The following protocol information is provided solely to describe how the authors conducted the research underlying the published report associated with the following article:

Multicenter, Randomized, Open-Label Phase III Trial of Decitabine Versus Patient's Choice, With Physician Advice, of Either Supportive Care or Low-Dose Cytarabine for the Treatment of Older Patients With Newly Diagnosed Acute Myeloid Leukemia

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2. OBJECTIVES

2.1 Primary Objective

To compare the overall survival in patients 65 years and older who have newly diagnosed *de novo* or secondary AML and either poor- or intermediate-risk cytogenetics who are randomly assigned to receive either decitabine or patient's choice with physician's advice of either supportive care or low-dose cytarabine.

2.2 Secondary Objectives

The secondary objectives of the study are:

- To compare complete remission rates between treatment arms.
- To characterize and compare the incidence and severity of toxicities in the two treatment arms.

2.3 Tertiary Objectives

The tertiary objectives of the study are:

- To compare cytogenetic complete remission rate between treatment arms.
- To compare patient quality-of-life measurements between treatment arms.
- To compare event-free survival and relapse-free survival between treatment arms.
- To determine the population pharmacokinetics of decitabine in poor- or intermediate-risk cytogenetic patients 65 years and older with AML when given this treatment regimen.

3. INVESTIGATIONAL PLAN

3.1 Summary of Study Design

This is a randomized, open-label, multicenter Phase 3 trial to compare low-dose decitabine to patient's choice with physician's advice of either supportive care or low-dose cytarabine in patients with newly diagnosed, histologically confirmed *de novo* or secondary AML. Patients must be at least 65 years of age and have poor- or intermediate-risk cytogenetics. All subtypes of AML are acceptable for this trial except acute promyelocytic leukemia (M3 classification).

It is anticipated that approximately 480 protocol-qualified patients will be randomly assigned in a 1:1 fashion to treatment arms in this study. Patients will be allocated to treatment groups by a central randomization procedure. Patients will be stratified according to ECOG performance status (0-1 versus 2), age (65-69 years versus 70+ years) and cytogenetics (poor-risk versus intermediate-risk).

Patients will be randomly assigned to either:

- Arm A: either supportive care OR 20 mg/m^2 cytarabine given subcutaneously once daily for 10 consecutive days repeated every 4 weeks; or,
- Arm B: 20 mg/m^2 decitabine as a 1-hour infusion once daily for 5 consecutive days every 4 weeks.

Supportive care (SC) for this trial is defined as treatment given with the intent to maximize quality of life without a specific antileukemic intent. SC specifically excludes surgery, immunotherapy, biologic therapy, radiotherapy (with the exception of palliative radiotherapy), anticancer hormonal therapy, and oral or systemic chemotherapy in which the goal is to either eradicate or slow the progression of the disease. Patients will receive SC as determined by their treating physician. Those therapies considered acceptable include, but are not limited to, treatment with antibiotics and antifungal agents, packed red blood cells or whole blood transfusions, fresh frozen plasma, platelet transfusions, nutritional support (enteral or parenteral), and/or focal external beam radiation given for symptomatic control of pain. Patients are allowed to receive erythropoietin and darbepoetin during this trial; however, use of G-CSF and GM-CSF is restricted to the treatment of severe infection. Patients are allowed to receive hydroxyurea until Cycle 1 Day 15. Hydroxyurea is not allowed after this time. For focal external beam radiation, the total dose delivered must be in the palliative range according to the institutional standards. For the purposes of this trial, palliative treatment is defined as treatments given

primarily to relieve pain. If it is unclear whether a therapy would be regarded as SC, the EMR study manager should be consulted. Additionally, it is recommended that patients be placed on a neutropenic diet, i.e. no fresh fruits or vegetables. All foods, especially meats, should be thoroughly cooked. Use of any SC therapy, including concomitant medications, hospitalizations, or palliative radiation, must be reported on the case report forms.

Patients on the SC arm will be considered on-study and will undergo the same study procedures (with the exception of study drug administration and associated procedures and timing of bone marrow aspirates after Cycle 5) required for patients receiving decitabine and cytarabine. For the SC arm, cycles will be defined as successive 4-week intervals, with the day of randomization defined as Day 1 of Cycle 1.

Patients will continue on study until: relapse or disease progression if on decitabine or cytarabine therapy; death; unacceptable toxicity; for patients on decitabine or cytarabine, no longer receiving clinical benefit from therapy; an intercurrent illness that prevents further administration of treatment; patient/physician request; or general or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator. Once the required number of events has been reached for the overall survival endpoint for the trial, data from the patients ongoing at that time will be censored; however, patients will be allowed to complete the study. Approximately 30 days after discontinuing from the study, patients will be assessed for adverse events, survival, and relapse or disease progression, if applicable. Thereafter, only treatment-related adverse events, new or continuing will be reported and/or followed until resolution, medically acceptable endpoint or the patient is lost to follow-up. After this 30-day period, patients will be followed for survival and disease progression (if applicable) every month for 2 years after date of randomization and every other month thereafter for 3 years or until death or lost to follow-up.

Bone marrow biopsies and aspirates must be performed at screening for all patients. Cytogenetic testing must be performed at screening. In addition, it is recommended that screening samples be analyzed by flow cytometry. Bone marrow aspirates must be performed within 7 days before Day 1 of Cycles 3 and 5 and at the end-of-study visit for all patients. Bone marrow aspirates must be performed at every 2nd cycle thereafter for patients receiving either decitabine or cytarabine and at every 3rd cycle thereafter for patients receiving supportive care. It is

recommended that bone marrow biopsies in addition to the aspirates be performed at the on-study visits, especially if a patient is suspected of having a complete or partial response to therapy. Patients having a documented complete remission or complete remission with incomplete platelet recovery are required to have additional bone marrow assessments with cytogenetics at the end of the first and third cycles post initial documentation of CR. After this, patients with CR are not required to have additional bone marrow assessments until clinically indicated. A complete blood count (CBC) with differential will be collected every 2 weeks. Patients will be assessed for remission rate according to the most current modified IWG criteria.[1] Bone marrow evaluations will be performed by a central reviewer in order to ensure consistency and accuracy of assessment criteria across all sites.

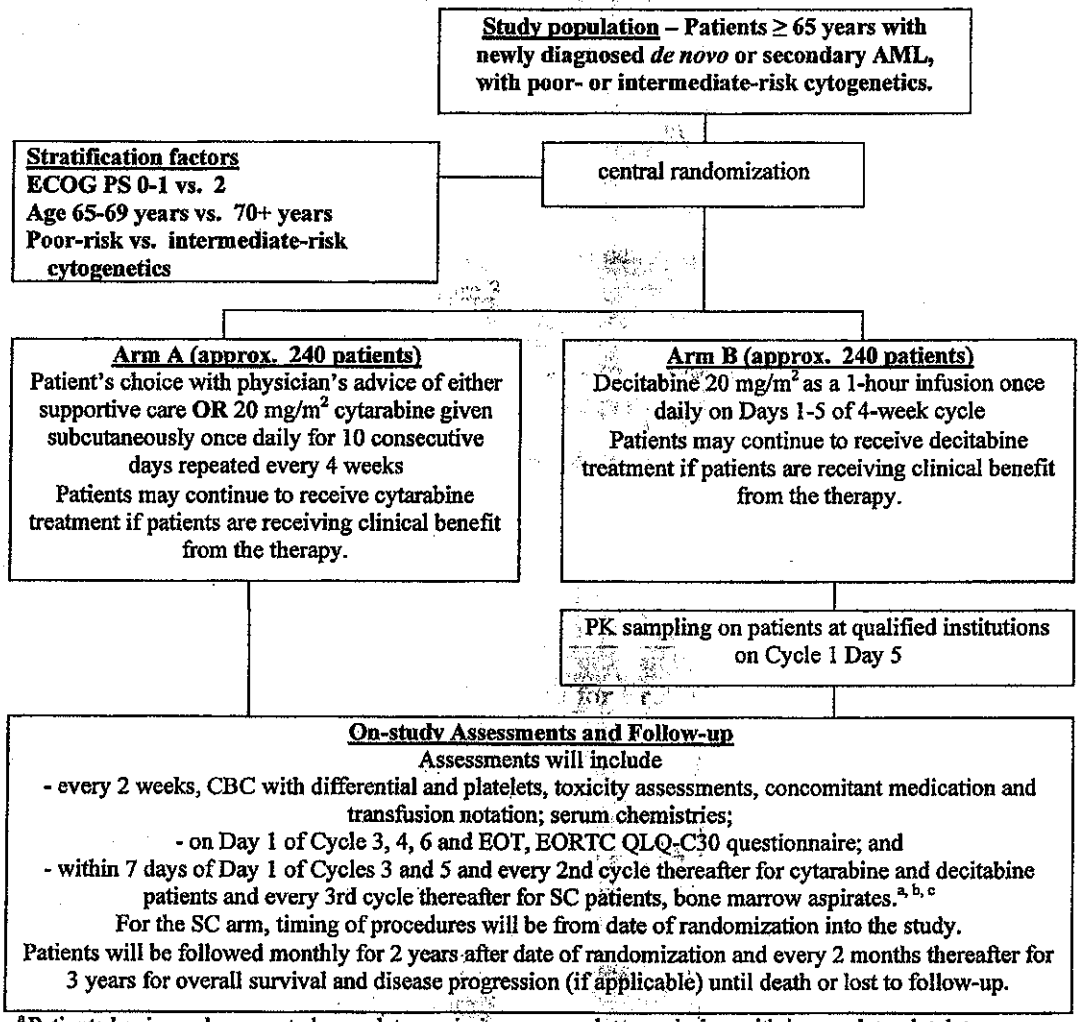
Arm B patients at qualified institutions will have PK sampling performed. Blood samples for PK evaluation will be collected on Cycle 1 Day 5 immediately before the start of the decitabine infusion (predose), 60 (immediately preceding the end of infusion), 90 minutes, 2 and 3 hours after the start of decitabine infusion.

All patients will complete at the site the EORTC QLQ-C30 quality-of-life questionnaire[50] at baseline, on Day 1 of Cycles 3, 4 and 6 (before first dose of study drug for patients receiving decitabine or cytarabine) and at the end-of-therapy visit.

Correlative studies with additional tissue and/or blood collection may be performed at some institutions on patients agreeing to this additional analysis. If studies are performed, a protocol defining the clinical and scientific rationale for this testing as well as the type of test and sample required will be submitted. Any such correlative study is likely to investigate predictive factors for success or failure of the therapy.

The study design is illustrated in Figure 3-1.

Figure 3-1 Illustration of study design for Protocol DACO-016



[a] Patients having a documented complete remission or complete remission with incomplete platelet recovery are required to have additional bone marrow assessments with cytogenetics at the end of the first and third cycles post initial documentation of CR. After this, patients with CR are not required to have additional bone marrow assessments until clinically indicated.
[b] It is recommended that bone marrow biopsies be obtained at each assessment.
[c] If a complete or partial response is suspected, a bone marrow biopsy in addition to the aspirate should be obtained for confirmation.

3.2 Discussion of Design and Control

3.2.1 Rationale for Study Design

A randomized, open-label, group sequential trial with a patient's choice with physician's advice control is appropriate for this population.

3.2.2 Rationale for Degree of Blinding

Due to the side effect profile and the method of administration of decitabine, and the patient's choice control arm, sufficient blinding is not feasible.

3.2.3 Minimization/Control of Bias

3.2.3.1 Randomization Procedures

Assignment of treatments to patients will be carried out using a stratified permuted block randomization method by way of an interactive voice response (IVR) system. Once a study candidate has been formally identified, participating centers are to call into the IVR system, follow the voice commands, and enter the required information to obtain a treatment assignment. Worksheets will be provided to facilitate this process. Relevant personnel at the Sponsor and at the participating center will receive a fax and/or email confirming the assigned treatment.

Randomization will be based on age (65-69 versus 70+), cytogenetics (intermediate versus poor), and ECOG score (0-1 versus 2). Each of the 8 schedules will be created by "stacking" randomly selected permuted block sizes of 2 or 4 until a sufficient number of assignments are generated. The maximum potential treatment imbalance is 16.

Age, cytogenetics, and performance status are significant prognostic factors.[7] Stratifying on these factors will help to achieve comparability between the treatment groups.

3.2.3.2 Other Steps to Minimize Potential Bias

Allocation concealment – The patient, investigator, site personnel, and sponsor personnel will only know the treatment assignment for a given patient after the patient has been randomized into the study.

Blinded outcome assessment – All bone marrow assessments used for response criteria will be centrally reviewed in a blinded fashion.

Dissemination of ongoing trial information – Any information about trial results must be subjected to review and approval by the Steering Committee in consultation with the independent Data Monitoring Committee prior to dissemination.

3.3 Study Population

Patients ≥ 65 years of age with newly diagnosed, histologically confirmed *de novo* or secondary AML who have intermediate- or poor-risk cytogenetics are eligible for this trial. *De novo* AML

is defined as AML with no prior history of MDS, myeloproliferative disorder, or exposure to potentially leukemogenic therapies or agents; secondary AML is defined as AML secondary to prior MDS, myeloproliferative disorder or the development of AML secondary to proven leukemogenic exposure.[1]

3.3.1 Criteria for Enrollment

3.3.1.1 Inclusion Criteria

Patients may be included in the study only if they meet **all** of the following criteria:

3. Must sign an institutional review board/ethics committee-approved informed consent.
4. Must have a newly diagnosed, histologically confirmed *de novo* or secondary AML using the World Health Organization classification (e.g., ≥20% blasts).[51]
5. Must be at least 65 years of age and have either poor- or intermediate-risk cytogenetics as categorized by Southwest Oncology Group.[52]
6. Must have a performance status of 0-2 on the Eastern Cooperative Oncology Group scale (see Protocol Appendix 1).
7. Must have adequate organ function, defined as:
   - Hematology: WBC ≤ 40,000/mm;
   - Hepatic: Bilirubin ≤ 1.5 times the upper limit of normal, AST or ALT ≤ 2.5 times the upper limit of normal; and
   - Renal: Creatinine clearance (calculated by the Cockroft and Gault method) ≥40 mL/min;
8. Must have a life expectancy of at least 12 weeks.

3.3.1.2 Exclusion Criteria

1. Must not have acute promyelocytic leukemia (M3 classification).
2. Must not have t(8;21), inv(16) or t(15;17) karyotype abnormalities.
3. Must not have known CNS leukemia.
4. Must not have any other active systemic malignancies.
5. Must not have unstable angina or New York Heart Association (NYHA) class 3 or 4 congestive heart failure (see Appendix 3).
6. Must not have inaspirable bone marrow.
7. Must not have received previous chemotherapy (except hydroxyurea), including azacytidine (Vidaza™), cytarabine or decitabine, for any myeloid disorder.
8. Must not have chronic respiratory disease that requires continuous oxygen use.
9. Must not have received any experimental drug within 4 weeks of randomization.
10. Must not be a candidate for a bone marrow or stem cell transplant within 12 weeks after randomization.
11. Must not have any concomitant medical or psychiatric disorders incompatible with the study (at the discretion of the investigator).
12. Must not have any comorbidity that causes organ dysfunction that is not related to leukemia.
13. Must not have an uncontrolled active infection(s) requiring IV antibiotics.
14. Must not have known HIV.

15. Must not have had radiotherapy for extramedullary disease within 2 weeks prior to study randomization.

3.3.2 Discontinuations

Patients may remain on study until one of the following occurs:

- death;
- for patients receiving decitabine or cytarabine, relapse or disease progression (see Section 3.5.5.);
- for patients receiving decitabine or cytarabine, no longer deriving clinical benefit from therapy;
- unacceptable toxicity;
- intercurrent illness that prevents further administration of treatment;
- patient/physician request; or,
- general or specific changes in the patient's condition that renders the patient unacceptable for further treatment in the judgment of the investigator.

When patients discontinue from the study or discontinue study treatment (cytarabine or decitabine), end-of-study procedures will be performed as described in Section 3.4.8 and in the Study Flow Chart (Appendix 2). However, all patients will be followed until death, lost to follow-up or 5 years post date of randomization.

3.4 Study Procedures

Study procedures and their timing are summarized in the Study Flow Chart, Appendix 2.

3.4.1 Prestudy Procedures (within 2 weeks prior to randomization)

1. Document patient adherence to all inclusion and exclusion criteria (see Sections 3.3.1.1 and 3.3.1.2).
2. Obtain informed consent for patient participation in this trial.
3. Bone marrow aspirate/biopsy assessment, including peripheral blood smear and cytogenetic analysis from aspirate. Peripheral blood smear, biopsy and aspirate are to be sent to central hematologic reviewer (see Section 3.7.2.1.1).
4. Hematology,[a] including RBC, hemoglobin, hematocrit, WBC, differential and platelets.
5. Blood chemistry,[a] including sodium, total protein, potassium, chloride, CO_2, calcium, blood urea nitrogen (BUN), creatinine, glucose, albumin, alkaline phosphatase, total bilirubin, aspartate transaminase (SGOT/AST) or glutamic pyruvic transaminase (SGPT/ALT), and lactate dehydrogenase (LDH).
6. Physical exam including height, weight, vital signs (blood pressure, pulse, respirations and temperature) and ECOG performance score.
7. ECG
8. Medical history.
9. Complete EORTC QLQ-C30 quality-of-life questionnaire.

6. Complete EORTC QLQ-C30 quality-of-life questionnaire.
7. Physical exam, including assessment of ECOG performance status, vital signs and weight.
8. Bone marrow aspirate, including peripheral blood smear. A bone marrow biopsy, in addition to the aspirate, is recommended. Samples are to be sent to central hematologic reviewer (see Section 3.7.2.1.1). If a patient is suspected of having a complete or partial remission, it is highly recommended that a bone marrow biopsy be obtained.

3.4.9 Post End-of-study Follow-up - All Patients

Approximately 30 days after discontinuing from the study, patients will be assessed for adverse events, survival and relapse or disease progression, if applicable. Thereafter, only treatment-related adverse events, new or continuing will be reported and/or followed until resolution, medically acceptable endpoint or the patient is lost to follow-up.

After this 30-day period, patients will be followed for survival and disease progression (if applicable) every month for 2 years after date of randomization and every other month thereafter for 3 years or until death or lost to follow-up.

3.5 Dosage and Administration

3.5.1 Materials and Supplies

Decitabine

Decitabine (5-aza-2'-deoxycytidine) is supplied as a lyophilized powder for injection, 50 mg in 20 ml vials. It is supplied by EMR, Ridgefield Park, NJ. The vials of decitabine must be stored at controlled room temperature per USP.

Each vial of decitabine is labeled with the following information:

- Study drug name
- Lot number
- Amount of drug per vial
- Name and address of sponsor
- Storage conditions
- Name of manufacturer
- Caution Investigational Drug statement

Cytarabine

EMR will supply the cytarabine used in this study. See the manufacturer's product information for further information.

3.5.2 Drug Inventory and Dispensing

Decitabine and cytarabine

An initial supply of decitabine and cytarabine will be shipped to each site's pharmacy when all initiation documents, including IRB/ethics committee (EC) approval and IRB/EC-approved Informed Consent, have been received and reviewed by the sponsor or designee and occurs upon activation of the site via the IVRS.

An accurate record of shipment and dispensing of the study drug will be maintained by the Principal Investigator or their designee. These records will be available for inspection by EMR, their representatives, and the regulatory authorities of applicable counties at any time. A copy of the study drug inventory will be provided to the sponsor at the conclusion of the study or as the record is completed.

Drug supplies for this study are to be used only in accordance with this protocol and under the supervision of the Principal Investigator. The Principal Investigator must insure that any unused study drug be returned to EMR or destroyed as authorized by EMR. Periodically throughout and at the conclusion of the study, supplies of decitabine or cytarabine will be inventoried by EMR or their designee.

An initial supply of decitabine and cytarabine will be provided to each site's pharmacy. Thereafter, it is the responsibility of the trial pharmacist to order a resupply.

The investigator and pharmacist of each site will be responsible for dispensing clinical supplies, for exercising accepted medical and pharmacy practices, and maintaining accurate records of receipt, dispensing and returning of decitabine and cytarabine, including date(s) received and total number of vials received.

In addition, accurate records must be kept regarding when and how much of each study drug (decitabine and cytarabine) was dispensed to and used by each individual patient in the trial. Reasons for departure from the expected dispensing regimen must also be recorded. Study drug inventory will be verified at each monitoring visit throughout the study. A copy of the study drug inventory will be provided to the sponsor at the conclusion of the study or as the record is completed. At the completion of the study, unopened decitabine and cytarabine vials will be returned to the sponsor for destruction or alternatively disposed of as authorized by the sponsor.

3.5.3 Study Drug Preparation and Administration

OSHA Guidelines for handling cytotoxic drugs outlined in the American Journal of Hospital Pharmacy must be followed.[53]

Decitabine

Decitabine (5-aza-2'-deoxycytidine) is supplied as a lyophilized powder for injection, 50 mg in 20-ml vials. When reconstituted with 10 ml of sterile water for injection, each ml will contain 5 mg of decitabine and 6.8 mg of KH_2PO_4. The reconstituted solution should be diluted to 0.1-1.0 mg/mL with pre-chilled 0.9% sodium chloride intravenous infusion, 5% glucose IV infusion, or lactated Ringer's injection USP, which has been cooled to 2 - 8 °C. Reconstitution of the powder results in a rapidly decomposing solution. Thus, the solution should be used as soon as possible. The diluted solution must be stored at 2 - 8 °C for a maximum of 7 hours prior to administration.

Decitabine at a concentration of 20 mg/m^2 should be given as a 1-hour IV infusion once daily on Days 1 through 5 of a 4-week treatment cycle.

Cytarabine

Cytarabine (cytosine arabinoside; Ara-C) for this study will be supplied by EMR. See the manufacturer's product information for the proper procedure for preparing and storing the product.

Cytarabine at a concentration of 20 mg/m^2 should be given subcutaneously once daily for 10 consecutive days of a 4-week treatment cycle.

3.5.4 Compliance

Decitabine and cytarabine will be administered intravenously or subcutaneously, respectively, at the investigational sites. Monitoring of patient compliance is ensured.

3.5.5 Cycle Delays and Therapy Discontinuation for Progressive Disease

All patients may receive hydroxyurea until Cycle 1 Day 15. Hydroxyurea may not be administered after this time. Study drug therapy will not be administered until the absolute blast count is less than 30,000/μL. If higher, see definition of progressive disease below.

Myelosuppression is a common toxicity resulting from the use of the study drugs. Treatment will be delayed at the discretion of the investigator if patients are experiencing disease-related

complications, most commonly infections or hemorrhages. Treatment may resume when the complications have improved or resolved.

Infections

Treatment will be delayed at the discretion of the investigator if patients are experiencing any of the following:

- Febrile neutropenia (defined as temperature at least 38.5 °C when the ANC is < 1000/μL)
- Infection documented clinically and/or microbiologically with Grade 3 or 4 neutropenia with a Grade 3 or 4 absolute neutrophil count (ANC < 1000/microliter)

Hemorrhage

Treatment will be delayed at the discretion of the investigator if patients are experiencing any of the following:

- Any hemorrhage occurring within the central nervous system
- Any gastrointestinal, genitourinary or pulmonary hemorrhage with Grade 4 thrombocytopenia (platelets < 25,000/microliter)

Renal/hepatic dysfunction

If renal dysfunction (creatinine clearance <50 mL/min) or hepatic dysfunction (bilirubin > 1.5 X ULN or AST/ALT > 2.5 X ULN for either cohort) occurs, therapy should not resume until the abnormalities have resolved, at the discretion of the investigator. Patients who are clinically benefiting from therapy and who develop renal or hepatic dysfunction as defined above may be retreated at the discretion of the investigator. If renal or hepatic dysfunction occurs during a treatment course, the next dose of decitabine or cytarabine may be held, and serum chemistries should be rechecked. Study therapy may be resumed when creatinine, bilirubin, and AST or ALT are below the limits listed above. If renal or hepatic dysfunction persists for > 4 days, that cycle of study drug should be held.

Retreatment or discontinuation for suspected progressive disease:

Progressive Disease During the First Cycle

Determination of progression during the first cycle will be based on peripheral blood counts, evidence of new extramedullary disease and/or the clinical judgment of the investigator. If the blast count in peripheral blood increases by > 50% over the pre-treatment value during the first cycle, the patient may be removed from the study for progressive disease. A bone marrow

assessment is recommended in order to determine disease progression for a patient having a >50% increase in peripheral blasts. A > 25% increase in the blast count from baseline on bone marrow aspirate is considered progressive disease. More than one cycle of treatment is likely to be required to achieve the full effectiveness of decitabine.

Progressive Disease During Subsequent Cycles

During the subsequent cycles, progressive disease will be defined as > 50% increase in peripheral blast count from baseline, > 25% increase in the blast count from baseline on bone marrow aspirate collected at least 7 days prior to every 2nd cycle beginning at Cycle 3 (and at the end of the first and third cycles post-documentation of CR) or as clinically indicated, evidence of new extramedullary disease or the clinical judgment of the investigator. Decision for discontinuation for disease progression should be based on bone marrow assessment. A > 25% increase in the blast count from baseline on bone marrow aspirate is considered progressive disease.

3.6 Concomitant Therapy

Hydroxyurea: Hydroxyurea will be allowed for all patients until Day 15 of Cycle 1. Thereafter, hydroxyurea will not be allowed for any patient on this study.

Packed red blood cells transfusion: Two units to be administered when the hemoglobin drops under 8 g/dl, or according to institutional standards. Transfusing with a hemoglobin of 8-10 g/dl is left to the investigator's clinical judgment.

Platelet transfusion: Five units (or one unit single-donor platelets) to be administered when platelets are < 10,000/μL or according to institutional standards.

Prophylactic antibiotics will be administered according to institutional standards.

Management of febrile neutropenia: Febrile neutropenia is defined as temperature at least 38.5 °C when the ANC is < 1000/μL. Febrile patients are to be evaluated by physical exam, CBC with differential, and blood culture. Patients with febrile neutropenia or suspected sepsis on the basis of physical exam are to be hospitalized for appropriate broad-spectrum antibiotic coverage, consistent with local pathogen sensitivities.

Growth Factors – All patients are allowed to receive erythropoietin and darbepoetin during this trial. Use of these agents should be guided by accepted practice or institutional guidelines.[54] G-CSF and GM-CSF are restricted to the treatment of severe infection.

Patients cannot have received chemotherapy, including azacytidine (Vidaza™), decitabine, cytarabine, for any myeloid disorder prior to study randomization.

Patients cannot have received any experimental therapy within 4 weeks or radiotherapy for extramedullary disease within 2 weeks before study randomization and must have recovered from any treatment-related toxicities. Other than the study agents, patients cannot receive chemotherapy during this trial.

3.7 Efficacy and Safety Measures

3.7.1 Primary Efficacy Measure – Overall Survival

Overall survival (OS) is measured from the date of randomization to the date of death from any cause.

3.7.1.1 Rationale for Choosing Endpoint

OS is an appropriate measure of clinical benefit in this population. It is readily measured and longer survival per treatment arm is a clear indication of treatment benefit.

3.7.1.2 Endpoint Threshold

The overall Type I error rate will be controlled at 0.025 using the Lan Demets alpha spending function of the O'Brien-Fleming type.[2]

3.7.2 Secondary Efficacy Measures

Patients will be assessed for remission rate and overall response to therapy according to the IWG criteria.[1] Disease assessments based on bone marrow evaluations and peripheral blood counts will be conducted by one central reviewer, blinded to the assessment of the investigator. Cytogenetics will be evaluated at the local site.

3.7.2.1 Complete Remission Rate and Duration of Complete Remission

Morphologic complete remission consists a morphologic leukemia-free state defined as less than 5% blasts in an aspirate sample with marrow spicules and with a count of at least 200 nucleated cells (there should be no blasts with Auer rods or persistence of extramedullary disease) PLUS ANC > 1,000/μL, platelet count ≥ 100,000/μL and independent of transfusions

for at least 1 week immediately before each assessment. There is no duration requirement for confirmation of this designation.

Morphologic complete remission with incomplete platelet recovery is defined as morphologic complete remission without the requirement of a platelet count of ≥100,000/μL.

Cytogenetic complete remission – Defined as morphologic complete remission plus a reversion to a normal karyotype. For this study, reversion to a normal karyotype is defined as no clonal abnormalities detected in a minimum of 20 mitotic cells.

Treatment Failure includes all patients who have died, developed progressive disease or discontinued due to a treatment-related adverse event.

Progressive Disease During the First Cycle

Determination of progression during the first cycle will be based on peripheral blood counts, evidence of new extramedullary disease and/or the clinical judgment of the investigator. If the blast count in peripheral blood increases by > 50% over the pre-treatment value during the first cycle, the patient may be removed from the study for progressive disease. Bone marrow assessments recommended in order to determine disease progression for a patient having an >50% increase in peripheral blasts should be considered. Patients have > 25% increase in the blast count from baseline on bone marrow aspirate should be considered to be progressing.

Progressive Disease During Subsequent Cycles

During the subsequent cycles, progressive disease will be defined as > 50% increase in peripheral blast count from baseline, > 25% increase in the blast count from baseline on bone marrow aspirate collected at least 7 days prior to every 2nd cycle beginning at Cycle 3 (and at the end of 1 and 3 cycles post-documentation of CR) or as clinically indicated, evidence of new extramedullary disease or the clinical judgment of the investigator.

Recurrence or morphologic relapse or a relapse from complete remission is defined as reappearance of blasts in the blood or the finding of ≥5% blasts in the bone marrow, not attributable to any other cause. If there are no blasts in the peripheral blood and 5-20% blasts in the bone marrow, bone marrow biopsy should be repeated in > 1 week to confirm relapse.

NOTE: The following two response assessments are included only as means of determining treatment duration and will not be used for efficacy assessment.

Partial remission is defined as a decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate (a value ≤ 5% may also be considered a partial remission if Auer rods are present), ANC > 1,000/μL and platelet count ≥ 100,000/μL.

Stable disease – includes all patients who fail to qualify for a complete or partial remission and who do not have progressive disease.

3.7.2.1.1 Shipping and Processing of Marrow Specimens for Central Pathology Review

Marrow and blood smears will be sent for central pathology evaluation and review. Bone marrow biopsies and aspirates are required at baseline. Bone marrow aspirates are required within 7 days before Day 1 of Cycles 3 and 5 and at the end-of-study visit for all patients; at every 2nd cycle thereafter for patients receiving either decitabine or cytarabine; and at every 3 cycles thereafter for patients receiving supportive care. Bone marrow biopsies (in addition to the required aspirates) are recommended at the on-study visits, especially if a patient is suspected of having a complete or partial response to therapy. Bone marrow assessments are required at the end of the first and third cycles post initial documentation of CR for patients having a documented complete remission or complete remission with incomplete platelet recovery. A bone marrow clot section is required for all assessments in which biopsies are not obtained.

Essential for evaluation:

1. Bone marrow aspirate smears (2 or more, stained or not stained with one iron stained slide or extra unstained slide)
2. Bone marrow core biopsy slide (or paraffin block)
3. Bone marrow clot section slide (or paraffin block)
4. Peripheral blood smear slide with corresponding CBC report.

Preferable (if available), but not essential for thorough evaluation:

1. Corresponding flow cytometry report

See Quest Study Manual for specific instructions.

3.7.2.2 Event-free Survival and Relapse-free Survival

Event-free survival is defined as the interval from the date of randomization to date of treatment failure, recurrence, death due to any cause, or lost to follow up.

Relapse-free survival is defined only for those patients achieving a complete remission. It is defined as the interval from the date of first documentation of a leukemia-free state to the date of recurrence, death due to any cause or loss to follow up.

3.7.3 Pharmacokinetic Measures

At qualified institutions, patients randomly assigned to Arm B may participate in the PK assessments. Plasma concentrations of decitabine will be measured on Cycle 1 Day 5 only.

3.7.3.1 Blood Samples

Blood samples should be drawn from a peripheral venous access from the arm opposite to the arm of study drug administration.

Timing of blood draws For Cycle 1, Day 5:

- immediately before the beginning of decitabine infusion (predose),
- 60 minutes after the beginning of decitabine infusion (immediately prior to end of infusion),
- 90 minutes after the beginning of decitabine infusion,
- 2 hours after the beginning of decitabine infusion, and
- 3 hours after the beginning of decitabine infusion.

If the end of infusion does not correspond (within 5 minutes) to 60 minutes after the start of decitabine infusion, it is preferred that that PK sample be taken just prior to the end of infusion, rather than 60 minutes after beginning of infusion. A total of 15 mL of blood will be drawn for the PK evaluations.

Blood samples for decitabine pharmacokinetic studies must be processed immediately (within 30 minutes) after collection. Approximately 3 mL of blood will be collected in a 3-mL K_3 EDTA tubes (purple top tubes). The K_3 EDTA tubes (3 mL) will be pre-loaded with 8 µL of tetrahydrouridine (THU-500 µg/mL). The K_3 EDTA tubes pre-loaded with THU are stable for 30 days at 4°C. Immediately upon collection, mix the sample by **gently** inverting the tube 8 to 10 times and then immediately place the tube in a container of wet ice. Sample must be kept on wet ice until centrifuged. Blood samples should be centrifuged (within 30 minutes after collection) at 1,800 x g for 10 min at 4°C. The resulting plasma is transferred into a screw top 2 mL cryovials and stored at -70°C or below until analyzed. Plasma tubes will be kept frozen at this temperature. Actual clock time and date of all blood draws will be accurately recorded in the CRF. A label will be securely affixed with the following information on the screw top cryovial: protocol number, patient's initials, patient's identification number, sample collection date and military time. Use a waterproof felt tip marker to write the required information prior to storing the plasma. Samples from each patient will be stored as a package for that patient.

3.7.3.2 Plasma Storage

Store the plasma samples for a minimum of 8 hours at -70°C or below until packaging for shipment to XenoBiotic Laboratories. Samples should be sent to Xenobiotic Laboratories within one week. Samples should **NOT** be stored longer than 10 days.

3.7.3.3 Plasma Sample Transport

Samples should be packaged for shipment in triple packaging (primary and secondary receptacles into outer packaging), which will be supplied by EMR, sealed, cushioned and containing a **sufficient** amount of crushed **dry ice** to last the duration of transport. The shipment will also include an itemized inventory by patient of all plasma tubes (using sample transmittal form) contained in the shipment. Clearly mark the package "Diagnostic Specimen," affix "Dry Ice" label and mail to

Ms. Pamela Stanley-Millner, SRO
XenoBiotic Laboratories, Inc.
107 Morgan Lane
Plainsboro, NJ 08536
Tel: 609-799-2295 x252, Fax: 609-799-7497

In addition, a notice of the expected shipment will be faxed **24 hours PRIOR** to shipment to the attention of Ms. Pamela Stanley-Millner, SRO at 609-799-7497. Pre-printed FedEx mailers will be supplied to the sites. Samples are to be sent via FedEx by overnight priority delivery and should not be sent over the weekend or the day before any observed holiday.

3.7.3.4 Plasma Sample Assay

At XenoBiotic Laboratories, plasma samples will be assayed for decitabine concentrations using a validated LC/MS/MS method.[55] The data from these analyses and the bioanalytical report will be electronically transferred to EMR for pharmacokinetic analyses. This complete bioanalytical report, describing the assay methodologies and results, will be included as an appendix to the pharmacokinetic report which is contained within the integrated study report.

3.7.4 Safety

Safety evaluation will include assessments of adverse events (AEs), medical history, physical examinations, vital signs, concomitant medications, and laboratory assessments at baseline and throughout the study period. A central laboratory (Covance [Indianapolis, IN]) will be used for scheduled laboratory assessments.

Investigators are responsible for monitoring the safety of patients who have entered this study and for alerting EMR or representative to any event that seems unusual, even if this event may be considered an unanticipated benefit to the patient.

The investigator is responsible for appropriate medical care of patients during the study and remains responsible for following Aes that are serious or that caused the patient to discontinue from the study.

All Aes including SAEs:

- will be recorded for all patients beginning immediately after the patient is randomly assigned to a treatment group until 30 days after the date of study discontinuation. The patient should be followed until the event resolves or reaches a medically acceptable outcome or the patient is lost to follow-up.
- After the 30-day period, only treatment-related events (new or continuing) will be reported and/or followed until resolution, acceptable medical outcome or the patient is lost to follow-up.

3.7.4.1 Toxicity Grading

All toxicities observed will be graded according to the NCI CTCAE v3.0.

For any toxicity not graded in the NCI CTCAE v3.0, intensity of the AE shall be recorded on the appropriate case report form pages based on the following definitions:

Mild: Sign or symptom noticeable, but does not interfere with normal daily activities.

Moderate: Sign or symptom sufficient to interfere with normal daily activities.

Severe: Sign or symptom is incapacitating with inability to perform daily activities.

Life Threatening: Sign or symptom significantly increases risk of death.

Fatal: Sign or symptom resulted in death.

3.7.4.2 Adverse Events

An AE is any change in physical signs, symptoms and/or clinically significant laboratory change occurring after randomization, regardless of its relationship to study drug. An abnormal laboratory value is considered to be an AE if the laboratory abnormality results in discontinuation from the study and/or is judged by the investigator to be of significant clinical importance. All AEs are to be recorded on the AE case report forms. The dates of duration,

intensity, relationship to the study drug, outcome, dose delays, seriousness and treatment will also be recorded on the case report form.

If treatment is discontinued as a result of an AE, study site personnel must clearly document the circumstances and data leading to discontinuation of treatment, using the appropriate CRF.

Relationship to Study Drug

The relationship between the administration of the study drug and the AE will be recorded as follows:

Not Related: This category applies to those AEs which, after careful consideration are clearly due to extraneous causes, e.g. disease, environment, etc.

Possibly Related: This category applies when there is modest suspicion that the AE may be related to study medication, but there is also suspicion that other etiologies such as concomitant illnesses, or other medications may be contributing to the event.

Probably Related: This category applies when the event seems to be related to study medication but there is a modest suspicion that it could be related to other causes. An example of this would be asthenia, which seems to get worse after dosing but which the investigator feels could be related to changes in the patient's underlying condition.

Definitely Related: This category applies when there is almost no consideration of other causality.

3.7.4.3 Serious Adverse Events

An AE will be classified as a serious adverse experience (SAE) when it refers to any event that results in any of the following:

- Death,
- Is life threatening,
- Requires inpatient hospitalization or prolongation of a hospitalization,
- Persistent or significant disability/incapacity,
- A congenital anomaly/birth defect, and/or
- An important medical event that may jeopardize the patient and may require medical or surgical intervention to prevent an outcome listed above.

Hospitalizations scheduled only for study treatment (decitabine or cytarabine) will not be reported or collected as SAEs. If a scheduled hospitalization is prolonged due to an AE or SAE for any patient on study, then it must be reported.

3.7.4.4 Reporting Requirements

All SAEs must be reported via fax to the appropriate safety officer (see contact information below) within 24 hours of discovery by the investigator. If a fax is not available, then the report must be made by phone. If the safety officer is not available, then the Medical Director (United States and Canada) or Medical Monitor (all countries other than the United States and Canada) must be contacted.

United States and **Canada** contacts:

Eisai Safety Center
Fax: 1-201-746-3207
Phone: 1-888-423-4636
E-mail: ESI_safety@eisai.com

All countries **other than** the United States and Canada:

Safety Officer	Medical Monitor
Christina Vogt	Elisabeth Oelmann, MD
Phone: +49-621-8782-154	Phone: +49-621-8782-725
E-mail: vogtchristina@praintl.com	E-mail: oelmannelisabeth@praintl.com

Fax #: +49-621-8782-181

A written report of any SAE will be provided to the sponsor on the appropriate SAE form and case report form. The investigator will then make an accurate and adequate report to the sponsor and to the reviewing IRB/EC on any serious and unexpected AE that may reasonably be regarded as being caused by or associated with the study drug and which was not previously anticipated (in nature, severity, or degree of incidence) in the written information or Investigator's Brochure provided to the investigator by the sponsor.

Copies of each report will be kept in the investigator's study file and adequate documentation will be provided to the sponsor, including documentation that the IRB/EC has been notified of such SAEs. Reports of all serious and unexpected AEs associated with the use of the study drug must be submitted to the applicable health authorities and IRB/EC within 15 calendar days after

their disclosure. However, fatal or life threatening experiences associated with the use of the study drug must be reported, via phone, by the sponsor to the applicable Health Authorities within 7 calendar days of disclosure. A written report must also follow within a 15-day timeframe.

Additional information relative to the patient's subsequent course must be submitted to the sponsor until the event has been resolved or until an acceptable medical endpoint has been reached.

The following SAEs or outcomes are to be reported to the sponsor if they become known to the investigator:

1a. All SAEs and deaths occurring within 30 days after study discontinuation,

1b. SAEs occurring after this 30-day time period that are considered by the investigator to be related to study treatment, or

2. The occurrence of a congenital anomaly up to 11 months after study completion.

SAEs should be followed by the investigator until resolution, a medically acceptable outcome is reached, or the patient is lost to follow-up.

4. STATISTICAL ANALYSIS METHODS AND SAMPLE SIZE

4.1 Analysis Populations

Intent-to-Treat - The intent-to-treat (ITT) population will be defined as all patients randomly allocated to a treatment group.

Modified Intent-to-Treat - The modified intent-to-treat (MITT) population will be defined as all patients randomly assigned to a treatment group who receive at least one dose of the assigned treatment.

Per-Protocol - The per-protocol (PP) population will be defined as all patients randomly assigned to a treatment group who receive at least two cycles of treatment with no major protocol deviations (e.g. ineligibility, missing assessments). Patients who die or discontinue for progressive disease prior to receiving two cycles will be included. Patients excluded from the PP cohort will be identified prior to a database lock.

Safety - The safety population will be defined as all patients who receive at least one dose study drug (decitabine or cytarabine). Patients who receive supportive care only will also be included.

4.2 Statistical Analysis

4.2.1 Descriptive Analyses

Patient Disposition: The number of patients discontinued, the reasons for discontinuation, and the number of cycles administered will be summarized by treatment arm.

Protocol Deviations: All significant deviations will be summarized by pre-determined categories (e.g. entry criteria, withdrawal criteria, concomitant therapies, etc.) for each treatment group. These categories will be listed in a statistical analysis plan and determined before database lock prior to the final analysis.

Demographics and Baseline Characteristics: Descriptive summary statistics will be provided for demographic and important baseline characteristics. For continuous variables, the number of patients, mean, standard deviation, median, minimum and maximum will be provided. For categorical variables, the number and percent of patients in each demographic/characteristic category will be summarized. The Wilcoxon rank sum test and Fisher's exact test will be used to flag differences between the treatment arms with regard to baseline demographics and characteristics for continuous and binary variables, respectively.

4.2.2 Primary Endpoint Analysis

The null hypothesis is that the overall survival experience of those patients treated with decitabine is not superior to treatment of the patient's choice (PC).

$$H_o : S_{decitabine} \leq S_{PC}$$

The alternative hypothesis is that decitabine is superior to treatment of the patient's choosing in terms of overall survival.

$$H_a : S_{decitabine} > S_{PC}$$

The primary analysis will be performed using a stratified log-ranktest, stratifying for baseline age, cytogenetic risk group, and ECOG. In addition, Kaplan-Meier product limit estimators will be used to describe overall survival and a stratified Cox's proportional hazards model will be used to calculate the hazard ratio and its corresponding confidence interval.

Assumptions for the statistical approach:

- Ratios of the treatment hazards are constant over time (proportional hazards)
- Censoring distributions are noninformative and the same between the two groups

4.2.3 Secondary Endpoint Analyses

Morphologic complete remission (CR) + CR without platelet recovery (CRp) rate – The incidence will be calculated with a corresponding confidence interval. Fisher's exact test will be used to compare the incidence between the two treatment arms. The p-value of this test will be compared to a significance level of 0.05.

The ITT population will be used in the primary analysis of the secondary endpoint.

4.2.4 Tertiary Endpoints

Ranked in order of importance as determined by Sponsor:

1. Cytogenetic complete remission (CRc) – The incidence will be calculated with a corresponding confidence interval. Fisher's exact test will be used to compare the incidence between the two treatment arms.
2. Event-free survival (EFS) – Kaplan-Meier product limit estimators will be used to describe EFS. An stratified log-rank test will be used to compare the EFS experience between the two arms. A Cox's proportional hazards model will be used to calculate the hazard ratio and the corresponding confidence interval.
3. Overall QoL score (from EORTC QLQ-C30) – The incidence of patients who have a stabilized or an improved QoL subscale score at Day 1 of Cycle 3 (> -10 point change

from pre-randomization) will be summarized and a Fisher's Exact test will be performed to compare between the treatment arms.[56-58]

4. Fatigue subscale score (from the EORTC QLQ-C30) – The incidence of patients who have a stabilized or an improved Fatigue subscale score at Day 1 of Cycle 3 (> -10 point change from pre-randomization) will be summarized and a Fisher's Exact test will be performed to compare between the treatment arms.
5. Nights hospitalized – The percentage of nights hospitalized (for medical or treatment reasons) on study will be summarized and a Wilcoxon rank sum test will be performed to compare between the treatment arms.
6. Relapse-free survival (RFS) – Kaplan-Meier product limit estimators will be used to describe RFS. Since RFS is calculated on the subset of patients experiencing a CR and the two treatment arms may not have baseline homogeneity, no formal statistical test will be performed for this endpoint.

4.2.5 Statistical Issues

Covariate Adjustment – A stratified log-rank test will be performed with regards to the primary endpoint.

Missing Data – For the primary analysis, patients lost to follow-up will be censored on the last date they were known to be alive.

Multiple Comparisons – With one comparison between two treatment groups, no adjustment for multiple comparisons is needed in the primary endpoint analysis.

4.2.6 Description of Planned Subgroup Analyses

Results from subgroup analyses will be considered exploratory in nature. The following subgroups (if sufficient numbers of patients) will be explored with respect to the overall survival and complete remission endpoints.

- *De novo* versus secondary AML
- Intermediate-risk versus poor-risk cytogenetics
- 65-69 versus 70+ years of age
- ECOG score (0-1 versus 2)
- North American versus Rest of World sites

4.2.7 Pharmacokinetic Analysis

The goals of population pharmacokinetics are:

- To characterize the pharmacokinetics of decitabine in patients with AML.
- To determine the influence of demographic variables, underlying disease, and concomitant medications on the pharmacokinetics of decitabine.
- To determine the inter-individual and intra-individual variability in pharmacokinetics of decitabine.

Plasma concentration-time data of decitabine will be analyzed by nonlinear mixed-effects modeling using the NONMEM program. For the base model, plasma concentration-time data will be fitted to one and two compartment body models with constant rate IV infusion as base models. Individual pharmacokinetic parameters such as clearance (CL) and volume of distribution (Vd) will be calculated by posterior conditional estimation technique (POSTHOC) of NONMEM. CL and Vd will be regressed against demographic variables such body weight, body surface area, age, etc. by performing generalized linear multiple regression to select potential significant covariates. Potential covariates identified in the screening process will be added to the base model separately and tested by NONMEM. Other covariates such as race, sex, disease, concomitant medications etc. will also be added to the model for their significance. All confirmed covariates will then be added to the base model incrementally and cumulatively to build the full fixed-effects model. For models and covariate evaluations, the maximum likelihood ratio test will be applied at a significance of 0.05. Diagnostic plots such as observed versus predicted concentrations, weighed residuals versus covariates will be visually evaluated, and parameter estimates and standard errors will be used for final model selection.

4.2.8 Safety Analysis

Extent of Exposure – Duration (number of cycles/infusions) of the assigned treatment and doses (cumulative and intensity) will be summarized by treatment group.

Adverse Events - All adverse events will be coded using MedDRA. The incidence of treatment-emergent adverse events (number and percent of patients reporting the adverse event at least once during the study) will be summarized by treatment arm. In addition, adverse events will be summarized by investigator attribution of relationship to study medication and by grade. Similar summaries will be provided for prevalence by treatment cycle. For each adverse event, Fisher's exact test will be used to compare the overall incidence between the two treatment arms.

Clinical Laboratory Evaluation – Applicable laboratory parameters will be graded according to NCI CTCAE version 3.0.[59] The incidence of maximum grade (number and percent of patients experiencing the maximum grade during the study) will be summarized for each laboratory parameter by treatment arm. Similar summaries will be provided by treatment cycle. For each laboratory parameter, Fisher's exact test will be used to compare the safety of the two treatment arms. Graphical displays of selected hematological parameters will also be provided.

Vital Signs, Physical Findings, and Other Safety Observations – Vital signs, findings from physical examinations, will be presented in listing format. Changes in vital signs may be explored by treatment arm.

4.2.9 Exploratory Analyses

A Cox proportional hazards model will be used to investigate the effects of important demographic and baseline characteristics on survival time. Additional exploratory analyses will be performed as appropriate.

4.3 Interim Analysis

4.3.1 Summary of Interim Analyses

Analyses will be focused on primary and key secondary efficacy measures, and safety endpoints. Additional analyses requested by the DMC will be provided as needed.

4.3.2 Interim Analysis Methods

The methods for interim analyses will be similar to that of the final analyses (see Section 4.2). The primary endpoint will be assessed using the Lan Demets alpha spending function of the O'Brien-Fleming type.[2] The first interim analysis is planned for when one-third (approximately 128) of the total number of deaths have occurred. The second interim analysis is planned for when two-thirds (approximately 257) of the total number of deaths have occurred.

4.3.3 Independent Data Monitoring Committee

An independent data monitoring committee (DMC), consisting of two expert physicians, and one expert biostatistician, will monitor this trial. The DMC will provide a suitable recommendation to the sponsor's steering committee for appropriate study direction. Such direction may include continuation of the trial as planned, early termination due to overwhelming treatment benefit, early termination due to inability to meet trial objectives, or modification to study conduct/design. A charter will be created prior to initiation of the protocol that will delineate the responsibilities of the DMC and its interactions with other trial components.

4.3.4 Timing of Interim Analyses

The interim analyses will take place shortly after the appropriate information fraction (number of events/deaths) is achieved. Based on assumptions in Section 4.4., Table 4-1 provides approximate dates of the planned analyses/DMC meetings.

Table 4-1 Projected Interim Analyses Dates

Look Number	Projected Interim Analysis Date
1	March 2008
2	November 2008
3 (Final)	June 2009

4.3.5 Interim Analyses Trial Adjustment(s)

Based upon recommendations from the DMC, the Steering Committee will provide appropriate trial direction to the Sponsor. Some of the DMC's recommendations may include one or more of the following trial adjustments (not inclusive):

Study termination due to inability to meet trial objectives – The DMC has determined that it is improbable to demonstrate a survival advantage on the decitabine arm. Secondary objectives were also considered in their decision.

Study termination due to overwhelming treatment benefit – The DMC has determined that enough evidence exists to conclude that decitabine has a significant survival benefit compared to the control arm. Secondary efficacy and safety endpoints were also considered in their decision.

Study termination due to safety issue – The DMC has determined a significant safety issue has been found that requires the study to be terminated.

Modifications to study conduct/design – The DMC has recommended modifications to the conduct and/or design of the study (e.g., extending follow-up). Appropriate changes will be made in a formal protocol amendment.

4.4 Sample Size

Number of Patients per Treatment Arm:

An estimated 480 patients will be enrolled in this study (approximately 240 patients per treatment arm). About 480 patients are required to observe 385 events (the minimum required) at the final analysis. According to trial design parameters, when 385 deaths have accrued, the study will have 80% power to detect a 25% reduction in mortality risk (median overall survival: 6 month to 8 month), and about 98% power to detect a 33.3% reduction in mortality risk (median overall survival: 6 month to 9 month).