Azacitidine compared to conventional chemotherapy in consolidation of elderly patients (≥ 65 years) with AML in first complete remission

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Signature of investigator and sponsor

The undersigned has read and understand the Study Protocol and agrees on the contents.

The Investigator has been given appropriate information about the study and agrees to conduct the study in accordance with the protocol, applicable local regulations, and Good Clinical Practice for Trials on Medicinal Products in the European Community.

Pa Bernell
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Signature

29-12/12
.....
Date

Per Bernell
Sponsor representative, the Swedish AML Group
and
Coordinating Investigator

Protocol synopsis

Sponsor	The Swedish AML Group
Full title	Azacitidine compared to conventional chemotherapy in consolidation of elderly patients (≥ 65 years) with AML in first complete remission
Study objectives	**Primary objective** ▪ Investigate whether consolidation treatment with repeated courses of azacitidine prolongs leukemia–free survival (LFS) as compared to standard consolidation chemotherapy in elderly (≥65y) patients with AML in CR1. **Secondary objectives** ▪ Study whether consolidation treatment with azacytidine, as compared with the control arm, improves overall survival (OS) ▪ Assess the relationship between azacitidine treatment and DNA-methylation patterns ▪ Assess the clinical outcome according to molecular mutations and cytogenetic aberrancies at diagnosis ▪ Assess safety, tolerability of azacitidine when given after standard induction chemotherapy (DA) in elderly patients with AML ▪ Assess quality of life in patients receiving consolidation with azacitidin and standard chemotherapy, respectively
Study Design	This is an open multi-center prospective randomized phase II study of the use of azacitidine as consolidation therapy compared to conventional chemotherapy consolidation in patients of 65 years of age or older. Patients will be included in the study once they have achieved first CR or CRp following treatment with one or two courses of intensive induction chemotherapy according to national guidelines. Study patients will be randomized between the two arms of treatment.
Background and rationale	AML can occur at any age but the incidence increases with age and the median age of onset is 70 years. It is the most common acute leukaemia in adults. Standard frontline therapy of acute myelogenous leukemia (AML), including cytarabine and daunorubicin, results in complete response (CR) rates of 40% to 80% and in cure rates of 5% to 60%, depending on patient and disease characteristics such as chromosomal and molecular aberrations, age, performance status and impaired organ functions. However, results in elderly patients (≥65y) with AML are substantially worse; approximately 50% of patients > 65 years of age with AML will achieve CR. However, the majority will eventually relapse and die from their disease despite consolidation treatment. There is lack of data confirming that the present strategy with repeated courses of intense chemotherapy is of clear clinical benefit in the

	elderly population. Thus, a better treatment to prevent relapse in the older AML population is greatly needed. In high risk myelodysplastic syndromes (MDS), subcutaneous (sc) azacitidine prolongs survival, reduces the risk of progression to AML and is associated with higher rates of complete remission (CR) and partial remission (PR) as compared to conventional therapy. Azacitidine as monotherapy can induce complete remission in AML, although the reported remission rate is rather low, 20-30%. In patients with high-risk MDS and MDS-AML azacitidine seems to prolong survival when given as maintenance treatment following induction chemotherapy. No clinical studies have previously been performed (or are ongoing) where randomization is performed between conventional consolidation chemotherapy and consolidation with azacitidine in patients above 65 years with AML in first CR.
Number of Subjects	130 patients.
Study drug	Azacitidine will be provided by Celgene free of charge.
Treatment	Induction treatment: Daunorubicin and cytarabine (DA) in accordance with the Swedish National treatment program (version May 2012; www.sfhem.se) will be given as induction treatment. Induction treatment is daunorubicin 60 mg/m^2 x 1 (iv infusion) day 1-3 and cytarabine 1000 mg/m^2 x 2 (iv infusion) day 1-5. Doses may be reduced to daunorubicin 45 mg/m^2 x 1 (iv infusion) day 1-3 and cytarabine 1000 mg/m^2 x 2 (iv infusion) day 1-4 if judged necessary by the treating physician. Consolidation treatment: **Arm A:** Azacitidin 75mg/m2/d for 5 days given every 5 th week for 8 cycles. **Arm B:** Two courses of DA in accordance with the Swedish National treatment program (reduced doses).
Study duration	Subjects will followed up yearly for 5 years and will be taken off the study when either of the following events occur: ▪ Disease relapse requiring treatment ▪ Death ▪ At the patient's request / withdrawn consent
Subject Selection	**Inclusion criteria:** 1. Subjects ≥65 years of age at the time of signing the informed consent form 2. A confirmed diagnosis of AML according to the 2008 WHO classification 3. A documented CR or CRp achieved after one or two induction courses. **Exclusion criteria:**

	1. Subjects who are not considered to be candidates to complete the consolidation therapy due to medical or psychological reasons. 2. Subjects who have taken any investigational drugs or participated in an interventional clinical trial within 30 days prior to screening. 3. Patients with acute promyelocytic leukemia 4. Patients with t(8;21) or inv(16) 5. CNS leukemia 6. Patients with a previous diagnosis of MDS, i.e. AML preceeded by a diagnosed MDS 7. Subjects who are candidates for allogeneic stem cell transplantation (SCT) 8. Another cancer diagnosis with a life expectancy of less than two years
Study Endpoints	**Primary endpoint** LFS at 12 months after start of induction chemotherapy (DA) **Secondary endpoints** ▪ OS 2 years after start of induction chemotherapy (DA) ▪ Analysis of a broad spectrum of molecular and cellular events relating to the epigenetic status of the malignant cells. These events will be used to identify biomarkers for successful consolidation and to address whether AML cases that relapse while on the study are molecularly and epigenetically different between those given azacitidine or DA consolidation ▪ Treatment related morbidity and mortality ▪ SAE until two months after the last course of consolidation ▪ No of consolidation courses actually given ▪ No of days in hospital during the period of 12 months from start of consolidation treatment as well as quality of life during and after consolidation
Adverse events	All AEs will be recorded by the Investigator(s) from the time of signing the informed consent through the end of the designated follow-up period. The sponsor will inform relevant Regulatory Authorities and the Ethics Committee: ▪ of all relevant information about serious unexpected adverse events suspected to be related to the study medication that are fatal or life threatening as soon as possible, and in any case no later than seven days after knowledge of such a case. Relevant follow-up information for these cases will subsequently be submitted within an additional eight days. ▪ of all other serious unexpected events suspected to be related to the study medication as soon as possible, but within a maximum of fifteen days of first knowledge by the investigator.
Ethics	The sponsor will be responsible for ensuring compliance with applicable legal

	guidelines. Investigator must agree with this protocol and know in detail the properties of the drug used in this clinical trial. Investigator must provide the patient with a patient information sheet and help him/her to understand the explanation provided. It is important to tell the patient that his/her participation in the study is completely voluntary and that it will not affect patient-physician relationship. In addition, it will be guaranted that all people involved in the study will observe the confidentiality of any information related to the patient. The investigator is also responsible for ensuring that the clinical study is performed in accordance with the protocol, current guidelines on Good Clinical Practice (GCP, ICH/135/95), EU-directive (2001/20/EC) and applicable regulatory requirements. All participants in the study are covered by their respectively countries National Pharmaceutical Insurance Pool.

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1. Study description

1.1 Background

AML can occur at any age but the incidence increases with age and the median age of onset is 70 years (1). It is the most common acute leukaemia in adults. The incidence of AML is approximately 12/100 000 at the age of 65 years and approximately 21/100 000 at the age of 80 years.

Standard frontline therapy of acute myelogenous leukemia (AML), including cytarabine and daunorubicin, results in complete response (CR) rates of 40% to 80% and in cure rates of 5% to 60%, depending on patient and disease characteristics such as chromosomal and molecular aberrations, age, performance status and impaired organ functions (2). However, results in elderly patients (≥65y) with AML are substantially worse. The low rate of durable remissions in this patient population is mainly related to two factors. Firstly, biological markers such as intermediate or high risk cytogenetics, previous hematologic disease such as myelodysplasia or myeloproliferative disorders are more common among elderly AML. Secondly, elderly patients frequently suffer from pronounced co-morbidity, excluding the possibility to offer intensive (and more toxic) chemotherapy.

Following recent progress in the understanding of AML, there is an increased interest in new drugs targeting molecular aberrations, which define different subgroups of AML. However, trials with “target therapy” (e.g. Flt3 inhibitors) have so far shown only modest clinical benefit.

There is lack of data confirming that the present strategy with repeated courses of intense chemotherapy is of clear clinical benefit in the elderly population. Cytarabine with varied dose intensification compared to no consolidation offered somewhat longer disease-free survival with no difference in total survival (3,4) Two other studies comparing 3 *vs.* 4 and 1 *vs.* 4 courses of combination chemotherapies showed no advantage for the more intense regimens (5,6). Thus new methods to prevent leukemia relapse are urgently needed, in particular in patients with high-risk cytogenetics or poor prognosis molecular markers.

DNA methylation plays a key role in the pathogenesis of myelodysplastic syndromes (MDS) and AML. Hypermethylation, associated with leukemic progression and arrest of differentiation, has been detected in MDS and acute leukemia (7). There is a trend towards higher methylation rate in the AML patients with unfavorable karyotype. Hypermethylation of several genes (e.g. CDKN2B), involved in apoptosis and cell differentiation, is quite common in AML. Aberrant hypermethylation is controlled by the recently identified DNA-methyltransferas genes (DNMTs). AML cells with methylated CDKN2B express elevated levels of DNMTs. Unregulated DNMTs are considered to contribute to the pathogenesis of AML due to regional aberrant methylation.

Azacitidine is a pyrimidine nucleotide analogue of cytidine. It is a cell cycle specific cytotoxic agent, that is incorporated into DNA and produce a marked decrease in the activity of DNMT. In vitro exposure of AML cell lines to azacitidine resulted in induction of differentiation (8). The capability of azacitidine to initiate differentiation is linked to its activity as a hypomethylating compound, i.e. by blocking DNMTs enzymes it inhibits the methylation of the genome. Inhibition of DNA-methylation is considered to restore antiproliferative signals by blocking hypermethylation.

In AML, clinical experience with azacitidine as monotherapy is limited. De novo AML patients in varied age groups treated with azacitidine monotherapy within small studies showed a

remission rate of 20 – 30%. Azacitidine in combination with conventional chemotherapy increased, as expected, the remission rate to around 60–80%.

In high risk MDS, subcutaneous (sc) azacitidine prolongs survival, reduces the risk of progression to AML and is associated with higher rates of complete remission (CR) and partial remission (PR) as compared to conventional therapy according to physician´s choice (10,11). Significantly more azacitidine treated patients achieved erythrocyte transfusion independence and azacitidine reduced the number of days in hospital compared to MDS patients treated with conventional therapy (11).

A recent subgroup analysis of patients classified as having AML according to the current WHO criteria (bone marrow blasts 20-30%) includes 113 patients that received sc azacitidine at 75mg/mg/d for 7 days of every 28 day cycle, for at least six cycles. At a median follow up of 20.1 months, median OS for azacitidine – treated patients was 24.5 months compared with 16.0 months for the control group treated according to doctor´s choice (12). Finally, azacitidine was associated with fewer total days in hospital ($P<0.0001$) (12).
Similar results were observed in two smaller trials (13,14). For example, Sudan et al reported a 60% PR (CR-20%, PR-25%, HR-15%) in a cohort 24 AML patients with 21-38% blasts treated with azacitidine. Equivalent results were recently observed in AML and high risk MDS patients harboring chromosome 5 and 7 abnormalities (15).

1.2 Clinical pharmacokinetics and metabolism

Azacitidine is a nucleoside analog of cytidine. The clinical development of azacitidine has been sparked by its strong in vitro and in vivo antileukemic activity at cytotoxic concentrations, and its differentiation-inducing potential at lower concentrations in hematopoietic and non-hematopoietic cell line models. The primary activation of azacitidine is by uridine-cytidine kinase. Following phosphorylation to a monophosphate, azacitidine is incorporated into newly synthesized RNA and DNA. As a ribonucleoside, azacitidine is incorporated predominantly into RNA. Incorporation of azacitidine into newly synthesized DNA produces hypomethylated strands. Azacitidine inhibits the methylation of DNA by covalent binding to DNA methyltransferase. Azacitidine is deactivated by enzymatic deamination by cytidine deaminase, which results in the production of 5-azauridine. 5-azauridine has been shown to be a potent inhibitor of de novo pyrimidine synthesis.

Limited clinical pharmacokinetic data are currently available. Early PK studies used ^{14}C-radiolabeled azacitidine to evaluate drug disposition, and more recently, the PK of SC azacitidine in patients with MDS was evaluated. Azacitidine was absorbed when given SC, with maximum concentrations found 0.5 to 2 hours after dosing. Azacitidine and/or its metabolites were then cleared by the kidneys, and amount of radioactivity recovered in urine was 50% to 98% of administered dose. The mean plasma elimination half-life was 0.69 hr $\pm$ 0.14 in the MDS patients.

In an in vitro metabolism study, incubation of azacitidine in human liver fractions indicated that azacitidine may be metabolized by the liver. The potential of azacitidine to inhibit cytochrome P450 is unknown. Azacitidine is not an inducer of the isoenzymes 1A2, 2C19, or 3A4/5.

1.3 Clinical safety

Treatment with azacitidine is associated with anemia, neutropenia and thrombocytopenia. The most frequently observed non-hematological adverse events (AE) are nausea, vomiting and injection site reactions.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

2. Rationale

Approximately 50% of patients > 65 years of age with AML will achieve CR. However, the majority will eventually relapse and die from their disease despite the consolidation treatments that are given with the aim to prevent recurrence of the disease. Elderly patients may not benefit from intensive consolidation treatment to the same extent as younger patients do. Thus, a better treatment to prevent relapse in the older AML population is greatly needed.

Azacitidine as monotherapy can induce complete remission in AML, although the reported remission rate is rather low, 20-30%. In patients with high-risk MDS and MDS/AML azacitidine seems to prolong survival when given as maintenance treatment following induction chemotherapy (12,16).

No clinical studies have previously been performed (or are ongoing) where randomization is performed between conventional consolidation chemotherapy and consolidation with azacitidine in patients above 65 years with AML in first CR. The study will be performed in compliance with Good Clinical Practice (GCP).

3. Study design

This is an open multi-centre prospective randomized phase II study of the use of azacitidine as consolidation therapy compared to conventional chemotherapy consolidation in patients of 65 years of age or older.

Patients will be included in the study once they have achieved first CR or CRp following treatment with one or two courses of intensive induction chemotherapy according to national guidelines. Study patients will be randomized between the two arms of treatment (see section 6).

4. Objectives of study and study endpoints

4.1 Primary objective

Investigate whether consolidation treatment with repeated courses of azacitidine prolongs leukemia–free survival (LFS) as compared to standard consolidation chemotherapy in elderly (≥65y) patients with AML in CR1.

4.2 Secondary objectives

Study whether consolidation treatment with azacitidine, as compared with the control arm, improves overall survival (OS)

Assess the relationship between azacitidine treatment and DNA-methylation patterns
Assess the clinical outcome according to molecular mutations and cytogenetic aberrancies at diagnosis
Assess safety, tolerability of azacitidine when given after standard induction chemotherapy (DA) in elderly patients with AML
Assess quality of life in patients receiving consolidation with azacitidine and standard chemotherapy, respectively

4.3 Primary end-point

LFS at 12 months after start of induction chemotherapy (DA)

4.4 Secondary end-points

OS 2 years after start of induction chemotherapy (DA)

Analysis of a broad spectrum of molecular and cellular events relating to the epigenetic status of the malignant cells. These events will be used to identify biomarkers for successful consolidation and to address whether AML cases that relapse while on the study are molecularly and epigenetically different between those given azacitidine or DA consolidation
Treatment related morbidity and mortality
SAE until two months after the last course of consolidation
No of consolidation courses actually given.
No of days in hospital during the period of 12 months from start of consolidation treatment as well as quality of life during and after consolidation.

5. Criteria for patient selection

5.1 General considerations

Patients of the age 65 years or older with newly diagnosed *de novo* AML or secondary AML who have achieved a first CR after one or two DA induction treatment and are judged fit for consolidation treatment are eligible for enrolment in the study. Patients will be randomized (1:1) between the two treatment arms. 130 patients will be recruited to the study.

The specific inclusion and exclusion criteria for enrolling subjects in this study are described in the following sections. An inventory of patients fulfilling inclusion criterias but not included in the study will be kept locally at each participating center and validated in retrospect using the Swedish AML registry (>97% coverage).

5.2 Inclusion criteria

Subject has signed the informed consent and must satisfy the following criteria to be enrolled in the study:

1. Subjects ≥65 years of age at the time of signing the informed consent form
2. A confirmed diagnosis of AML according to the 2008 WHO classification
    - This includes: Bone marrow aspirate with a good clot or biopsy for morphology, flow cytometry, cytogenetic analysis, and in case of normal cytogenetics, molecular genetic analyses including FLT3-ITD, NPM-1 and CEBPA
    - Biobanking of leukemic cells at diagnosis performed (according to guidelines of the Swedish National AML biobank).
3. A documented CR or CRp achieved after one or two induction courses.

- CR is defined as
    - bone marrow blasts < 5% with regenerating hematopoiesis
    - no extramedullary leukemia
    - independency of erythrocyte transfusions
    - neutrophils > 1.0 x 10(9)/L
    - platelets > 100 x 10(9)/L

- Criteria for complete remission with low platelet count (CRp):
    - A patient fulfilling bone marrow and peripheral blood criteria for CR in AML but who has a lower platelet count than 100 x 10(9) may also be considered CR (CRp) provided platelet count is stable and greater than 30 x 10(9) and there is no bleeding symptoms.

5.3 Exclusion criteria

1. Subjects who are not considered to be candidates to complete the consolidation therapy due to medical or psychological reasons.
2. Subjects who have taken any investigational drugs or participated in an interventional clinical trial within 30 days prior to screening.
3. Patients with acute promyelocytic leukemia
4. Patients with t(8;21) or inv(16)
5. CNS leukemia
6. Patients with a previous diagnosis of MDS, i.e. AML preceeded by a diagnosed MDS
7. Subjects who are candidates for allogeneic stem cell transplantation (SCT)
8. Another cancer diagnosis with a life expectancy of less than two years

6. Induction treatment plan and dosing

Daunorubicin and cytarabine (DA) in accordance with the Swedish National treatment program (version May 2012; www.sfhem.se) will be given as induction treatment. Induction treatment is daunorubicin 60 mg/m^2 x 1 (iv infusion) day 1-3 and cytarabine 1000 mg/m^2 x 2 (iv infusion) day 1-5. Doses may be reduced to daunorubicin 45 mg/m^2 x 1 (iv infusion) day 1-3 and cytarabine 1000 mg/m^2 x 2 (iv infusion) day 1-4 if judged necessary by the treating physician.

If the criteria for CR or CRp are obtained after first or second induction, the patient can be included in the study and randomized between the two treatment arms. Randomization will be stratified according to risk groups;

Intermediate (non-adverse karyotype)
Adverse (abn 3q, -5, 5q$^-$, -7, 7q$^-$, abn 11q23, t(6;9) and/or complex karyotype, defined as ≥3 unrelated abnormalities). FLT3-ITD positive.

6.1 Consolidation treatment plan and dosing (see section 10.1)

Arm A: Azacitidine 75mg/m2/d (sc injection, 5 min) for 5 days given every 5th week for 8 cycles.

Arm B: Two courses of DA in accordance with the Swedish National treatment program (reduced doses):

In case one induction course was given:
First consolidation course: daunorubicin 45 mg/m^2 x 1 (iv infusion) day 1-3 and cytarabine 1000 mg/m^2 x 2 (iv infusion) day 1-4.

Second consolidation course: daunorubicin 45 mg/m^2 x 1(iv infusion) day 1-2 and cytarabine 200 mg x 2 (fixed dose sc injection) day 1- 5.

In case two induction courses were given:
First consolidation course: daunorubicin 45 mg/m^2 x 1(iv infusion) day 1-2 and cytarabine 200 mg x 2 (fixed dose sc injection) day 1- 5.

Second consolidation course: cytarabine 200mg x 2 (fixed dose sc injection) day 1-5.

6.1.1 Dose modification of azacitidine

Treatment with azacitidine is associated with anemia, neutropenia and thrombocytopenia. The most frequently observed non-hematological adverse events are nausea, vomiting and injection site reactions.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

Hematological Toxicity:
In case of cytopenia before planned cycle of azacitidine, with platelet count < 30 x 10^9, WBC < 1.0 x 10^9 or ANC < 0.5 x 10^9, the dose may be reduced according to the following scheme:

1. 60 mg/m^2
2. 45 mg/m^2
3. 30 mg/m^2

In case of stabilized levels of platelets, WBC and ANC, a return to the higher dose may be done. Any patient who experiences a hematological toxicity with platelet count < 15 x 10^9 or ANC < 0.5 x 10^9, that is an escalation from the status at baseline (prior to the first dose) should have azacitidine temporarily discontinued until the toxicity grade returns to the baseline value. G-CSF can be used to increase the ANC.

In any case of worsened cytopenia during azacitidine treatment, it is important to exclude relapse.

Non-Hematological Toxicity:
After any dose of azacitidine, subsequent cycles may be delayed if a subject experiences a non-hematological AE with NCI CTC toxicity grade 3 or 4, that is an escalation from the status at Baseline (prior to the first dose). Subject should have azacitidine temporarily discontinued until the toxicity grade returns to the baseline value. Azacitidine should be permanently discontinued if the non-hematological toxicity persists for more than 21 days, despite the temporary interruption of the study drug, or if the toxicity is life threatening.

7. Study evaluations

7.1 Study procedures

In general the patients are treated and monitored, including repeated blood samplig, according to local routines during active AML treatment. The following laboratory examinations are however mandatory.

At diagnosis

Bone marrow aspirate with a good clot or biopsy for morphology, cytogenetic analysis and in case of normal karyotype molecular genetic analyses including FLT-3-ITD, NPM-1, CEBPA. Flow cytometry, and biobanking according to the national AML biobank guidelines (see addendum) should be performed in all patients.

At inclusion (i.e. at remission evaluation) the following evaluations should be done

- General medical and hematological history
- Concomitant medication
- Physical examination and vital signs
- WHO performance status
- ECG

- Bone marrow aspirate with a good clot or biopsy for morphology, flow cytometry, and biobanking

- Hematology (Hemoglobin, white blood cell count (WBC) and differential, absolute neutrophil count (ANC), reticulocytes, platelet count).

- Serum chemistry (Total bilirubin, alkaline phosphate (ALP), ALT, AST, , serum creatinine, glucose, albumin, uric acid, lactose dehydrogenase (LDH), and electrolytes (sodium, potassium, calcium)

- Quality of life (QoL) questionnaire

After consolidation treatment (4 weeks after the last consolidation course) or at relapse

- Bone marrow aspirate with a good clot or biopsy for morphology, and bio bank.

- Hematology (Hemoglobin, (WBC) and differential, platelet count).

- Days of hospitalization during consolidation treatment.

- Number of blood and platelets transfusions

- Quality of life (QoL) questionnaire

- Patients shall be monitored according to local clinical routines but with a minimum of full blood counts every 4th week during two years after the start of induction therapy.

At the one and two year follow-ups

- Hematology (Hemoglobin, (WBC) and differential, platelet count).

- Days of hospitalization during consolidation treatment.

- Number of blood and platelets transfusions

- Quality of life (QoL) questionnaire

Yearly follow-up

- Survival and relapse status will be followed yearly five years after inclusion

Relapse definition and treatment

Bone marrow blasts ≥5%; or reappearance of blasts in the blood;
or development of extramedullary disease
Treatment of relapse is at the discretion of the investigator.

8. Study flow chart

	Screening	8 weeks (1)	39 weeks (2)	12 months	24 months	Yearly	At suspected relapse
General medical and hematological history	*						
Concomitant medication	*						
Physical examination and vital signs	*						
WHO performance status	*						
ECG	*						
Bone marrow aspirate	*	*(6)	*(6)				*
Biobank	*						*
Hematology (3)	*			*	*		*
Serum chemistry (4)	*						
Quality of life (QoL) questionnaire	*	*	*	*	*		
Cumulative days of hospitalization (5)		*	*	*	*		*
Number of blood and platelets transfusions (5)		*	*	*			*
Survival and relapse status				*	*	*	*

Patients will be monitored according to local clinical routines but with a minimum of full blood counts every 4th week during two years after the start of induction therapy

(1) In the DA-group at approximately 4 weeks after the last consolidation course

In the Azacitidine group 8 weeks after consolidation start

(2) In the Azacitidine group at approximately 4 weeks after last consolidation

In the DA-group at 39 weeks after the start of the first consolidation

(3) Hematology=Hemoglobin, white blood cell count (WBC) and differential, absolute neutrophil count (ANC), reticulocytes, platelet count

(4) Serum chemistry=Total bilirubin, ALP, ALT, AST, serum creatinine, glucose, albumin, uric acid, lactose dehydrogenase, and electrolytes (sodium, potassium, calcium)

(5) Since start of the first consolidation

(6) Remission bone marrow aspirate **once per patient** at approximately 4 weeks after the last consolidation course, i.e approximately week 8 in the DA-group and week 39 in the Azacitidine group.

9. Study termination/End of trial definition:

Subjects will be taken off the study when either of the following events occur:

- Disease relapse requiring treatment
- Death
- At the patient's request / withdrawn consent

The trial will be terminated 5 years after the inclusion of the last patient, unless closed prematurely at the interim analysis.

10. Statistical considerations:

This is an exploratory randomized phase II open label study. Based on data from the Swedish Adult Acute Leukemia Register a LFS of 50% at one year can be expected in the patients in the control arm. With 60 patients in each arm, a 76% LFS in the azacitidine arm is needed to reach a power of 80% at a significance level of 0.05. (To detect an increase in LFS from 50 to 60% with the same power and significance level, 410 patients would need to be recruited to each arm. The corresponding number of patients to detect an increase in LFS from 50 to 67% is 145.)

The sample size was based on the ability to detect, with a two-sided alfa of 0.05, an improvement in the 12 months leukemia free survival from 50 % in the standard arm to 76 % in the experimental arm (corresponding to 30 events in the standard arm versus 14 in the experimental arm) which would be a clinically meaningful improvement. With a power of 80 % the patient number would be 60 in each arm.

Evaluation of the primary end point (LFS) and of OS will be made on an intention to treat basis. All patients that were randomized will be included in the analysis. Prematurely withdrawn patients will be censored. LFS and OS will be compared using the Kaplan Meier method and the Log-Rank test.

Patients who are prematurely withdrawn from the study will not be replaced. With a calculated drop out rate of 8 % the total number of patients will be 130.

10.1 Randomization

Patients will be randomly assigned in blocks to consolidation with Azacitidine (arm A) or conventional DA (arm B) in a 1:1 ratio. The randomization list will be generated using www.randomization.com. Block size will be documented in the clinical trial report. Randomization will be conducted by a nurse at the Karolinska University Hospital, Solna. Randomization requests should be sent by fax to +46-8-517 719 10

11. Safety and adverse event reporting

11.1 Adverse events

An adverse event (AE) is any noxious, unintended, or untoward medical occurrence occurring at any dose that may appear or worsen in a subject during the course of a study. It may be a new inter current illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria below), regardless of etiology. Any medical condition that was present prior to study treatment and that remains unchanged or improved should not be recorded as an AE.

If there is a worsening of that medical condition this should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the Case Report Form rather than the individual signs or symptoms of the diagnosis or syndrome.
All AEs will be recorded by the Investigator(s) from the time of signing the informed consent through the end of the designated follow-up period.

11.2 Abnormal laboratory values defined as adverse events

An abnormal laboratory value is considered to be an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study.
- Requires treatment, modification/interruption of study drug dose, or any other therapeutic intervention.
- Is judged by the Investigator(s) to be of significant clinical importance.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE.

11.3 Serious adverse event

A serious adverse event (SAE) is any AE which:

- Results in death
- Is life-threatening (i.e., in the opinion of the Investigator(s) the subject is at immediate risk of death from the AE)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions)
- Constitutes an important medical event

Important medical events are defined as those occurrences that may not be immediately life threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

Events not considered to be SAEs are hospitalizations which: were planned before entry into the clinical study; are for elective treatment of a condition unrelated to the studied indication or its treatment; occur on an emergency outpatient basis and do not result in admission (unless fulfilling other criteria above); are part of the normal treatment or monitoring of the studied indication and are not associated with any deterioration in condition.

If an AE is considered serious, both the AE pages of the CRF and the SAE Report Form must be completed.

For each SAE, the Investigator(s) will provide information on severity, start and stop dates, relationship to study drug, action taken regarding study drug, and outcome.

11.4 Classification of severity

For both AEs and SAEs, the investigator(s) must assess the severity of the event. The severity of adverse events (AEs) will be graded on a scale of 1 to 5 according to the National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events Version 3.0 (NCI CTCAE). The NCI CTCAE V3.0 can be viewed on-line at the following NCI web site:

http://ctep.cancer.gov/reporting/ctc.html. If a specific event is not included in the NCI CTCAE toxicity scale, the following scale should be used to grade the event

Grade	Definition
1	**Mild** Awareness of sign, symptom, or event, usually transient, requiring no special treatment and generally not interfering with usual daily activities
2	**Moderate** Discomfort that causes interference with usual activities; usually ameliorated by basic therapeutic manoeuvres
3	**Severe** Incapacitating with inability to do usual activities or significantly affects clinical status and warrants intervention. Hospitalization may or may not be required
4	**Life-threatening** Immediate risk of death; requires hospitalization and clinical intervention.
5	**Death**

11.5 Classification of relationship/causality of adverse events (SAE/AE) to study medication

The Investigator(s) must determine the relationship between the administration of study drug and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below:

Not suspected: The temporal relationship of the adverse event to study drug administration makes a causal relationship unlikely or remote, or other medications, therapeutic interventions, or underlying conditions provide a sufficient explanation for the observed event

Suspected: The temporal relationship of the adverse event to study drug administration makes a causal relationship possible, and other medications, therapeutic interventions, or underlying conditions do not provide a sufficient explanation for the observed event.

11.6 Monitoring and reporting of adverse events

All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject's clinical symptoms; laboratory, pathological, radiological, or surgical findings; physical examination findings; or other appropriate tests and procedures.

AEs that cause a subject to discontinue study participation must be followed until either the event resolves, stabilizes or returns to baseline (if a baseline assessment is available).

11.7 Severe adverse event (SAE) reporting

Report of Adverse Events to Regulatory Authorities and the Ethics Committee

The sponsor will inform relevant Regulatory Authorities and the Ethics Committee:

- of all relevant information about serious unexpected adverse events suspected to be related to the study medication that are fatal or life threatening as soon as possible, and in any case no later than seven days after knowledge of such a case. Relevant follow-up information for these cases will subsequently be submitted within an additional eight days.
- of all other serious unexpected events suspected to be related to the study medication as soon as possible, but within a maximum of fifteen days of first knowledge by the investigator.

11.8 Immediate reporting by Investigator to Sponsor and Celgene

The investigator will inform the sponsor and Celgene of any serious adverse event. This applies to all SAEs, regardless of relationship to the study medication, that occur during the study, those made known to the Investigator(s) within 30 days after a subject's last dose of study drug, and those made known to the investigator(s) at any time that are suspected of being related to the study medication. This must be documented on an SAE form. This form must be completed in English and supplied to Celgene Europe Drug Safety within 24 hours/1 business day or at the latest on the following working day. The initial report must be as complete as possible, including details of the current illness and serious adverse event, and an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up SAE form.

All adverse event reports must include the patient number, age, sex, weight, severity of reaction, relationship to study drug, date and time of administration of test medications and all concomitant medications, and medical treatment provided

Contact details for Sponsor:

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11.8.1 Sponsor reporting to Celgene

The sponsor will provide Celgene with a copy of the annual safety report at the time of the submission to the regulatory authority and the Ethics Committee.

11.9 Adverse event updates/IND safety reports

Celgene shall notify the principle investigator and the sponsor via an IND Safety Report of the following information:

- Any AE associated with the use of study drug in this study or in other studies that is both serious and unexpected.
- Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

The principle investigator will forward the information to other investigators involved in the trial.

The sponsor shall notify the EC and the relevant regulatory authorities of any new significant risks to subjects as required.

11.10 Interim analysis

An interim analysis will be performed of primary outcomes and AE:s after 60 included patients. Recruitment will be paused until an independent safety committee evaluates and approves of study continuation.

11.11 Safety committee

A safety committee of three independent senior hematologists will be appointed at the start of the study.

12. Study medication materials and management

12.1 Supplier

Celgene Corporation will supply azacitidine (Vidaza®) for the duration of this trial.

12.2 Dosage Form

Azacitidine is a white powder. It is provided in vials containing 100 mg of azacitidine and 100 mg mannitol ready for reconstitution with water or aqueous solution. The reconstituted product is a homogenous, cloudy suspension that is free of agglomerates. Azacitidine should be reconstituted as described in the Vidaza® SmPC (17).

Reconstituted azacitidine will be injected subcutaneously into the upper arm, thigh, or abdomen. The site of injection should be rotated and subsequent injections should be at least 2,5 cm from the previous site. Azacitidine should not be injected at a site that is tender, bruised, red or hard. Doses greater than 4 ml should be divided equally into two syringes and injected at two different sites.

Azacitidine is a cytotoxic drug, and caution should be exercised when handling and preparing azacitidine suspensions.

12.3 Study Medication Packaging and Labeling

Colourless type I 30 ml glass vial sealed with butyl elastomeric stopper and aluminium seal with polypropylene plastic button.
Pack size: 1 labeled vial of 100 mg azacitidine.

12.4 Study Medication Recipient and Storage

The investigator(s) or designee(s) is responsible for taking an inventory of each shipment of azacitidine received, and comparing it with the accompanying azacitidine accountability form. The investigator(s) will verify the accuracy of the information on the form, sign and date it, retain a copy in the study file, and return to Celgene.

At the study site, all investigational study medications will be stored in a locked, safe area to prevent unauthorized access.

This medicinal product does not require any special storage conditions. For storage conditions of the reconstituted medicinal product, see SmPC (17)

12.5 Record of Administration

Accurate recording of all study medication administration (including dispensing and dosing) will be made in the appropriate section of the subject's CRF and source documents.

12.6 Study Medication Accountability

The investigator(s) or designee(s) is responsible for accounting for all study medication that is issued to and returned by the subject during the course of the study.

12.7 Study Medication Handling and Disposal

Unused study medication will be destroyed. If any study medication is lost or damaged, its disposition should be documented in the subject's CRF and source documents. Study medication supplies will also be destroyed at the end of the study.

13. Ethical aspects

In accordance with the Declaration of Helsinki/Tokyo/Venice (14) patients have the right to withdraw from the protocol at any time for any reason. The investigator also has the right to withdraw patients from the protocol in the event of inter current illness, adverse events and treatment failure after a prescribed procedure, protocol violence, cure, administrative reasons or other reasons. If a patient decides to withdraw from the protocol, all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation at the time of the patient's withdrawal should be made together with the reason.

The clinical trial sponsor is the physical person or legal entity which is interested in the performance of the trial, signs requests for authorization addressed to the Independent Ethics Committee (IEC) and regulatory authorities in respective countries and is responsible for the trial, including its performance, initiation and completion. The sponsor will be responsible for ensuring compliance with applicable legal guidelines.

Investigator must agree with this protocol and know in detail the properties of the drug used in this clinical trial. Investigator must provide the patient with a patient information sheet and help him/her to understand the explanation provided. It is important to tell the patient that his/her participation in the study is completely voluntary and that it will not affect patient-physician relationship. In addition, it will be guaranteed that all people involved in the study will observe the confidentiality of any information related to the patient. The investigator is also responsible for ensuring that the clinical study is performed in accordance with the protocol, current guidelines on Good Clinical Practice (GCP, ICH/135/95), EU-directive (2001/20/EC) and applicable regulatory requirements. All participants in the study are covered by their respectively countries National Pharmaceutical Insurance Pool.

14. References

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