20. Kabbinavar F,Hurwitz HI, Fehrenbacher L et al Phase II randomized trial comparing Bevacizumab plus fluuoracil/ Leucovorin with FU/LV alone in patients with metastatic colorectal cancer J Clin Oncol 2003; 21:60-65
21. Yang JC, Haworth L , Sherry RM et al A randomised trial of bevacizumab, an antivascular endothelial cell growth factor for metastatic renal cell cancer N Engl J Med 2003 349;427-434
22. Hurwitz H et al Bevacizumab plus Irinotecan, Fluorouracil and Leucovorin for metastic colorectal cancer N Engl J Med 2004,350:2335-2342
23. Karp JE, Gojo I, Pili R, Gocke CD, Greer J, Guo C, Qian D, Morris L, Tidwell M, Chen H, Zwiebel Targeting vascular endothelial growth factor for relapsed and refractory adult acute myelogenous leukemias: therapy with sequential 1-beta-d-arabinofuranosylcytosine, mitoxantrone, and bevacizumab.J. Clin Cancer Res. 2004;10:3577-85.
24. Cheson BD, Bennett JM, Kantarjian H et al. Report of an International Working Group to standardize response criteria for myelodysplastic syndromes. Blood 2000; 96: 3671-3674.
25. Cheson BD, Bennett JM, Buchner JK et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003; 21: 4642-9.
26. Greenberg P et al. International scoring system for evaluating prognosis in myelodysplastic syndrome. Blood 1997, 89: 2079-2088.

A. FAB classification of AML

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

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

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

Cytological Criteria for the diagnosis of RAEB and RAEB-t

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

International Prognostic Score System (IPSS) for MDS[26]

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

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

Low : 0

Int-1 : 0.5-1.0

Int-2 : 1.5-2.0

High : ≥ 2.5

* Karyotype

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

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

Intermediate : all other (or not done)

**Cytopenias

Hb < 6.2 mmol/l

ANC < 1.5×10^9/l

Platelets < 100×10^9/l

C. Response criteria for AML and MDS

HOVON-AML/MDS Response criteria (modified from CALGB-CRITERIA for AML and according to the International Working Group Criteria[24,25] for MDS)

1. DISEASE STATUS CRITERIA

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

1.1 Bone Marrow

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

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

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

A3 Failure to meet criteria for A1 or A2

1.2 Peripheral Blood

B1 Peripheral Blood Recovery (PBR): ANC ≥1.0 x 10^9/l or 1000/mm³, transfusion independent platelet count ≥ 100 x 10^9/l (i.e. 48 h after last transfusion); **and** no leukemic blasts in the peripheral blood and no dysplasia (*)

B2 Failure to meet the criteria for B1

(*) The presence of mild megaloblastoid changes may be permitted if considered to be consistent with chemotherapy effect. However, persisting pretreatment abnormalities (e.g. pseudo-Pelger-Hüet cells, ringed sideroblasts, dysplastic megakaryocytes) are not consistent with CR or PR.

1.3 Extramedullary Disease

C1 None

C2 Any

2 RESPONSE CRITERIA

2.1 Complete remission (CR)

Attainment of A1 marrow status and B1 peripheral blood recovery and C1 extra-medullary disease status without evidence of relapse within 28 days.

2.2 Complete remission with delayed platelet recovery (CRp)

Similar to complete remission (CR), but without complete platelet count recovery. The platelet count must be in the range of 20 – 100 x 10^9/l, transfusion independent (more than one week since last platelet transfusion).

2.3 Treatment failure

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

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

2.4 Relapse Criteria

Relapse after complete remission for patients with AML, RAEB / RAEB-t is defined as:

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

D. 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.

For the present protocol in all cases bone marrow sampling includes diagnosis, in complete remission (CR) after cycle I and cycle II and at relapse.

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

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.

References:

1. Venditti, F. Buccisano F, G. Del Poeta, L. Maurillo, A. Tamburini, C. Cox, A. Battaglia, G. Catalano, B. Del Moro, L. Cudillo, M. Postorino, M. Masi, S. Amadori. Level of minimal residual disease after consolidation therapy predicts outcome in acute myeloid leukemia. Blood 96: 3948-3952 (2000).
2. J.F. San Miguel, M.B. Vidriales, C. Lopez-Berges, J. Diaz-Mediavilla, N. Gutierrez, C. Canizo, F. Ramos, M.J. Calmuntia, J.J. Perez, M. Gonzalez, A. Orfao. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood 98: 1746-1751 (2001).
3. E.L. Sievers, B.J. Lange, T.A. Alonzo, R.B. Gerbing, I.D. Bernstein, F.O. Smith, R.J. Arceci, W.G. Woods, M.R. Loken. Immunophenotypic evidence of leukemia after induction therapy predicts relapse: results from a prospective Children's Cancer Group study of 252 patients with acute myeloid leukemia. Blood 101: 3398-3406 (2003).
4. W. Kern, D. C. Voskova, W. Schoch, S. Hiddemann, T. Schnittger, Haferlach T. Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. Blood 104: 3078-3085 (2004). Epub 2004 Jul 29.

5. N. Feller, M.A. van der Pol, A. van Stijn, G.W.D. Weijers, A.H. Westra, B.W. Evertse, G.J. Ossenkoppele, G.J. Schuurhuis. MRD parameters using immunophenotypic detection methods are highly reliable in predicting survival in acute myeloid leukemia. Leukemia 18: 1380-1390 (2004).
6. N. Feller, G.J. Schuurhuis, M.A. van der Pol, G. Westra, G.W.D. Weijers, A. Van Stijn, P.C. Huijgens, G.J. Ossenkoppele. High percentage of CD34-positive cells in autologous AML peripheral blood stem cell products reflects inadequate in vivo purging and low chemotherapeutic toxicity in a subgroup of patients with poor clinical outcome. Leukemia 17: 68-75 (2003). See also Commentary by T de Witte. Leukemia 17: 39-40 (2003).
7. N. Feller, P.C. Huijgens, A Kelder, A.H. Westra, G.J. Ossenkoppele, G.J. Schuurhuis. Immunophenotypical sequential MRD assessment in AML for prediction of relapse and definition of putative future intervention time. Blood 104 (ASH 2004), in press.
8. Venditti, L. Maurillo, F. Buccisano, G. Del Poeta, C. Mazzone, A. Tamburini, M.I. Del Principe, M. Consalvol, P. De Fabritiis, L. Cudillo, A. Picardi, A. Franchi, F. Lo-Coco, S. Amadori, F.L. Coco. Pretransplant minimal residual disease level predicts clinical outcome in patients with acute myeloid leukemia receiving high-dose chemotherapy and autologous stem cell transplantation. Leukemia 17: 2178-2182 (2003).

E. Safety profile of Bevacizumab

Safety Issues

The overall safety profile of bevacizumab is based on 1132 patients with metastatic carcinoma of the colon or rectum (691), locally advanced or metastatic non small cell lung (85), metastatic breast cancer (304), hormone-resistant prostate cancer (15) and patients with various advanced malignancies in phase I trials (37), who received bevacizumab either as a single agent or in combination with chemotherapy in clinical trials.

In the pivotal phase III, randomised, double-blind, active-controlled study in metastatic carcinoma of the colon or rectum (Study AVF2107g), 396 patients were treated with IFL (Irinotecan/5-FU/Leucovorin) plus placebo (Arm 1), 392 patients were treated with IFL plus bevacizumab (Arm 2), and 109 patients were treated with 5 FU/LV (5-Fluorouracil/Leucovorin) plus bevacizumab (Arm 3). Enrollment in the 5 FU/LV plus bevacizumab arm of the study was discontinued, as pre specified in the protocol, once the safety of combination of bevacizumab with IFL regimen was established and considered safe by an independent monitoring committee viewing an unblinded interim analysis.

In another phase II, randomised, double-blind, active-controlled study (Study AVF2192g), the safety of bevacizumab was investigated in 204 patients with metastatic carcinoma of the colon or rectum who were not optimal candidates for first-line irinotecan. Patients had to be either more susceptible to irinotecan toxicity (≥ 65 years, with prior radiotherapy to pelvis or abdomen) or less likely to benefit from irinotecan treatment (PS ≥ 1, baseline albumin < 3.5 g/dl) in order to be eligible for enrolment. Of these patients, 104 were treated with 5-Fluorouracil/Leucovorin (5-FU/LV) + placebo (Arm 1) and 100 patients were treated with 5-FU/LV + AVASTIN (Arm 2).

Hypertension

An increased incidence of hypertension was observed in patients treated with bevacizumab. Hypertension was generally treated with oral anti-hypertensives such as angiotensin converting enzyme inhibitors, diuretics and calcium-channel blockers. It rarely resulted in discontinuation of therapy (0.7% of all patients treated with bevacizumab) or hospitalisation. A hypertensive encephalopathy was reported in one case (0.1%) only. The risk of bevacizumab associated hypertension did not correlate with the patients' baseline characteristics, underlying disease or concomitant therapy.

In the phase III, randomised, double-blind, active-controlled study in metastatic carcinoma of the colon or rectum (AVF2107g), hypertension of any grade (NCI-CTC, version 2.0) occurred in 22.4% of patients receiving IFL (Irinotecan/5-FU/LV) plus bevacizumab compared with 8.3% of patients receiving IFL alone. Grade 3 hypertension (requiring oral anti hypertensive medication) was reported in 11.0% of patients receiving IFL plus bevacizumab compared with 2.3% of patients receiving IFL alone. At week 24 of treatment, the mean change of blood pressure (BP) from

baseline was diastolic BP +4.1 mmHg and systolic BP +5.5 mmHg in patients treated with bevacizumab.
In Study AVF2192g, hypertension of any grade occurred in 32.0% of patients treated with 5-FU/LV plus bevacizumab (Arm 2) compared to 4.8% of patients treated with 5-FU/LV plus placebo (Arm 1). Grade 3 hypertension was observed in 16.0% of patients in Arm 2 compared to 2.9% of patients in Arm 1. At week 24 of treatment, the mean change of BP from baseline was diastolic BP +5.4 mmHg and systolic +8.4 mmHg in bevacizumab-treated patients. Hypertension did not lead to death or study drug discontinuation in this study. No hypertensive crisis (Grade 4) was reported.
There is no information on the effect of bevacizumab in patients with uncontrolled hypertension at the time of initiating bevacizumab therapy. Therefore, caution should be exercised before initiated bevacizumab therapy in these patients. Monitoring of blood pressure is recommended during bevacizumab therapy.

Proteinuria
In study AVF2107g, proteinuria was reported as an adverse event in 21.7% of patients receiving IFL alone and 26.5% of patients receiving IFL plus bevacizumab. There was no Grade 4 (NCI-CTC, version 2.0) proteinuria, and incidences of Grade 2 and 3 proteinuria were similar in both arms. Proteinuria, reported as adverse event, was observed in 23.3% of all patients treated with bevacizumab. It ranged in severity from clinically asymptomatic, transient, trace proteinuria to nephrotic syndrome with the great majority as Grade 1 proteinuria. The proteinuria seen in bevacizumab clinical trials was not associated with renal dysfunction and rarely required permanent discontinuation of bevacizumab therapy.
In Study AVF2192g, proteinuria was reported as adverse event in 38.0% of patients receiving 5-FU/LV plus bevacizumab (Arm 2) and 19.2% of patients receiving 5-FU/LV plus placebo (Arm 1). The majority of these events was Grade 1 (30.0% vs. 15.4%). There was no Grade 4 proteinuria (nephrotic syndrome) and only one case of Grade 3 proteinuria was reported in Arm 2. No proteinuria resulted in death or study drug discontinuation.
Patients with a history of hypertension may be at increased risk for the development of proteinuria when treated with bevacizumab. There is evidence suggesting Grade 1 proteinuria. Monitoring of proteinuria by dipstick urinalysis is recommended prior to starting and during bevacizumab therapy. Bevacizumab should be discontinued in patients who develop NCI-CTC (version 3.0) Grade 4 proteinuria (nephrotic syndrome).

Gastrointestinal Perforation
Bevacizumab has been associated with serious cases of gastrointestinal perforation in patients with metastatic carcinoma of the colon or rectum. In study AVF2107g in patients with metastatic colorectal cancer, there were six reports of gastrointestinal perforation in the IFL plus bevacizumab arm and one report in the 5 FU/LV plus bevacizumab arm compared with none events in the IFL plus placebo arm. In two patients this event had a fatal outcome; the remaining five recovered but

three patients resumed bevacizumab therapy. The presentation of these events varied in type and severity, ranging from free air seen only on the plain abdominal X-ray, which resolved without treatment, to a colonic perforation with abdominal abscess and fatal outcome. The common feature among these cases was **intra abdominal inflammation**, either from gastric ulcer disease, tumour necrosis, diverticulitis or chemotherapy-associated colitis. Nevertheless, a causal association of an intra-abdominal inflammatory process and gastrointestinal perforation to treatment with bevacizumab has not been established. However, caution should be exercised when treating patients with intra-abdominal inflammatory process with bevacizumab.

In study AVF2192g, two cases of gastrointestinal perforation were observed in patients metastatic colorectal cancer treated with 5-FU/LV plus bevacizumab arm compared to none in 5-FU/LV plus placebo arm. One case had fatal outcome whereas the other resolved but study treatment was discontinued due to the event. In both cases, perforation occurred at the site of sigmoid colon diverticulum.

Patients with metastatic carcinoma of the colon or rectum may be at increased risk for the development of gastrointestinal perforation when treated with bevacizumab and chemotherapy. No gastrointestinal perforation has been observed in any other bevacizumab clinical trials.

Wound Healing

As bevacizumab may adversely impact wound healing, patients who had major surgery within the last 28 days prior to initiation of bevacizumab therapy were excluded from participation in study AVF2107g. In this study, 173 patients in IFL plus bevacizumab arm and 180 patients in IFL plus placebo arm underwent cancer-related surgery between 28 and 60 days prior to starting bevacizumab therapy. There was no increased risk of post-operative bleeding or wound healing complications observed in these patients.

Forty patients in the IFL plus bevacizumab arm underwent major surgery while receiving bevacizumab, of which 4 patients experienced an adverse event consistent with post-operative bleeding or wound healing complications. There were no similar complications observed in the 25 patients from the IFL plus placebo arm who also underwent major surgery.

In Study AVF2192g, 39 patients in 5-FU/LV plus placebo arm (Arm 1) and 43 patients in 5-FU/LV plus bevacizumab arm (Arm 2) underwent cancer-related surgery between 28 and 60 days prior to starting study drug. No patients experienced Grade 3/4 wound healing and bleeding complications within 60 days after prior major surgery.

Fifteen patients in Arm 2 underwent major surgery while receiving bevacizumab, of which 3 experienced Grade 3/4 wound healing or bleeding complications within 60 days of surgery. Three patients in Arm 1 underwent major surgery during study treatment and none experienced Grade 3/4 wound healing or bleeding complications.

Haemorrhage

Overall, 4.0% of NCI-CTC (version 2.0) Grade 3 and 4 bleeding events were observed in all patients treated with bevacizumab. In Study AVF2107g, there was no significant difference in the incidence of Grade 3 and 4 bleeding events observed in IFL plus bevacizumab arm (3.1%) and IFL plus placebo arm (2.5%). A similar observation was noted in study AVF2192g; the overall incidence of Grade 3 and 4 bleeding events was 5.0% in 5-FU/LV plus bevacizumab arm (5.0%) and 2.9% in 5-FU/LV plus placebo arm.

The haemorrhagic events that have been observed in bevacizumab clinical studies were predominantly tumour-associated haemorrhage (see below) and minor mucocutaneous haemorrhage.

Tumour associated haemorrhage was observed in phase I and phase II bevacizumab studies. Six serious events, of which 4 had fatal outcome, were observed in patients with non small cell lung cancer receiving bevacizumab. These events occurred suddenly and presented as major or massive haemoptysis in patients with either squamous cell histology and/or tumours located in the centre of the chest in close proximity to major blood vessels. In five of these cases, these haemorrhages were preceded by cavitation and/or necrosis of the tumour.

Tumour-associated haemorrhage was also seen rarely in other tumour types and locations, including central nervous system (CNS) bleeding in a patient with hepatoma with occult CNS metastases and continuous oozing of blood from a thigh sarcoma with necrosis.

In Study AVF2107g, five haemorrhagic events in IFL plus bevacizumab arm (three rectal haemorrhages, one gastrointestinal haemorrhage and one melaena) were assessed as tumour-associated haemorrhages. The addition of bevacizumab did not result in significant increase in the incidence or severity of Grade 3 or 4 haemorrhagic events in this study. .

In study AVF2192g, three patients in 5-FU/LV + Avastin arm (Arm 2) experienced Grade 3 and 4 gastrointestinal haemorrhages that were assessed as tumour-associated.

Across all bevacizumab clinical trials, ***mucocutaneous haemorrhage*** has been seen in 20% - 40% of patients treated with bevacizumab. These were most commonly Grade 1 epistaxis that lasted less than 5 minutes, resolved without medical intervention and did not require any changes in bevacizumab treatment regimen. In Study AVF2107g, epistaxis was reported in 35.3% of patients receiving IFL plus bevacizumab compared with 10.2% of patients receiving IFL alone.

In study AVF2192g, epistaxis (all Grade 1) was observed in 22.0% of patients receiving 5-FU/LV + Avastin arm (Arm 2) compared to 16.3% of patients receiving 5-FU/LV + placebo (Arm 1).

There have also been less common events of minor mucocutaneous haemorrhage in other locations, such as gingival bleeding and vaginal bleeding.

The risk of CNS haemorrhage in patients with CNS metastases receiving bevacizumab could not be evaluated, as patients with history or evidence upon physical examination of central nervous system (CNS) metastases were excluded from all clinical trials. The use of bevacizumab is contraindicated in patients with untreated CNS metastases.

Patients with metastatic cancer of the colon or rectum might have an increased risk of tumour-associated haemorrhage.

There is no information on the safety profile of bevacizumab in patients with congenital bleeding diathesis, acquired coagulopathy or in patients receiving full dose of anticoagulants for the treatment of thromboembolism prior to starting bevacizumab treatment, as such patients were excluded from clinical trials. However, patients who developed venous thrombosis while receiving bevacizumab therapy did not appear to have increased rate of serious bleeding when treated with full dose of warfarin and bevacizumab concomitantly.

Thrombosis/Embolism

In Study AVF2107g, 16.2% of patients receiving IFL plus placebo (Arm 1) and 19.4% of patients receiving IFL plus bevacizumab (Arm 2) experienced thromboembolic events. In study AVF2192g, the overall incidence of thromboembolic events was 18.0% in 5-FU/LV plus bevacizumab arm (Arm 2) and 18.3% in 5-FU/LV + placebo arm (Arm 1).

Arterial Thromboembolism

In study AVF2107g, the incidence of arterial thromboembolic events (including CVAs, MIs, TIAs, and other arterial thromboembolic events) was higher in patients receiving IFL plus bevacizumab (3.3%) compared to patients receiving IFL plus placebo (1.3%). In study AVF2192g the incidence of arterial thromboembolic events was also reported to be higher in the 5-FU/LV plus bevacizumab arm (10.0%) compared to the 5FU/LV arm (4.8%).

In five randomised trials including AVF2107g and AVF 2192g (N=1745), arterial thromboembolic events including CVAs, MIs, TIAs, and other thromboembolic events occurred in 4.9% (49/1004) of patients treated with bevacizumab in combination with chemotherapy compared to 2.3% (17/741) of patients treated with chemotherapy alone. In patients treated with bevacizumab plus chemotherapy, arterial thromboembolic events led to a fatal outcome in 1.1% (11/1004). In patients treated with chemotherapy alone, a fatal outcome from arterial thromboembolic events was reported in 0.8% (6/741). CVAs (including TIAs) occurred in 2.2% of patients treated with bevacizumab in combination with chemotherapy and 0.5% of patients treated with chemotherapy alone. MI occurred in 2.2% of patients treated with bevacizumab in combination with chemotherapy compared to 1.3% of patients treated with chemotherapy alone.

Bevacizumab should be permanently discontinued in patients who develop arterial thromboembolic events.

A history of arterial thromboembolic events or age greater than 65 years was associated with an increased risk of arterial thromboembolic events during bevacizumab therapy. Patients receiving Avastin plus chemotherapy with a history of arterial thromboembolism and age greater than 65 years have a higher risk. Caution should be taken when treating these patients with bevacizumab.

Venous Thromboembolism

In Study AVF2107g, venous thromboembolic events, including deep venous thrombosis, pulmonary embolism and thrombophlebitis, occurred in 15.2% and 16.4% of patients in Arms 1 and 2,

respectively. It could not be determined if these events were due to the patients' underlying cancer, their cytotoxic chemotherapy, bevacizumab or other risk factors.
In study AVF2192g, the incidence of venous thromboembolic events was lower in the 5-FU/LV plus bevacizumab arm compared to that in control (9.0% vs. 13.5%).

Congestive Heart Failure

In the phase III controlled clinical trial of metastatic breast cancer, there were 7 reports (3%) of congestive heart failure (CHF) in patients treated with bevacizumab compared with two (1%) seen in the controlled group. These events varied in severity from asymptomatic declines in left ventricular ejection fraction to symptomatic CHF requiring hospitalisation and treatment. All the patients treated with bevacizumab were previously treated with anthracyclines (doxorubicin cumulative dose range 240 – 360mg/m2). Many of these patients also had prior radiotherapy to the left chest wall. Most of these patients showed improved symptoms and/or left ventricular function following appropriate medical therapy. There was no information on patients with pre-existing CHF of NYHA II – IV at the time of initiating bevacizumab therapy, as these patients were excluded from clinical trials.
Prior anthracyclines exposure and/or prior radiation to the chest wall may be possible risk factors for the development of CHF. Caution should be exercised before initiating bevacizumab therapy in patients with these risk factors.
No increased incidence of CHF in patients treated with bevacizumab was observed in other clinical trials apart from metastatic breast cancer.

Elderly Patients

In five randomised clinical trials, age > 65 years was associated with an increased risk of developing arterial thromboembolic events including cerebrovascular accidents, transient ischaemic attacks and myocardial infarction as compared to those aged <65 years when treated with AVASTIN (see sections 3.4 and 3.8 under Thromboembolism). No increased incidence of other AVASTIN-related events including gastrointestinal perforation, wound healing complications, hypertension, proteinuria, haemorrhage and congestive heart failure was observed in elderly patients (>65 years) with metastatic cancer of the colon or rectum receiving AVASTIN compared to those aged ≤ 65 years treated with AVASTIN.
In study AVF2107g, 114 out of the 392 patients who received IFL plus bevacizumab were older than 65 years. A difference of greater or equal to 5% occurred only for Grade 3/4 leukopenia in the elderly patients (age >65 years) compared to those patients aged ≤ 65 years.

Pregnancy

Bevacizumab has been shown to be embryotoxic and teratogenic when administered to rabbits. Observed effects included decreases in maternal and foetal body weights, an increased number of foetal resorptions and an increased incidence of specific gross and skeletal foetal alterations. Adverse foetal outcomes were observed at all tested doses of 10–100mg/kg.

Angiogenesis has been shown to be critically important to foetal development. The inhibition of angiogenesis following administration of bevacizumab could result in an adverse outcome of pregnancy.

There are no adequate and well controlled studies in pregnant women. IgGs are known to cross the placental barrier, and bevacizumab may inhibit angiogenesis in the foetus. Therefore, bevacizumab should not be used during pregnancy. In women with childbearing potential, appropriate contraceptive measures are recommended during bevacizumab therapy. Based on pharmacokinetic considerations, contraceptive measures are recommended for at least 6 months following the last dose of bevacizumab.

Physeal Development

In studies of up to 26 weeks duration in cynomolgus monkeys, bevacizumab was associated with physeal dysplasia. Physeal dysplasia was characterised primarily by thickened growth plate cartilage, subchondral bony plate formation and inhibition of vascular invasion of the growth plate. This effect occurred at doses ³ 0.8 times the human therapeutic dose and exposure levels slightly below the expected human clinical exposure, based on average serum concentrations. It should be noted, however, that physeal dysplasia occurred only in actively growing animals with open growth plates. Because bevacizumab will most likely be administered to adult patients with closed growth plates, physeal dysplasia is not expected to occur in the clinical population.

It is not known whether bevacizumab is excreted in human milk. As maternal IgG is excreted in milk and bevacizumab could harm infant growth and development, women should be advised to discontinue nursing during bevacizumab therapy and not to breast feed for at least 6 months following the last dose of bevacizumab.

F. 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.info.nih.gov/reporting/ctc.html
http://www.hovon.nl (under Studies > Documents)

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

G. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

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

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

4 Unable to get out of bed

5 Dead